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Banding studies on spontaneous and threatened abortion

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Abstract
Chromosome studies were performed on 288 consecutively collected early spontaneous abortuses and their parents and on 103 cases of threatened abortions by means of Q- and C-banding techniques. The frequency of chromosome anomalies was 61% in first trimester abortions and 55% in the entire series. In the parents the frequency of major chromosome anomalies and chromosome variants was 0.76% and 26.9%, respectively. The mean maternal age was increased in abortions with trisomy 9, 15 and 21. The origin of the extra chromosome or chromosome set was determined in trisomy 13 (1 case), trisomy 16 (11 cases) and in triploidy (9 cases). The recurrence risk following an abortion was 14.2% if there had been no previous abortions. If there had been previous abortions the frequency of a subsequent abortion was 45% in cases with a karyotypically normal index abortus and 20% in cases with a karyotypically abnormal index abortus. Of the mothers with threatened abortion, 43% proceeded to term, while 57% aborted. All infants had a normal karyotype whereas 56% of the abortuses were karyotypically abnormal.

Keywords: spontaneous and threatened abortion, karyotype, cytogenetic analysis, chromosome anomalies

1. Introduction
It is estimated that about 15% of all recognized pregnancies terminate in spontaneous abortion (Reid *et al*., 1972) [12].
Morphological studies of more than 1,000 spontaneous abortuses led Mall and Meyer (1921) [20] to the conclusion that an abnormal uterine environment was the main cause of spontaneous abortion. On the other hand Streeter (1931) [19] and Hertig and Rock (1949) [18] advanced the hypothesis, based on pathological-anatomical examinations of surgically removed early fertilized ova, that the principal aetiological factor in spontaneous abortion was an intrinsic anomaly in the fertilized ovum, a so-called "germ-plasm defect". Evidence in support of this hypothesis has recently been provided by the observation that a high proportion of early spontaneous abortuses are chromosomally abnormal.
Penrose and Delhanly (1961) [17] were the first to describe a spontaneously aborted fetus with an abnormal karyotype. Since then, a large number of studies have demonstrated the importance of chromosome anomalies in the fetus as causative factors in spontaneous abortion (Hamerton, 1971) [15].
The early chromosome investigations on spontaneous abortion were carried out using conventional staining techniques. However, the chromosome banding technique introduced by Caspersson *et al*. (1970) [16] made it possible to identify each chromosome and to study the aberrations in greater detail, and in some instances also to establish their origin (Jonasson *et al* 1972) [13].
In the present report the frequency of chromosome anomalies in spontaneous abortuses and their parents is estimated. The various abnormal karyotypes, and in some cases their origin, are described by means of banding techniques. In addition, an analysis is given of the recurrence risk in pregnancies following an abortion in which the fetus had been karyotyped. Finally, the results obtained in spontaneous abortions are compared with those in a series of threatened abortions occurring during the same period of time.

2. Material and Methods
The study includes a total of 288 consecutive spontaneous abortions occurring during the first 16 weeks of pregnancy and 103 consecutive cases of threatened abortion admitted to hospital during the same period of pregnancy. The 288 abortuses comprised cases in which pure embryonic tissue was available for karyotyping, and the 103 threatened abortions comprised cases from a single hospital over the period from Aprel, 2013 to May, 2014. A follow-up study of the parents of spontaneous abortions, involving 97% of the couples, was carried out in November 2015, i.e.1,5 years after the karyotyped abortion had occurred.

3. Results
Cytogenetic analysis of the abortuses
Successful cell growth and karyotyping were obtained in 255 of the 288 aborted specimens, i.e. The success rate of analysis was 89%. Of the 33 cases in which cultivation failed, 19 (58%) were contaminated; if these cases are excluded the success rate is 95%.
Chromosome anomalies were diagnosed in 140 of the 255 abortuses (55%). The type of aberrations and their relative frequencies are given in Table I. The most common chromosome anomaly in the series was autosomal trisomy (65 cases) which comprised 46% of the abnormal karyotypes. The relative frequency of each type of trisomy, however, varied considerably, as seen from Table I. Trisomy 16 (21 cases) thus accounted for 32% and trisomy 15(11 cases) for 17% of the autosomal trisomies. Monosomy X (45,X) was second in frequency among the identified chromosome anomalies: it occurred in 40 cases, i.e. 29% of the abnormal abortuses. X

chromatin preparations were available in 39 of the cases, all of which were negative. A translocation was diagnosed in four abortuses (3%). It was familial in one case only, at (13; 14) (p11 ;q 11) pat. Robertsonian translocation.

