Chromosome Abnormalities in Early Spontaneous Abortions*†

DAVID T. ARAKAKI and SORRELL H. WAXMAN

From the Department of Genetics, School of Medicine, University of Hawaii, Honolulu, Hawaii

The reports in 1959 of the chromosomal anomalies in Turner's, Klinefelter's, and Down's syndromes were followed by a period of rapid advancement in human medical cytogenetics. Clinical cytogenetic research continued on living subjects, while a large area of human development remained relatively uncommitted to study. This area, spontaneous abortion, results in foetal wastage estimated at 10% of recognized pregnancies (Potter and Adair, 1963) or up to 20% of all conceptions. The Geneva Conference (1966) concluded that chromosomal abnormalities were a significant factor in spontaneous abortions, since 19% of nearly 800 abortuses were found to have a chromosome anomaly.

In clinical patients a variety of chromosomal rearrangements and mosaicism is found. Theoretically even larger numbers of chromosomal aberrations would be predicted and expected to be found in spontaneous abortions. However, there have been little or no reports of abnormalities such as monosomy, deletion, duplication, translocation, and ring in abortions.

A study on the cytogenetics of spontaneous abortions was initiated in 1964 at the Kapiolani Maternity and Gynecological Hospital in Honolulu. The findings on the abnormal chromosome constitution of the abortuses are presented in this report.

Materials and Methods

The aborted specimens were obtained from a single large maternity hospital, the Kapiolani Maternity and Gynecological Hospital located in Honolulu, Hawaii. The aborted material was examined by the hospital pathologist who submitted specimens under 20 weeks' gestation for the cytogenetic study. The samples were representative of this geographical area, being multi-racial, multi-ethnic, and multi-religious. A cross-

section of the socio-economic groups was well represented also.

Since the gestational ages of our samples ranged from 4 to 18 weeks, it was felt that the earliest recognized aborted pregnancies were being collected. Specimens of foetal membrane and decidua were collected in sterile Hanks' balanced salt solution containing no antibiotics. Foetal membrane was differentiated from the decidua and both were separately analysed. The chromosome technique was described elsewhere (Arakaki, Waxman, and Smith, 1967).

Results

During this project, specimens from 127 spontaneous abortions, under 20 weeks' gestation, were successfully cultured for chromosome study. This represents 35.5% of all specimens submitted for analysis. Specimens which were severely macerated and autolysed or obviously contaminated were included in the total number of specimens put into culture. The details of the first 25 successfully analysed specimens have been published elsewhere (Waxman, Arakaki, and Smith, 1967) in a preliminary report of this current study.

Sixty-three (49.5%) of the specimens were found to have a chromosome abnormality (Tables I and II). There were 24 (38.1%) sex chromosome anomalies, 31 (49.2%) autosomal anomalies, and 8 (12.7%) polyploids. The pure X-monosomy was the most common specific anomaly, with a frequency of 28.5%. The second most common specific anomaly was trisomy-16, with a frequency of 22.2%, followed by triploidy, with a frequency of 11.1%. Deletions, translocations, a monosomy, and a ring chromosome were among the karyotypes found in our group of chromosomally abnormal conceptuses.

Sex chromosome abnormalities. There were 18 specimens with monosomy of the X chromosome, which were confirmed by sex chromatin studies. 45,X abortuses were found in 14.2% of

Received 28 November 1969.

* Request reprints from the Cell Genetics Laboratory, Kauaikeolani Children's Hospital, Honolulu, Hawaii 96813.

† Supported by a USPHS grant, HD-01133, from the National Institutes of Health.

FIG. 1. Karyotype showing a 46,XXp- sex chromosome anomaly from a 46,XX/46,XXp- mosaic.

all successful analyses, 28.5% of all abnormal specimens, and 75% of all sex chromosome anomalies. There were three additional mosaic XO's (one 45,X/46,XX and two 45,X/46,XY) and one mosaic with a deletion of the short arm of an X (46,XX/46,XXp-, Fig. 1). Two specimens were 46,XX/47,XXY.

