

CHROMOSOME ANALYSIS OF 52 SPONTANEOUS ABORTUSES IN BRAZIL

Denise Pontes Cavalcanti¹, Iris Ferrari² and Walter Pinto Jr.¹

ABSTRACT

Chromosome analysis of 52 spontaneous abortuses from first and second-trimester pregnancies showed that 56.7% of them had an abnormal chromosome constitution. Autosomic trisomies were the most common finding among the chromosomally abnormal abortuses. Evaluation of the origin of the 46,XX normal karyotypes through chromosome polymorphisms showed a high level of maternal cell contamination in the cultured material. On the basis of the present data and of a review of other reports, we conclude that a 46,XX karyotype in a first-trimester abortus can only be considered real after a comparative study of chromosomal polymorphisms in the abortuses and their respective parents.

INTRODUCTION

It is generally accepted that chromosome abnormalities are the major cause of spontaneous abortions. The majority of these abortions, about 90%, occur during the first trimester of pregnancy. There is a tendency for specimens with abnormal chromosome constitutions to be retained *in utero* for several weeks. Thus, although gestational age is usually known, it does not necessarily reflect the stage of embryo development because of the variable time of *in utero* retention (for a review, see Boué *et al.*, 1985).

About 60% of first-trimester abortuses are chromosomally abnormal. This rate, however, may be even higher if we take into account undetected abortions,

¹ Departamento de Genética Médica, Faculdade de Ciências Médicas, UNICAMP, Caixa Postal 1170, 13081 Campinas, SP, Brasil. Send correspondence to D.P.C.

² Departamento de Genética e Matemática Aplicada à Biologia, Faculdade de Medicina de Ribeirão Preto, USP, 14049 Ribeirão Preto, SP, Brasil.

especially the earlier or subclinical spontaneous abortions which occur at home, and the fact that part of chromosomally normal 46,XX spontaneous abortuses may be due to contamination with maternal tissue in the culture.

Approximately 50% of chromosomally abnormal abortuses are autosomic trisomies, though the most common finding is monosomy X (Cavalcanti, 1986).

In this report we present the results of a chromosomal study in a sample of 52 spontaneous abortuses in Brazil. Maternal contamination of cultures is also discussed.

MATERIAL AND METHODS

Samples from 52 spontaneous abortuses with less than 20 weeks of gestational age were collected by amniocentesis or uterine curettage. Gestational age was calculated from the first day of the last menstrual period until the day of the abortion.

Amniotic fluid was obtained by amniocentesis from seven patients with inevitable abortions during ultrasound examination. About 10 ml of amniotic fluid was collected in disposable syringes, and cultured by the method of Nazareth *et al.* (1981), with slight modifications.

Tissue samples usually consisting of placental tissue were obtained by uterine curettage from the remaining patients with inevitable, incomplete or missed abortions. The specimens were placed in a sterile bottle with 0.9% saline and carried to the cytogenetic laboratory. The tissue was washed two or more times with sterile saline to remove excess erythrocytes and clots. The chorionic villi were separated from the maternal decidua by dissection, minced and transferred to an Erlenmeyer flask containing about 10-15 ml of a 1:250 trypsin solution (Difco). This solution was shaken in an electromagnetic stirrer at room temperature for 10-15 minutes. A 10-15 ml volume of Ham F-10 complete medium (Cultilab) containing 20% fetal calf serum (Cultilab) was added to the solution. The cell suspension thus obtained was distributed among different tubes and centrifuged at 1000 rpm for 10 minutes. The cell pellet from each tube was resuspended in 2 ml complete medium, distributed among Leighton culture tubes and incubated at 37°C. Usually 6-12 Leighton tubes were started from each abortus.

When a sufficient number of cells had grown, and the cultures showed adequate mitotic activity, they were sequentially exposed to 0.05 ml of 4×10^{-5} M colchicine solution for 2-4 hours, trypsinized, centrifuged and submitted to the progressive hypotonia technique. This technique, developed by one of us (Pinto Jr.), consists of the addition of 1 ml of hypotonic solution (0.075 M KCl) to the cell pellet every 3 minutes at 37°C until a 4 ml volume is obtained. Three minutes after the addition of the last ml, the hypotonic treatment is stopped by the addition of 0.5 ml of fixative solution (3:1 methanol/glacial acetic acid). After centrifugation, the pellet is submitted to several changes of fixative.

At least three cells from each specimen were analyzed. Standard banding methods (QFQ, GTG and CBG) were used for chromosome analysis and polymorphism identification.

RESULTS

Of the 52 specimens used for tissue culture, 34 (65.4%) showed satisfactory growth, permitting chromosome analysis. Amniotic fluid cultures showed a lower growth rate (42.8%) than placental or fetal tissue cultures (68.8%) (Table I). No bacterial contamination occurred in any culture.

Table I - Growth rate of spontaneously aborted specimens according to cultured tissue.

Cultured tissue	Total number	Growth		%
		Negative	Positive	
Amniotic fluid	07	04	03	42.8
Chorionic villi/fetal membrane	45	14	31	68.8
Total	52	18	34	65.4

The 46,XX karyotype was constant among the 23 chromosomally normal abortuses. Analysis of chromosome polymorphisms by QFQ and CBG banding in 11 of these specimens and their respective parents permitted us to identify seven in which the maternal and fetal polymorphisms were identical, suggesting that the 46,XX cells were clearly maternal.

