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Spontaneous abortion and confined chromosomal mosaicism

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Summary. Confined placental chorionic mosaicism is reported in 2% of viable pregnancies cytogenetically analyzed on chorionic villi samplings (CVS) at 9–12 weeks of gestation. In follow-up studies this mosaicism has been shown to be associated with increased frequency of second and third trimester pregnancy loss or intrauterine fetal growth retardation. We have studied 54 spontaneous abortions (SA) for the detection of confined placental mosaicism and found 11 of them to be mosaic. All mosaic cases were identified among first trimester spontaneous abortions, and the mosaicism was confined to specific placental or embryonic/fetal cell lineages. These results indicate that the previously reported mosaicism in SA represents both confined and generalized types of mosaicism and that its accepted frequency of 5%–10% in SA will likely be higher. Over the whole gestational period, the confined placental mosaicism is more common than the reported rate of 1%–2% seen in viable pregnancies at CVS, and a higher proportion of pregnancy complications than previously suspected may be associated with confined placental mosaicism.

Introduction

Spontaneous abortion is a common event in human reproduction. Almost half of all conceptions and 15%–20% of clinically recognized pregnancies are estimated to be lost (Jacobs and Hassold 1987; Warburton and Fraser 1964). The majority of clinically recognized spontaneous abortions occur during the embryonic stage of development, and over 60% of these abortions are cytogenetically abnormal. The most commonly observed cytogenetic defects are trisomies, monosomy, polyploidies. Less than 10% of late spontaneous abortions show chromosomal defects (Craver and Kalousek 1987; Kalousek et al. 1990).

Traditionally cytogenetic studies of spontaneous abortions have been based on analysis of a single cultured tissue sample of embryonic, fetal, or placental origin and usually only a low number of cells have been analyzed (Boué et al. 1975; Creasy et al. 1976; Kajii et al. 1980; Hassold 1986). Several investigators focusing on chromosomal mosaicism (Warburton et al. 1978; Hassold et al. 1980; Bernert et al. 1988; Eiben et al. 1987, 1990) have concluded that approximately 5%–10% of spontaneous abortions are mosaic. In their studies although only one tissue type was analyzed, any observed mosaicism was assumed to be generalized and therefore affecting the whole conceptus.

Chromosomal mosaicism is the presence of two or more cell lines having different chromosomal complements in the same individual. Mosaicism is the result of postzygotic nondisjunction, anaphase lag, or structural rearrangement. In the conceptus the extent of the resultant mosaicism depends on the timing of chromosomal mutation occurrence, the cell lineage affected, and the viability of the mutation (Kalousek 1985). If the error occurs before blastocyst formation and affects both the progenitors of embryo and placental, the resultant mosaicism is generalized (Fig. 1A). Confined placental mosaicism (CPM) is most likely to occur as the result of the unequal distribution of the embryonic and placental progenitor cells in a mosaic blastocyst (Fig. 1B, C). Confined embryonic mosaicism originates from a mutation occurring in progenitor cells of the embryo proper (Fig. 1D).

There are three types of confined mosaicism in placenta (Kalousek 1990) defined by the involvement of trophoblast, villus stroma, or both (Table 1). CPM has been demonstrated at different stages of pregnancy. Analysis of chorionic villi samples for prenatal diagnosis at 9–12 weeks of gestation has shown confined chorionic mosaicism of all three types to occur in 1%–2% of pregnancies (Simon et al. 1985; Mikkelsen and Ayme 1987). CPM type I has also been demonstrated in viable aneuploid pregnancies at 20–40 weeks of gestation (Kalousek et al. 1989). All three types of CPM have been confirmed in term placentas of infants with prenatally diagnosed CVS mosaicism and among infants with intrauterine growth retardation (IUGR); (Kalousek and Dill 1983; Kalousek et al. 1991). In infants with prenatally diagnosed placental mosaicism a higher frequency of adverse