Autosomal trisomies. Autosomal trisomy (Tables I and II) was the most frequently occurring

anomaly (38%). The group E16 trisomy was the most common (14/24), with 12 trisomy-16 plus 2

TABLE II
TYPES AND FREQUENCIES OF CHROMOSOMAL ABNORMALITIES IN SPONTANEOUS ABORTUSES

Karyotype	No.	Frequency (%)
Sex chromosomes		
XO	18	28.5
XO/XY	2	
XO/XX	1	
XX/XXY	2	
XX/XXp-	1	
Group A		
Trisomy-2	1	1
Monosomy-3	1	
Normal/ring-3	1	
Group B		
Trisomy-B/trisomy-B, monosomy-G	1	1
Group C		
Trisomy-C6	1	
Normal/trisomy-C (9-10)	1	1
Normal/trisomy-?C	1	
Group D		
Trisomy-D	1	1
Deletion-D/translocation-D	1	
Group E		
Trisomy-16	12+	22.2
Normal/trisomy-16	2	
Trisomy-17	1	
Trisomy-18	1	
Group F		
Trisomy-F	2	11.1
Group G		
Normal/trisomy-G	1	
Triploidy		
XXX	3	11.1
XXY	3	
XXY	1	
Tetraploidy		
XXYY	1	1
Others		
Large ?translocation +	1	
?Fragment +	1	1
49-51 chromosome count	1	

TABLE I SUMMARY OF CHROMOSOME FINDINGS IN SPONTANEOUS ABORTUSES			
	No.		Frequency (%)
			Sex, Autosomal and Polyploid
			Common Anomalies
Normal	64		
Abnormal	63		
(a) Sex			
XO	24	18	38.1
Mosaic		6	28.5
(b) Autosomal			
Trisomy	31	24	49.2
Pure		19	38.0
Mosaic		5	
Miscellaneous	7		
Monosomy		1	
Translocation		1	
Ring mosaic		1	
Deletion			
translocation		1	
Fragment		1	
Others		2	
(c) Polyploidy	8		12.7
Triploidy	7		
Tetraploidy	1		11.1
	127	63	100

normal/trisomy-16 mosaics for a frequency of 58.3% of all trisomies.

Trisomy of each autosomal group was found though not all autosomes were involved. Trisomy-C was differentiated from triplo-X by the sex chromatin study. One of the trisomy-C's was a trisomy-6. The trisomy-A involved chromosome 2 according to morphology and autoradiography. We were unable to identify the autosomes involved in the trisomies of groups B, C (2 out of 3), D, F, and G.

Polyploidy. Eight polyploid specimens were found with seven specimens being triploid and one specimen a tetraploid. The triploids consisted of 69,XXX (3), 69,XXY (3), and 69,XXYY (1). The tetraploid was 92,XXYY.

Mosaicism. In our study, mosaicism was common, comprising over 10% of the whole series and 20% of the chromosomally abnormal specimens. This indicates a high degree of post-fertilization non-disjunction in the abortuses of this Hawaiian population. The sex chromosomes and the autosomes were equally affected. No mosaicism was found in the polyploid specimens. The incidence of mosaicism in the Hawaiian livebirth population is, of course, unknown.

There were 14 cases of mosaicism, 6 involving the sex chromosomes and 8 involving the autosomes. Of the autosomal mosaics, one had a ring chromosome No. 3: 46,XY/46,XY,3r; one was a deletion/translocation: 46,XY,Dq-/46,XY,D-,t(DqDq)+; and one was a 47,XY,B+/46,B+,G-. The others were trisomic mosaics composed of one normal cell line and a trisomic cell line involving chromosomes of group C, E16, and G. 25% of all sex chromosome anomalies and 26% of all autosomal anomalies were mosaics.

Discussion

It became evident as larger series of abortions were studied cytogenetically that most chromosome abnormalities were of the same type as described in livebirths, with unique types being observed especially in early abortions. In most cases whether a chromosome anomaly occurs in livebirths or abortions is a matter of the degree of viability of the foetus *in utero*. Some unique chromosomal defects may never be found in conceptuses simply because of their lethality in the gametic stage.

Sex chromosome abnormalities. In a neonatal clinical study by Miller (1964), the incidence

of 45,X babies was calculated to be about 0.4 per 1000 livebirths. If non-disjunction is a major cause of sex chromosome anomalies then the incidence of 45,X and 47,XXY anomalies should be equal. In livebirths, the 47,XXY anomaly is at least 5 times greater than the 45,X anomaly. This suggests a high mortality for 45,X embryos and a low mortality for 47,XXY embryos. The evidence accumulated thus far supports this view. 45,X's have been found in 4.1% of all abortuses and no 47,XXY has been reported in nearly 800 examined aborted specimens (Geneva Conference, 1966). The Lyon hypothesis has been implicated by Carr (1965) as the cause of the high mortality of the 45,X anomaly. During early embryogenesis, both X chromosomes in an XX embryo were considered to be active and might be needed for critical differentiation. The 45,X embryo would thus be at a disadvantage since during this critical stage only one X would be active, and the consequent loss of full activity in 45,X embryos might be lethal.