Table I: Distribution of 140 karyotypically abnormal spontaneous abortuses

Karyotype of abortus	No.	%
Tetraploidy	12	9
Triploidy	14	10
Monosomy X	40	29
Trisomy 2	1	-
Trisomy 4	2	-
Trisomy 7	2	-
Trisomy 8	3	2
Trisomy 9	3	2
Trisomy 10	2	-
Trisomy 13	1	-
Trisomy 14	4	3
Trisomy 15	11	8
Trisomy 16	21	15
Trisomy 18	6	4
Trisomy 21	4	3
Trisomy 22	1	-
Trisomy X	2	-
Double trisomy	1	-
Mosaicism	1	-
Translocations	4	3
Miscellaneous	5	4

Grouping ot the karyotyped abortuses according to gestational age showed that 164 (64%) occurred in the first trimester of pregnancy and the remaining 91 during the 13th to 16th gestational week. The first trimester was defined as the first 90 days of pregnancy, calculated from the first day of the last menstrual period. The frequency of chromosome anomalies in first trimester abortions was 61% as compared with 44% in abortions of 13-16 weeks' gestational age. The difference is significant

$$(\chi_1^2 = 6.8469; P<0.01).$$

Cytogenetic analysis of the parents of spontaneous abortions

Major chromosome anomalies were found in four parents, i.e. 0.76%. A translocation was found in the father of the abortus with the 13/14 translocation mentioned above. One mother had a balanced t(5; 15) (q13;q25) translocation which was also found in her only child, and another mother had a balanced t(X;5) (p11;pl5) translocation, but in both cases cultivation of the abortuses failed. Finally, one mother with a 47,XXX karyotype had a 45,X abortus. She had previously given birth to three healthy children, all with normal karyotypes.

Eighty couples were studied in detail with respect to the occurrence of marker chromosomes or so-called chromosome variants by means of the Q-banding technique A marker chromosome was defined as a chromosome which can be readily recognized by its homologous partner by two individual observers independently. The Ychromosome was measured according to the method of Nielsen and Friedrich (1972) [14].

The frequency of chromosome variants in the parents was 26.9%. No significant difference in the overall frequency of chromosome variants was found between parents of abortuses with normal and abnormal karyotypes (27.8% and 26.2%, respectively). There was, however, a significant difference in the distribution of the various types of chromosome variants between the two groups. Thus the frequency of parents in w hom one was a carrier of an enlarged secondary constriction of chromosome No. 9 (9qh+) was significantly higher (P<0.05) among parents of abortuses with abnormal karyoty pes (14.8%) than among parents of abortuses with normal karyotypes (5.6%).

Parental age

In Table II the abortuses arc grouped according to karyotype, and the mean maternal and paternal ages are stated. The mean maternal age was higher in trisomy 9, trisomy 15 and trisomy 21-22 than in karyotypically normal abortuses. The difference between the class frequencies was, however, significant only for mothers of abortuses with trisomy 15 (χ_1^2 = 10.3821; P<0.01). No significant difference in the age distribution was observed with regard to paternal age in trisomy 15 (χ_1^2 = 1,4589; P=0.23). In contrast, the paternal age in abortuses with trisomy 16 was significantly higher than that in abortuses with normal karyotype (χ_1^2 = 10.4458; P<0.01).

