Am J Hum Genet 25:539–547, 1973

Banding Analysis of Abnormal Karyotypes in Spontaneous Abortion

TADASHI KAJII,[1] KOSO OHAMA, NORIO NIIKAWA, ARIANE FERRIER, AND SUGANDHI AVIRACHAN

Chromosome anomalies are frequent among spontaneous abortuses. Most of them are numerical aberrations such as monosomy X, autosomal trisomies, triploidy, and tetraploidy. However, it had not been possible until recently, due to technical limitations, to identify the exact nature of anomalies in most instances. Only four autosomal pairs (nos. 1, 2, 3, and 16) and frequently the Y can be identified in conventional chromosome preparations. Phenotype of the abortus is of little help in the identification of chromosome anomalies, since the embryo in most instances is resorbed or very stunted at the time of abortion. The recent development of banding pattern techniques—fluorescence, Giemsa, and others—has made it possible to analyze these chromosome anomalies in more detail. We have attempted to apply the trypsin banding technique [1] on spontaneous abortion material, and reported the first few of them [2–4]. Lauritsen et al. [5] recently described the result of fluorescence analysis among 34 chromosomally abnormal abortuses. The present preliminary report will deal with the result of trypsin banding analysis among 82 heteroploid specimens derived from 152 karyotyped abortuses.

MATERIALS AND METHODS

Consecutive specimens of spontaneous abortuses from one particular maternity clinic were collected. The aborted and evacuated material was collected under sterile conditions. Specimens were washed in Ringer's solution and embryonic and extraembryonic tissues were selected. All the specimens were cultured irrespective of the degree of maceration, except for those where only decidua were recovered and those that were 100 mm (corresponding to 90 days of gestational age) or more of crown-rump length. Whenever possible, two different tissues, amnion and cord in most instances, were cultured from each case. A culture technique developed in our laboratory was employed: finely minced pieces of tissue, around 100 per slide, were directly seeded onto glass slides, cultured, and harvested without being trypsinized. The hypotonic solution used was 1% sodium citrate. After being fixed in 3:1 methanol acetic acid, the slides were dipped in 75% acetic acid for 10 sec, dried on a spirit flame, and stained with Giemsa. Chromosome counts and photography

Received February 7, 1973; revised March 26, 1973.

This study was supported by the Ford Foundation Population Program, grant no. 640-0411B and by the World Health Organization. Sugandhi Avirachan is the recipient of a World Health Organization research training grant.

[1] All authors: Laboratory of Embryology and Cytogenetics, University Clinic of Gynecology and Obstetrics, Geneva, Switzerland.

were done on unmounted slides. Details of trypsin banding technique have been described elsewhere [1]. In short, the slides were destained after storage for 3–7 days from preparation, treated with 0.25% trypsin solution, and restained in Giemsa. A minimum of five cells from each case was analyzed for banding patterns. The X chromatin test was done on tissues in favorable condition, amnion in most instances.

RESULTS

Chromosome analysis was successful in 152 specimens (including three pairs of twins) of 216 which were accepted and set up in culture (70%; see table 1). There

TABLE 1

SPECIMENS COLLECTED

CLASSIFICATIONS OF SPECIMENS	*N*
Total specimens received	323
Specimens excluded:	
Maternal tissue	86
⩾100 mm crown-rump length	21
Total	107
Failures (12 from chorion)	64
Specimens studied:	
With normal karyotype:	
46,XY (one from chorion)	28
46,XX (nine from chorion)	42
With abnormal karyotype (eight from chorion)	82(54%)
Total	152

were 70 chromosomally normal specimens and 82 with chromosome abnormalities (54%). Trypsin banding analysis was done in 36 specimens with abnormal karyotypes. One case of trisomy C, four cases of trisomy D, two cases of trisomy 17–18, and two cases of trisomy G were not analyzed with banding technique since they had been karyotyped before the technique became available. The trypsin-banded specimens included monosomies X and 21, and trisomies 2, 3, 4, 6, 7, 8, 9, 10, 14, 15 (fig. 1), 16, 21, and 22 (table 2). Of the 22 pairs of possible autosomal trisomies, 13 were detected, while there was only one autosomal monosomy. Assuming that monosomies occur with the same frequency as trisomies as a consequence of nondisjunction during meiosis, it can be deduced that most instances of autosomal monosomies are lost in earlier stages and so escape detection.