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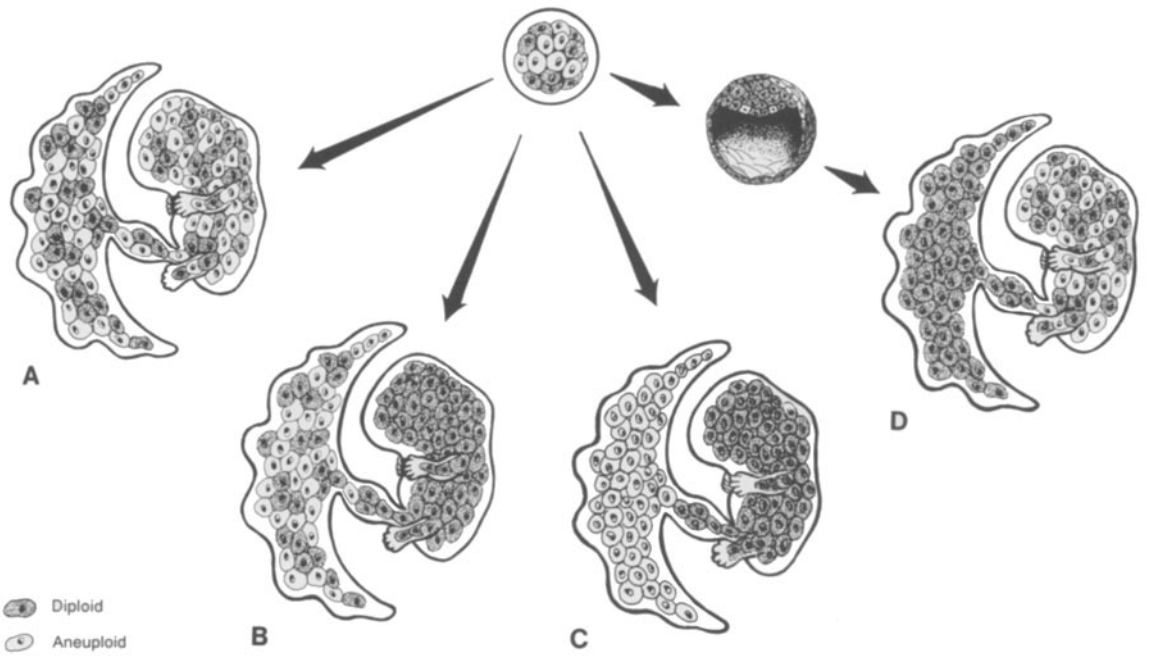

Fig. 1A–D. Diagrammatic representation of *(A)* generalized mosaicism, *(B)* confined placental mosaicism with both cell lines represented in placenta, *(C)* confined placental mosaicism with complete dichotomy between placenta and embryo/fetus, *(D)* confined embryonic mosaicism. Note that confined embroynic mosaicism originates during blastocyst development while both generalized and confined placental mosaicism develop earlier

Table 1. Types of confined placental mosaicism in gestations with a diploid embryo/fetus[a]

Tissue	Type I	Type II	Type III
Cytotrophoblast	Mosaic or non-mosaic aneuploidy	Normal (diploidy)	Mosaic or non-moasic aneuploidy
Chorionic stroma	Normal (diploidy)	Mosaic or non-mosaic aneuploidy	Mosaic or non-mosaic aneuploidy
Embryonic/fetal tissues	Normal (diploidy)	Normal (diploidy)	Normal (diploidy)

[a] For aneuploid embryo/fetus, the same types of confined placental mosaicism (CPM) apply but aneuploidy/diploidy are reversed

outcome has been documented and may include IUGR or an increased rate of spontaneous abortion (Guerneri et al. 1989; Johnson et al. 1990, Kalousek et al. 1991).

The purpose of this study is to assess both frequency and type of confined mosaicism occurring in spontaneous abortions.

Materials and methods

Specimens included fresh products of conception, which were either spontaneously lost or therapeutically removed following ultrasound diagnosis of missed abortion, incomplete abortion, or blighted ovum.

Based on gross morphological examination and clinical history, the specimens were categorized as early (up to 9 weeks of development) or late (between 9–18 weeks of development) spontaneous abortion (Kalousek et al. 1990). Early spontaneous abortions were further subdivided into specimens with well-developed embryos, growth-disorganized embryos, and ruptured or fragmented chorionic sacs without identifiable embryos. Histological examination of all chorionic sacs or placentas was also performed.

For each specimen chorionic villi, chorionic plate and amnion were sampled for cytogenetic analysis. Primary cultures were established from all of the tissues; in addition a direct peparation of cytotrophoblast was prepared on the chorionic villi (Simoni et al. 1983; Simoni 1987; Eiben et al. 1986). From each cultured specimen multiple in situ cultures were harvested and trypsin-Giemsa banded, and 15 metaphases for each tissue were analyzed. The tissue was classified as mosaic if three or more cells had an identical karyotype that differed from the rest of the cells analyzed. The analysis of multiple in situ cultures established from one tissue sample was used to exclude cultural artifacts.

DNA flow cytometry was performed on cell suspensions from amnion, chorionic plate, and chorionic villi of three cases identified as 2n/4n mosaics.