One of the sex chromosome mosaics had a structural aberration (46,XX/46,XXp-, Fig. 1). The frequency of cells with the deleted X chromosome differed considerably in cord and amnion cultures. In the cord, 98% of the cells had the deleted X chromosome, while in the amnion only 29% of the cells had this deletion. The defect is similar to a short arm X-deletion found in Turner's syndrome.

In the present series 14.2% of all the specimens were 45,X. This is almost three times higher than Inhorn's (1967) 5.5% or Carr's (1965) 5.3%. In Carr's series, however, 45,X specimens represented 24% of all chromosomally abnormal specimens as compared to 28.5% in our series.

There were 6 sex chromosome anomalies in which mosaicism was involved. Two of these mosaic specimens contained an XXY cell line. 47,XXX abortuses have not previously been reported in spontaneous abortion studies.

Autosomal abnormalities. There were 31 specimens with an autosomal chromosome anomaly; 24 (38.1%) were trisomic making trisomy the most frequently occurring chromosome anomaly in this as well as in other series (Geneva Conference, 1966).

Seven cases of trisomy-A have been reported (Hall and Källén, 1964; Carr, 1965; Kerr and Rashad, 1966; Thiede and Metcalfe, 1966). No trisomy for chromosome No. 1 has been reported. One of our specimens was a trisomy-2. The large autosomes are believed to contain many genes vital to normal development, so that aberrations involving these chromosomes are generally thought to be non-existent or lethal. However, in our series, five

(12.6%) trisomic specimens of groups A, B, and C were found as compared to only two trisomic specimens of group F. In the Geneva study, 13 (11.1%) trisomic specimens of group A to C were found as compared to only one trisomic specimen of group F.

Anomalies of group B chromosomes in clinical cases have been deletions of the short arms of chromosome 4 (Wolf's syndrome) and of chromosome 5 (the cri-du-chat syndrome). A case of an effective trisomy of the long arm of a B chromosome was described by Shaw, Cohen, and Hildebrandt (1965). Only two specimens reviewed by the Geneva group contained an extra chromosome B (Carr, 1965; Sato, 1965). In our study a trisomy-B mosaic was seen in one specimen (47,XY,B+/46,XY,B+,G-). The original cell line in this conceptus was probably a trisomy-B since 78% of the cells had this karyotype. Non-disjunction or chromosome lag of a G chromosome would have produced the 46,XY,B+,G- cell line. The specimen was obtained from a 34-year-old mother. The gestational age was 9 weeks.

Trisomy-C was found in 4 of the specimens reviewed by the Geneva Conference. These cases were confirmed as being of autosomal origin by sex chromatin determinations (Clendenin and Benirschke, 1963; Carr, 1965; Szulman, 1965). Three additional cases of trisomy-C were shown in the present series.

It is often difficult to tell whether the D trisomy found in foetal abortions is the same as the D₁ trisomy syndrome found in livebirths. Nine cases of D trisomy were reported by the Geneva Conference. In our study one specimen with a D trisomy was found in a 9½ week conceptus obtained from a 43-year-old mother. An embryo was not present. Attempts to identify the extra D chromosome by autoradiography were not successful.

There have been only a few reports of trisomy-16 in living subjects. An extra chromosome 16 was found in the blood cells of a 59-year-old woman with multiple anomalies (Lewis *et al.*, 1963). An analysis of her skin fibroblast cells was not reported so that mosaicism was not ruled out. In two other cases, mosaicism was found in skin and/or blood (Schmidt *et al.*, 1963; Arakaki and Waxman, 1969). Other investigations have revealed aberrations of presumptive chromosome 16 (Tips *et al.*, 1964); isochromosome-16 (F. Hecht, J. Relnyk, and H. Thompson, 1968, personal communication); or non-homology of chromosome 16 (J. D. Rowley, 1968, personal communication).

In our series nearly 22% of all anomalies were trisomy-16, making it the second most frequent

chromosome anomaly. Four of the 14 specimens were empty intact amniotic sacs. Six of the other gestational sacs were ruptured and no embryo or cord was seen. Two contained a badly macerated embryo, while two others had remnants of the umbilical cord. One specimen appeared to be a dichorial twin: one sac contained a macerated embryo and the other was empty. Thus only four specimens had any foetal tissue. Two other E trisomies were identified morphologically as trisomy-17 and trisomy-18. In the Geneva Conference there were 21 specimens with trisomy-E.