There were 12 specimens with monosomy of the X chromosome, which were supported by X chromatin studies. The possibility of some of them being 45,XY,—G was also considered. Both the 45,X and 45,XY,—G karyotypes are X chromatin negative and have four G-group chromosomes. The notion is supported by the fact that all the three G-monosomic abortuses with known sex in the literature had a 45,XX,—G karyotype [4]. It is most likely that some 45,XY,—G abortuses were overlooked, being mistaken as 45,X. Fluorescent Y chromatin body, however, was negative in alcohol-fixed amnion in all the 12 specimens in the present series. Thus

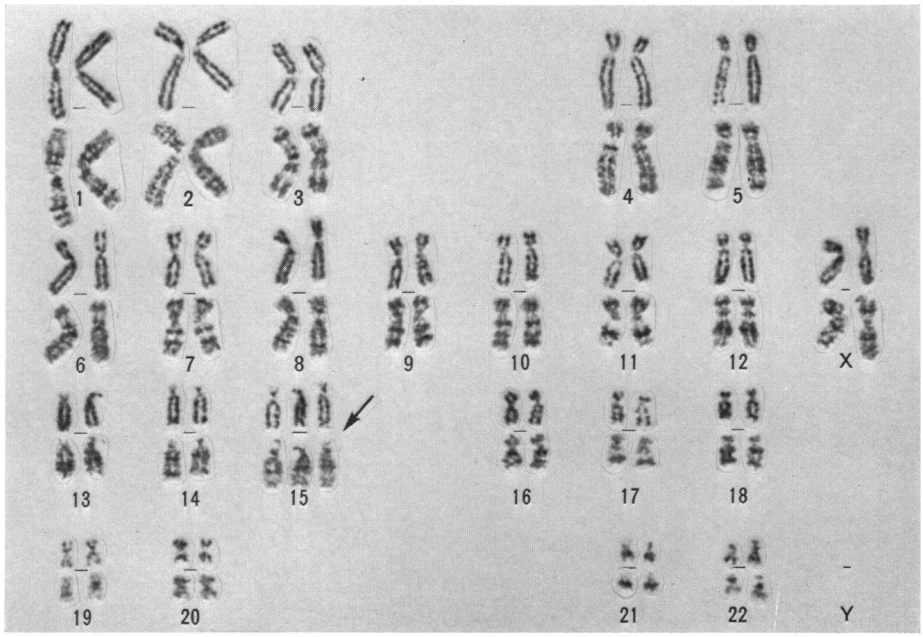

FIG. 1.—Double karyotype from an abortus with trisomy 15 (*arrow*) before (upper row) and after (lower row) trypsin treatment.

the possibility of having a G-monosomic specimen mixed among them was ruled out.

There were two instances of double heteroploidy: 46,XY,t(13q14q),+18 and 48,XXY,+9. Organized embryos and fetuses were more often found among those with chromosome abnormalities which could be compatible with live births (monosomy X, trisomies 17–18, and 21) than among those with abnormalities 100% lethal in utero.

Analyses of C-trisomic specimens revealed that a variety of C autosomes was involved, that is, pairs 6–10 (fig. 2). The finding is in contrast with that in liveborns, where only trisomy 8, mosaic or nonmosaic, has been identified [6]. There was an instance of 47,XXY. This is the anomaly found in the majority of subjects with Klinefelter's syndrome. The diagnosis in the case was supported by positive X and Y chromatin bodies, and the male genital organs.

It was not always possible in the past to identify trisomy 16, one of the most frequent chromosome anomalies in spontaneous abortion, from other trisomies, especially trisomy 17 or 18. In the present study, banding analysis was done whenever the diagnosis of trisomy 16 was uncertain. One case, which was diagnosed as trisomy F by conventional karyotyping, turned out to have an extra chromosome 16 with a condensed long arm.

An extra G-like chromosome was found in 11 specimens. Five had trisomy 21, two had trisomy 22 (fig. 3), one was 47,XXY, one had an extra chromosome 13 with long arm deletion (13q—), and two were unidentified. The phenotype of the embryos and fetuses with trisomy 21 was variable (fig. 4).