Table 2: Mean maternal and paternal ages among different groups of spontaneous abortions

Karyotype	No. of abortuses	Mean maternal age (years±S.D.)	Mean paternal age (years±S.D.)
Karyotype normal	115	27.1±5.5	28.2±5.7
Karyotype abnormal	140	27.3±5.6	29.3±7.9
Tetraploidy	12	26.7±4.6	26.4±3.2
Triploidy	14	25.7±4.8	27.6±2.6
45,X	40	26.6±4.6	28.3±4.7
Trisomy 8	3	27.7±6.4	28.7±7.3
Trisomy 9	3	31.7±9.6	32.0±8.2
Trisomy 14	4	23.8±2.5	25.3±2.7
Trisomy 15	11	32.8±8.6	32.7±9.3
Trisomy16	21	27.3±4.1	31.4±7.1
Trisomy 18	6	27.2±5.3	31.2±8.0
Trisomy 21-22	6	30.2±5.8	30.4±4.0

The age distribution of the parents of the entire series of spontaneous abortions is considered to be fairly representative of the age distribution of the parents in the population from which the abortions were drawn. Thus the mean maternal and paternal age of the 255 karyotyped abortuses was 27.2 years and 28.8 years, respectively. In comparison, the mean ages of mothers and fathers of children born in Baku during the period of study was 26.4 years and 29.6 years, respectively.

Origin of trisomy and triploidy

The meiotic and mitotic mechanisms leading to trisomy 13-15 were investigated using fluorescence marker chromosomes, whereas the mechanisms leading to trisomy 16 and triploidy were studied by means of sequential Q-and C-banding techniques.

In one trisomy 13 abortus non-disjunction seemed to have occurred in the second meiotic division in the mother. This was the only case of trisomy D in which the origin of the non-disjunction could be determined by the analysis of

fluorescence markers.
Eleven of the trisomy 16 cases (Table I) were informative with respect to the origin of the supernumerary 16-chromosome. In eight cases (72%) the extra chromosome originated in the mother, and in three cases (28%) in the father. Of the eight cases of maternal origin, seven (87%) were due to non-disjunction during the first meiotic division, and the last was caused by a failure in the second division. In the three cases of paternal origin, nondisjunction had occurred during the first meiotic division in two cases, and during the second division in one case.
The origin of the supernumerary haploid chromosome set was determined in nine of the triploid cases (Table I). In seven cases (78%) non-reduction during the first meiotic division in the mother seems to be the mechanism. In two cases non-reduction during the first meiotic division in the father could be the mechanism. However, fertilization by dispermy was also a possibility.

Recurrence risk
The1^{1}/2 - 3^{1}/2 years' follow-up study has shown that a couple's risk of having another abortion following the case leading to their ascertainment was correlated with the number of previous abortions and with the karyotype of the index abortus of a total of 134 couples with no history of previous abortion other than the karyotyped one, 19(14.2%) had another abortion in the following pregnancy. Forty couples had experienced one or more spontaneous abortions prior to the karyotyped one. In 20 of these the index abortus was karyotypically normal and the frequency of subsequent abortion was 45%, whereas in the remaining 20 couples in whom the index abortus was karyotypically abnormal the frequency of subsequent abortion was 20%. (In the follow-up study each woman is only represented by one index abortion. One woman with an ectopic pregnancy and 65 who had not wanted to, or not been able to, conceive have been excluded.

Analysis of threatened abortions
A total of 103 mothers were admitted because of threatened abortion. Of these 44 (43%) gave birth to 39 full-term, and five to premature livebom infants. One of the premature infants died after two days. Karyotyping was performed in 34 infants and all were normal. The remaining 59 (57%) of the mothers miscarried. The aborted fetuses were chromosomally abnormal in 56% of these cases.