The results of cytogenetic analysis of 34 successful cultures are summarized in Table II. Excluding the 7 cases with maternal cell contamination, the frequency of chromosomal abnormalities was 56.7%. More than one-half (62.6%) of the chromosomally abnormal abortuses showed autosomal trisomies. The remaining chromosomal abnormalities were a monosomy X, a double trisomy, a triploidy and a tetraploidy.

The mean gestational age of chromosomally normal and abnormal abortuses was 12.6 [s(x) = 4.3] and 11.3 weeks [s(x) = 1.3], respectively, i.e. gestational age was slightly lower for abnormal abortuses.

Mean maternal age at the time of abortion for normal and abnormal abortuses was 27.8 [s(x) = 4.3] and 30.4 years [s(x) = 6.5], respectively. Although the standard deviation is large, an effect of maternal age may be suggested.

Table II - Chromosome constitution of 34 cultured abortuses.

Karyotype	Number		%
Normal			
46,XX	23	23	
46,XY	—		67.6
Abnormal		11	32.3
Monosomy X	01	01	9.1
Trisomy			
47,XX,+2	02		
47, +9	02		
47,XY,+16	02	07	63.6
47,XX,+18	01		
Double trisomy	01	01	9.1
Triploidy			
(69,XXX)	01	01	9.1
Tetraploidy			
(92,XXXX)	01	01	9.1

DISCUSSION

The rate of successful cell culture in the present study was 65.4%, which is higher than the 53% rate reported by Creasy *et al.* (1976), but lower than the 91.8% rate described by Hassold *et al.* (1978). The low growth rate of amniotic fluid, chorionic villi and fetal membrane cultures may reflect longer periods of *in utero* retention, which reduces cell viability (Cavalcanti, 1986).

The rate of chromosomal abnormalities found among the specimens studied (32.3%) was low, but when corrections were made for cases due to maternal cell contamination the rate became 56.7%. Another factor that may have influenced this rate is gestational age, since 17% were second-trimester abortuses. The proportion of chromosomally abnormal abortuses depends on the gestational age at expulsion. Among second-trimester abortuses the frequency of chromosome abnormalities is relatively

low, i.e. 44% between 13 and 17 weeks (Lauritsen, 1976) and 11.7% beyond 20 weeks (Creasy *et al.*, 1976).

Despite the small sample, the rates of autosomal trisomies were slightly higher (63.6%) than in previous reports (about 50%). Coincidentally we found two trisomies of chromosome 2, whose frequency in chromosomally abnormal abortuses is lower than 2%.

Based on our rates, even though a reduced sample was used, 7 of 11 (63.6%) were due to maternal cell contamination, a fact calling our attention to this very controversial point, mainly because contamination conceals the real frequency of chromosomal abnormalities in studies of spontaneous first-trimester abortuses. The rates of maternal cell contamination in cultures of spontaneous abortuses was evaluated by Hassold *et al.* (1980), who found a rate of 21.4% using chromosome heteromorphisms. Based on the proliferation, morphology and cytogenetic data (46,XX karyotype) of cultured cells, Boué *et al.* (1967) found that 38 of the 132 successful cultures of spontaneous abortuses were of maternal origin.

Several studies have reported an overall excess of females among chromosomally normal abortuses (Kajii *et al.*, 1973; Lauritsen *et al.*, 1976; Hassold *et al.*, 1980; Procter *et al.*, 1986). However, an excess of males has been observed in other studies (Kajii *et al.*, 1980; Creasy *et al.*, 1976). This different sex ratio is improbable since all the studies are remarkably similar concerning the other variables, including the sex ratio among trisomic abortuses (Hassold *et al.*, 1980). In addition, there is evidence to suggest that the differences in sex ratio may reflect variable levels of maternal cell contamination. Hassold *et al.* (1983) estimated the sex ratio at 1.32 among chromosomally normal abortuses, assuming that all of the studies were affected to some degree by maternal cell contamination.

Based on our experience and suggestive data in the literature concerning maternal cell contamination in cultures of spontaneous abortuses, we conclude that a 46,XX karyotype for a first-trimester spontaneous abortus may be considered real only after taking into account the possibility of maternal cell contamination through a comparative study of chromosomal polymorphisms in the abortuses and their respective parents.

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RESUMO

O estudo cromossômico em uma série de 52 abortos espontâneos dos primeiro e segundo trimestres gestacionais mostrou que 56.7% apresentavam anormalidades cromossômicas. As tris-

somias autossômicas representaram o achado mais comum entre os abortos cromossomicamente anormais. Uma avaliação, através de polimorfismos cromossômicos, da origem dos abortos normais, 46,XX, mostrou um alto índice de contaminação com células maternas no material cultivado. Considerando os nossos dados e os da literatura, concluímos que um cariótipo 46,XX em um aborto espontâneo do 1^o trimestre só pode ser considerado verdadeiro após afastar-se a possibilidade de contaminação materna através do estudo comparativo de polimorfismos cromossômicos do aborto com seus respectivos pais.

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