TABLE 2

KARYOTYPE ANALYSIS OF SPONTANEOUS ABORTUSES

KARYOTYPE	*N*
Monosomy:	
X	12(1)*
21	1(1)
Total	13(2)
Trisomy:	
A:	
2	1(1)
3	1(1)
Total	2(2)
B:	
4	1(1)
C:	
6	1(1)
7	1(1)
8	1(1)
9	1(1)
10	1(1)
C	1
XXY	1
Total	7(5)
D:	
14	4(4)
15	3(3)
D	4
Total	11(7)
E:	
16	18(8)
17-18	2
Total	20(8)
G:	
21	5(5)
22	2(2)
G	2
Total	9(7)
Double trisomy, +9,XXY	1(1)
Triploidy:	
69,XXY	5
69,XXX	3(1)
70,XXY,+C	1
70,XXX,+14	1(1)
Total	10(2)
Tetraploidy:	
92,XXYY	1
92,XXXX	2
94,XXYY,+17-18,+F	2(twins)
Total	5
Structural abnormalities:	
47,XY,+13q—pat	1†
46,XX,13q—pat	1†
46,XY,t(13q14q),+18	1(1)
Total	3(1)

* Number analyzed with banding technique.

† Successive abortions.

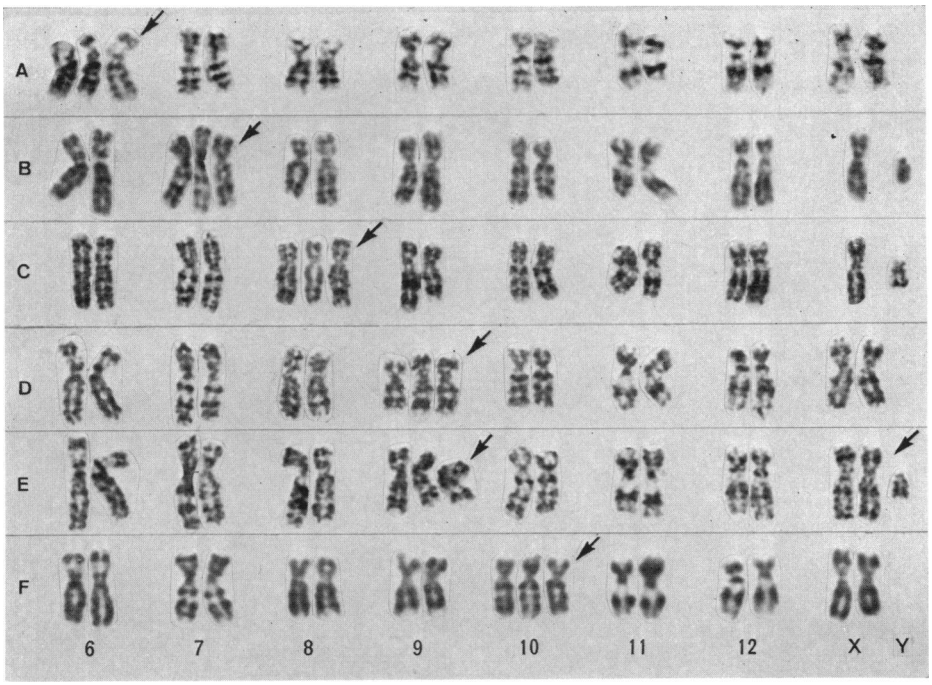

FIG. 2.—Collection of partial karyotypes of C-group anomalies: trisomies 6 (*A*), 7 (*B*), 8 (*C*), 9 (*D*), double aneuploidy with trisomy 9 and XXY (*E*), and trisomy 10 (*F*).

Chromosome analysis of two successive abortuses was possible in three pairs, and all of them showed chromosome anomalies. They were: (1) 45,X and 47,XY, +4; (2) 47,XY,+16 and 47,XY,+16; and (3) partial trisomy and partial monosomy of chromosome 13 (47,XY,+13q— and 46,XX,13q—) resulting from a paternal balanced translocation, 46,XY,t(13q—;18q+).

The sex ratio for the present series was 28 male and 42 female among the chromosomally normal specimens, and 40 male and 30 female in chromosomally abnormal specimens, excluding 45,X. There were 10 specimens with apparently normal karyotypes in which the culture was started from chorion. Culture of chorion is often contaminated with maternal cells (K. Ohama and T. Kajii, unpublished data). On karyotype analysis the presence of maternal cells would lead to a false diagnosis, and therefore increase the frequency of apparent female karyotypes. If these 10 specimens are excluded, there will remain 27 male and 33 female in chromosomally normal specimens. The corrected overall sex ratio is 67 male and 63 female, which is close to the expected 1:1 ratio.