Results

We classified 48 specimens as early spontaneous abortions, represented by 30 intact chorionic sacs with or without embryos and 18 ruptured or fragmented chorionic sacs. Only 6 late spontaneous abortions were studied. Cytogenetically, of the total 54 spontaneous abortions 12 were normal, 31 nonmosaic abnormal, and 11 mosaic abnormal complements (Table 2). The overall rate of confined mosaicism was 11/54 (20%). In the 11 mosaics there were 7 chromosomal trisomies, 3 polyploidies, and 1 chromosomal monosomy with structural rearrangement (Table 3). The distribution of abnormal cells and diploid cells among different tissues of the conceptus for each mosaic abortus is shown in Table 4. All mosaic abortuses (11/54) were shown to represent a con-

fined type of mosaicism. Of the 6 that showed type-I CPM involving tetraploidy and aneuploidy, 3 abortions showed a normal karyotype on direct preparation and tetraploidy in all cultures of chorionic villi, chorionic plate, and amnion, and in the other 3 a cell line in trophoblast showed the loss of a trisomic chromosome from the aneuploid constitutional karyotype. In 2 cases a normal cell line in trophoblast was derived from trisomy 3 and 16 and in 1 case a loss of chromosome 13 in a diploid karyotype with structural rearrangement of chromosome 18 resulted in monosomy 13. Three cases of type-II CPM involving mosaicism confined to chorionic stroma were detected. In 3 of them the conceptus was aneuploid (trisomy 2, 7, and 16) with a normal diploid cell line in villus stroma (trisomy 2, 7, 16) and chorionic plate (trisomy 7 and 16). In 1 case the conceptus was diploid with trisomy 4 confined to the stroma of chorionic villi.

Cell suspension from noncultured amnion and chorionic villi of 3 mosaic cases with 2n/4n showed on DNA flow cytometry a high proportion of tetraploidy. Similar to other cytogenetic studies of spontaneous abortion the most commonly observed abnormal chromosomal complement was that of trisomy 16. Of 7 abortuses with trisomy 16, 3 were mosaic and 4 were nonmosaic. Abortuses with trisomy 2 and trisomy 4 showed 1 mosaic and 1 nonmosaic chromosomal complement.

Of the 18 growth-disorganized embryos 8 were mosaic. This represents 44% mosaicism in this morphological category compared to a significantly lower rate of mosaicism for the other first trimester abortion specimens (3/30 or 10%).

Table 2. Specimen morphology and mosaicism detection in 54 spontaneous abortions

Specimen morphology	Cytogenetic findings			Total
	Nonmosaic complement		Mosaic complement	
	Normal	Abnormal		
Intact chorionic sac with or without embryo	2	19	9	30
Fragmented or ruptured chorionic sac	4	12	2	18
Fetus and placenta	6	0	0	6
Total	12	31	11	54

Table 3. Specimen morphology and type of chromosome defect in 11 mosaic cases

	Autosomal trisomy	Polyploidy	Autosomal trisomy
Well-formed embryo	1	0	0
Growth-disorganized embryos	5	2	1
Fragmented chorionic sacs	1	1	0
Total	7	3	1

Discussion

Previous studies of spontaneous abortions have estimated the frequency of mosaicism in spontaneous abortions to be approximately 5%–10% (Eiben et al. 1990; Warburton et al. 1978; Hassold et al. 1980). As in these studies only one tissue was examined, the distinction between generalized mosaicism affecting the entire conceptus and

Table 4. Number of cells and tissues involved in 11 mosaic specimens[a]. NA, Not analyzed; E. mosaicism confined to embryo (see Fig. 2)

Mosaicism	Direct preparation Villi	Cultured tissues			Mosaicism type
		Villi	Chorionic plate	Amnion	
46,XY/47,XY,+2	0/15	4/11	0/15	NA[b]	2
46,XX/47,XX,+3	8/ 3	0/15	0/15	NA[b]	1
46,XX/47,XX,+4	6/ 0	0/11	20/ 0	20/ 0	2
46,XX/47,XX,+7	0/15	6/ 9	5/ 7	0/15	2
46,XY/47,XY,+16	5/ 7	0/15	0/15	0/12	1
46,XX/47,XX,+16	0/15	3/16	3/ 7	NA[b]	2
46,XX/47,XX,+16	0/15	NA	0/15	13/26	E
46,XY/92,XXYY	17/ 0	0/12	0/ 3	0/ 8	1
46,XY/92,XXYY	16/ 0	0/20	0/16	0/31	1
46,XY/92,XXYY	13/ 2	0/15	0/15	NA[b]	1
45,XX,−13,del(18p) /46,XX,del(18p)/ 46,XX,i(18q)	14/11/10	NA	0/10/11	NA[b]	1