4. Discussion
Chromosome anomalies in the fetus are the principal cause of early spontaneous abortion. They were found in 61% of the first trimester abortions and in 55% of all abortions in the present study. The success rate of analysis in the present study was 89%. The success rates in the two above-mentioned reports were 70% and 68%, respectively.
Based on these data the overall frequency of chromosome anomalies in clinically recognizable first trimester abortions is estimated at 60%. It should be pointed out that this estimate is restricted to cases admitted to hospital. If very early spontaneous abortions could be included, the frequency of chromosome anomalies would undoubtedly be much higher. This view is supported by the fact that gestational age and frequency of chromosome anomalies among abortuses are inversely related.
The mutagenic factors which cause the chromosome anomalies in the fetus are largely unknown. Chromosome anomalies are rarely observed in parents of spontaneous abortions (Bingol B., Abike F., 2012; Kim J.W, Lee W.S, 2010) [1, 4]. In the present survey the frequency of chromosome aberrations in one of the parents was 0.76%. In comparison, the frequency was 0.58% in a combined series of 54,749 liveborn infants. On the other hand, there is evidence that for parents one of whom is a carrier of a balanced translocation, there is an increased risk of recurrent spontaneous abortion(Alcázar J.L, Ruiz-Perez M.L, 2000; Patel B., Trivedi V., 2000) [10, 11].
In the present series a significantly higher frequency of carriers of an enlarged secondary constriction of chromosome No. 9 (9qh+) was found among parents of abortuses with abnormal karyotypes as compared with parents of abortuses with normal karyotypes. A significantly increased frequency of the 9qh+ variant was also observed in mothers of newborn infants with chromosome. The series mentioned are all relatively small and it is therefore, at present, very difficult lo assess the significance of the chromosome variant 9qh + in the origin of chromosome anomalies in the fetus.
Advanced maternal age has been reported in cases of trisornic abortuses, particularly in those with acrocentric chromosomes. No increased mean maternal age has been found in abortuses with tetraploidy, triploidy, monosomy X and translocations. The mean maternal age was increased in the present study for 9, 15 and 21 trisomies but the increase was only significant for mothers of trisomy 15. A significantly increased paternal age was found for 16 trisomy (Park I.Y, 2006; Tannirandorn Y., Sangsawang S., 2003) [6, 9].
An attempt was made, by means of the banding techniques, to test the significance of increased paternal age as a predisposing factor in the aetiology of trisomy 16. However, in only three of the 11 informative cases did the extra chro-mosome No. 16 originate in the father, and, consequently, no definite answer to that question can be given at present. In seven out of nine informative triploid cases, non-reduction during the first meiotic division in the mother seems to be the underlying mechanism (Wyatt P., Owolabi T., 2005; Mulik V., Bethel J., 2004.) [7, 8] The data presented indicate that failure of the first meiotic division of the mother seems to be the most frequent mechanism leading to both trisomy 16 and triploidy.
A couple's risk of having another abortion following the case leading to their ascertainment was correlated with the number of previous abortions and with the karyotype of the index abortus. In couples with no history of previous abortion other than the katyotyped one, the frequency of abortion in the following pregnancy was about 15%,. On the other hand, if there had been an nbotuon previous to the karyotyped one, the recurrence risk was correlated with the karyotype of the index abortus(Baranov V.S, Kuznetsova T.V., 2007; Martínez M., Méndez C., *et al*. 2010) [5, 3] In couples in whom the index abortus was karyotypically abnormal the frequency of a subsequent abortion was 45'% whereas n couples in whom the index abortus was karyotypically abnormal the frequency of subsequent abortion was 20%.

Spontaneous abortion seems to represent an effective protective mechanism against the birth of children with chromosome abnormalities (Werner M., 2012) [2]. This view is sup ported by the investigation of patients with threatened abortions in the present series. Of the aborted fetuses- 56% were chromosomally abnormal, but the karyotypes of the liveborn infants were normal in all cases.

5. Conclusion

The frequency of chromosome anomalies was 61% in first trimester abortions and 55% in the entire series. In the parents the frequency of major chromosome anomalies and chromosome variants was 0.76% and 26.9%, respectively. The mean maternal age was increased in abortions with trisomy 9, 15 and 21. The origin of the extra chromosome or chromosome set was determined in trisomy 13 (1 case), trisomy 16 (11 cases) and in triploidy (9 cases). The recurrence risk following an abortion was 14.2% if there had been no previous abortions. If there had been previous abortions the frequency of a subsequent abortion was 45% in cases with a karyotypically normal index abortus and 20% in cases with a karyotypically abnormal index abortus. Of the mothers with threatened abortion, 43% proceeded to term, while 57% aborted. All infants had a normal karyotype whereas 56% of the abortuses were karyotypically abnormal.

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