Specimens from the first trimester of pregnancy comprised 64% of karyotyped abortuses. Among them, the incidence of chromosomally abnormal specimens was 59% as compared with 51% in the second trimester abortions (table 3); but the difference was not significant. The frequency of chromosomally abnormal specimens from the second trimester was higher than any reported in the literature. This

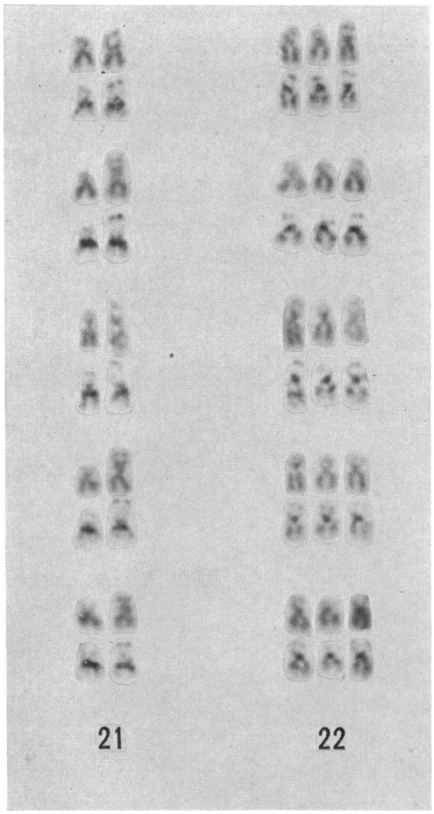

FIG. 3.—Collection of partial karyotypes of trisomy 22 and elongation of the short arm of a chromosome 21, a minor structural change.

would in part be due to the fact that those of 100 mm or more crown-rump length were excluded from the study. The mean gestational age was 77.7 days in the chromosomally normal abortuses and 73.9 days in the abnormal abortuses.

DISCUSSION

Banding pattern analysis of chromosomally abnormal spontaneous abortuses proved to be useful in identifying chromosome anomalies. Lauritsen et al. [5], using fluorescence technique, identified a series of anomalies including trisomies 2, 15, 18, 21, and XXY. Trypsin banding analysis enabled us to identify monosomy 21 and trisomies 3, 4, 6, 7, 8, 9, 10, 14, and 22, in addition to those found using fluorescence technique.

All seven D-trisomic abortuses analyzed with trypsin banding were either trisomy 14 or 15, contrary to the findings in liveborn infants, in whom the extra D chromosome is a no. 13. It seems that trisomy 13 is infrequent, or absent, among early spontaneous abortuses. The finding is in conflict with that by Roux [7], who reported that half of a group of eight embryos with trisomy D had a gross facial

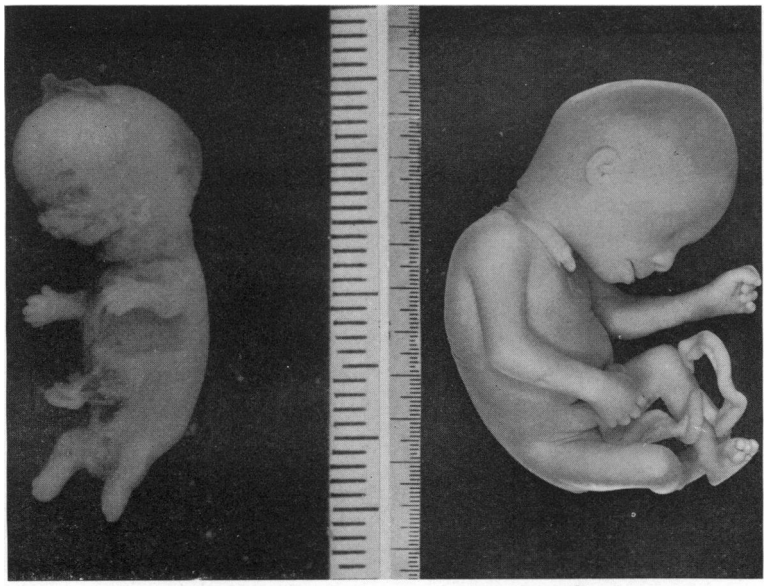

FIG. 4.—Abortuses with trisomy 21. *A*, An embryo with 21-mm crown-rump length; note webbing of the toes. *B*, A 90-day-old fetus without apparent external malformations, 95-mm crown-rump length.

anomaly, comparable to that commonly found in infants with trisomy 13. It should be borne in mind, however, that Roux's series was selected for specimens with embryos. Those with embryos would have a higher incidence of chromosome anomaly compatible with live birth—trisomy 13 in this case. Collection of a larger number of abortuses is necessary to determine whether or not trisomy 13 can be found among early spontaneous abortuses.