[a] Morphologically all mosaics were growth-disorganized embryos except for trisomy 4 and one tetraploidy, which presented as fragmented chorionic sac, and one trisomy 16 with morphology of a well-formed embryo

[b] In these specimens the amnion was not formed and therfore could not be sampled for tissue culture and cytogenetic analysis

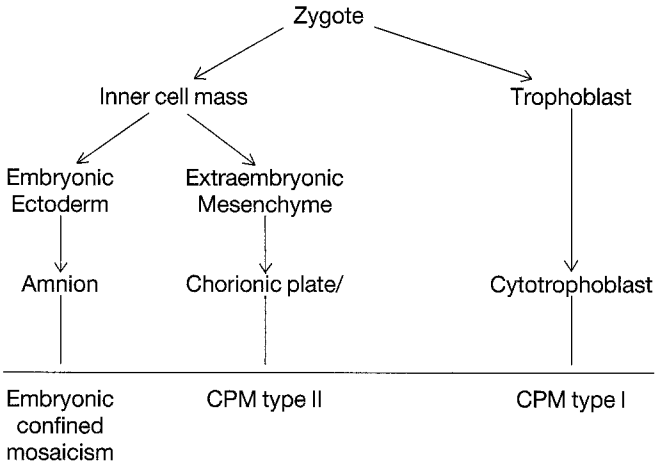

Fig. 2. Diagram of embryological derivation of placental tissues and corresponding confined mosaicism type when second isolated cell line is identified

mosaicism confined to a specific cell lineage could not be made. Also examining only one tissue reduces the possibility of detecting mosaicism. The results of the present study provide evidence for a higher frequency of mosaicism in spontaneous abortion and suggest that the majority of mosaic spontaneous abortions represent a confined placental mosaicism.

Cell division errors producing the mosaic cases in this study involve nondisjunction or anaphase lag giving rise to diploid/aneuploid mosaicism and also cleavage errors resulting in diploid/tetraploid cell lines. The detected mosaicism is confined to a specific cell lineage, such as trophoblast, extraembryonic mesenchyme, or embryonic ectoderm (Fig. 2). Thus for each of the mosaic cases the approximate timing of mutational events during early embryonic development can be deduced based on the involvement of specific developmental cell lineage(s) and under the assumption that the viability of the mutation is the same in all embryonic tissues.

The postzygotic mutational errors in the 11 mosaic abortion specimens shown in Table 4 consist of 8 events chromosomal segregation error and 3 cleavage errors. For segregation errors the original karyotype can be implied from the predominant karyotype present in multiple cell lineages. For cleavage errors the cytotrophoblast, being one of the early delineated cell lineages, likely represents the constitutional karyotype. Of the 11 mosaic specimens, 6 were trisomic and 4 were diploid. For the last case in Table 4 DNA studies of the parental origin of chromosome 13 would be needed to establish whether the zygote was monosomic or disomic for this chromosome. Cleavage errors were more common than nondisjunction or anaphase lag in diploid zygotes. Although placental cultures seem to be particularly prone to clonal growth of tetraploid cells (Hunt and Jacobs 1985a, b), the high proportion of tetraploidy in noncultured amnion and chorionic villi from our mosaic tetraploidy specimens indicates that detected tetraploidy in cultures represents a true finding and not a culture artifact.

A large proportion of the mosaic conceptions are growth-disorganized embryos. To date cytogenetic studies, utilizing conventional tissue culture and cytogenetic analysis of conceptions with the morphology of growth-disorganized embryos have revealed the entire range of cytogenetic abnormalities that are usually observed in spontaneous abortion studies (Kalousek 1987). Histological examination of these specimens typically shows abnormal villus morphology with hydropic degeneration, fibrosis and poor cytotrophoblast development. The prolonged intrauterine retention of some of these conceptuses and the continued growth and development of the chorionic sac in the absence of a developing embryo may be related to the presence of a normal cell line facilitating the continued survival of the gestational sac. A similar survival advantage has been observed in viable advanced trisomy 13 and 18 gestations that have a normal cell line confined to cytotrophoblast (Kalousek et al. 1989).

The most interesting is a case of trisomy 16 in which both direct preparation and chorionic cultures showed trisomy 16 in nonmosaic form, but amnion revealed 2 cell lines, trisomy 16 and a diploid cell line. Morphologically the conceptus showed normally developed embryo, which is a very unusual finding in trisomy 16. The morphology therefore supports the diagnosis of true confined embryonic mosaicism for diploidy and trisomy 16 cell lines.

The frequency of confined mosaicism in the first trimester abortion specimens is high. It is even higher when specimens with the morphology of growth-disorganized embryo are singled out. The mechanisms of contribution of confined placental mosaicism to the spontaneous loss is not understood.

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