The incidence of proven trisomy 21 in our material was five of 152 karyotyped abortuses. This is a minimum estimate, because there were two other cases of trisomy G unidentified with banding technique. Assuming that spontaneous abortion occurs in 15% of recognizable pregnancies, the overall frequency of trisomy 21

TABLE 3

DISTRIBUTION OF ABORTUSES BY GESTATIONAL AGE

TRIMESTER	KARYOTYPE		TOTAL
	Normal	Abnormal	
First	37	53(59%)	90
Second	24	25(51%)	49

is one in every 200 conceptions. If the incidence of trisomy 21 at birth is taken as one in 668 [8], the figure for abortion suggests that no more than one of every five 21-trisomic conceptuses reaches term.

Trisomy 18 has a grossly disturbed sex ratio of four females to one male in liveborn infants [9]. One of the possible explanations for this situation would be the preferential loss of male embryos and fetuses through spontaneous abortion. It would be of interest in this connection to note that the three cases of trisomy 17–18 (including a case of double aneuploidy with 13q14q translocation) in the present study and one analyzed with fluorescence technique [5] all had XY sex chromosomes.

Banding analysis in the present study was restricted to specimens which had been preselected for chromosome abnormalities. Banding analysis of consecutive specimens is needed to determine whether there is any degree of latent chromosome aberrations which are not detectable by conventional methods. Such a study is now being carried out in our laboratory. Sixteen specimens with apparently normal karyotypes have so far been analyzed with trypsin banding technique, and no structural abnormalities have been detected.

SUMMARY

Chromosome analyses of 152 spontaneous abortuses showed 82 with chromosome anomalies (54%). The application of trypsin banding technique on 36 heteroploid specimens resulted in the identification of a number of chromosome anomalies including monosomies X and 21, trisomies 2, 3, 4, 6, 7, 8, 9, 10, 14, 15, 16, 21, and 22 and double heteroploidies. There was one XXY specimen. Survival rate of conceptuses with trisomy 21 was estimated as one in every five.

ACKNOWLEDGMENTS

Appreciation is expressed to Profs. H. de Watteville and W. Geisendorf, and Dr. J. Meylan for help and interest devoted to the study; to our gynecologic staff for supplying specimens and clinical data; and to Mr. T. Myit, Miss M.-F. Blanc, and Mrs. M.-F. Berthon for technical assistance. The authors are also indebted to Prof. K. Mikamo for his revision of the manuscript.

REFERENCES

1. KAJII T, OHAMA K, SUGANDHI AVIRACHAN, AVIRACHAN TT: Trypsin banding of Giemsa stained chromosomes. *Lancet* 2:1311–1312, 1972
2. KAJII T, OHAMA K, FERRIER A: Trisomy 14 in spontaneous abortus. *Humangenetik* 15:265–267, 1972
3. OHAMA K, KAJII T: Monosomy 21 in spontaneous abortus. *Humangenetik* 16:267–270, 1972
4. SUGANDHI AVIRACHAN, KAJII T: Double heteroploidy 46,XY,t(13q14q),+18 in a spontaneous abortus. *Clin Genet* 4:101–104, 1973
5. LAURITSEN JG, JONASSON J, THERKELSEN ÅJ, LASS F, LINDSTEN J, BRUNN PETERSEN G: Studies on spontaneous abortions. Fluorescence analysis of abnormal karyotypes. *Hereditas* 71:160–163, 1972
6. CASPERSSON T, LINDSTEN J, ZECH L, BUCKTON KE, PRICE WH: Four patients with

trisomy 8 identified by the fluorescence and Giemsa banding techniques. *J Med Genet* 9:1–7, 1972

7. ROUX CH: Etude morphologique des embryons humains atteints d'aberrations chromosomiques. *Presse Méd* 78:647–652, 1972
8. HAMERTON JL: *Human Cytogenetics,* vol 2: *Clinical Cytogenetics.* New York, Academic Press, 1971
9. TAYLOR AI: Autosomal trisomy syndromes: a detailed study of 27 cases of Edwards' syndrome and 27 cases of Patau's syndrome. *J Med Genet* 5:227–252, 1968