Two cases of trisomy-F were found in this series. Only two other cases of a trisomy-F have been reported in abortion studies (Inhorn, Therman, and Patau, 1964; Carr, 1967). In livebirths trisomy-F is also rare, having been described in only one patient (Civantos, 1961). Due to their extreme rarity in livebirths and in abortions, chromosomes 19 and 20 must contain factors having great biological significance, which affect development in early embryogenesis, unless trisomy-F is induced less frequently than other chromosomes.

The G chromosomes found in abortuses may or may not be the same chromosome responsible for Down's syndrome. Since trisomy-G is quite common in livebirths, a low incidence of trisomy-G would be expected in abortion studies. Except for one 46,XY/47,XY,G+ mosaic, no pure trisomy-G was found in this series. In contrast, group G trisomies have been found in 12.4% (Geneva Conference, 1966) and 9% (Carr, 1965) of abnormal abortion.

Miscellaneous karyotypes

Translocations. Many unbalanced translocations have been reported in livebirths but very few translocations have been found in abortions. Clendenin and Benirschke (1963) described a possible translocation in a long chromosome No. 1, and O. J. Miller and D. Warburton (1966, personal communication to Inhorn, 1967) reported a case of a t(GqGq) translocation. One of our specimens, an 8½-week-old gestation, was found to be 47,XX with a large submetacentric translocated chromosome of undetermined origin (Waxman *et al.*, 1967). This chromosome is larger than chromosome No. 1 and was thought to have originated by a combination of exchanges between chromosomes of group B (4-5) and/or group C (6-12).

In a deletion/translocation specimen, a count of 46 was obtained with mosaicism in the form of a structural abnormality of a chromosome D. The abnormality is believed to be a deletion of the long

arm of a D chromosome in one cell line and the translocation of this long arm portion to the short arm of another D chromosome in the other cell line, resulting in a 46,XY,Dq- / 46,XY,D-,t(DqDq) + mosaic (Fig. 2 and 3). The foetus, which otherwise looked normal, had polydactyly of the left hand and syndactyly of the left foot. The maternal age was 23 years and the gestational age was 17 weeks.

Deletions. Deletions are found in clinical patients with aberrations of chromosomes 4, 5, 18, and X. No deletions have been reported in abortion studies. Deletions were shown in three of our abortuses. Two have been discussed above, an Xp- and a Dq- deletion. In the third specimen an analysis of several tissues revealed a chromosome count of 47 with the extra chromosome being about the size of a G chromosome. This abnormal chromosome resulted from a deletion of an unknown chromosome. The chromosome fragment could be any suitable chromosome which when broken at the centromere would give rise to a G-size chromosome.

Monosomy. Autosomal monosomy, assuming that gametic selection does not occur, would be expected in a high proportion of abortions since trisomies due to meiotic non-disjunction occur in

large numbers. Autosomal monosomy due to possible meiotic non-disjunction has been found in 3 spontaneous abortions. Inhorn *et al.* (1964) reported 2 cases of proven monosomy-C. Kelly *et al.* (1965) found a monosomy-1 in a 9-week embryo with no apparent deformity. One of our specimens was a monosomy-3 (Waxman *et al.*, 1967). The few monosomies found in either spontaneous abortions or livebirths seem to indicate either inviability of the nullisomic gamete, zygotic selection at the time of fertilization, or very early abortion.

Rings. A ring chromosome 3 mosaic was found in this series. One cell line was normal whereas the other cell line had a missing chromosome 3 replaced by a large monocentric ring chromosome (46,XY/46,XY,3r) (Arakaki, Waxman, and Nonomura, 1969). The ratio of normal cells to abnormal cells was 3:1. The mother had intermittent vaginal bleeding since her last normal menstrual period and was being treated concurrently with progesterone for the vaginal bleeding and with antibiotics, cortisone, and expectorants for chronic bronchitis. No information was available concerning exposure to x-rays before or after conception. The maternal age was 33 years and the gestational age was 6½ weeks.

FIG. 2. Karyotype showing a 46,XY,Dq- chromosome constitution.

FIG. 3. Karyotype showing a 46,XY,D-t(DqDq)+ chromosome constitution.

Polyploidy

Triploidy. The third most common chromosomal anomaly in spontaneous abortions is triploidy. Triploidy was found in 3.3% of 788 karyotyped specimens or nearly 17% of all specimens with abnormal karyotypes (Geneva Conference, 1966). In our series, triploidy was found in 5.5% of all karyotyped specimens and in 11% of our chromosomally abnormal specimens. Kerr and Rashad (1966) have cautioned that polyploidy derived from placental and amniotic cultures may not indicate the true chromosomal complement of the foetus, since polyploidy may occasionally be found in amnion cultures. Polyploidy would thus be acceptable only if found in true embryonic tissues. Eighteen of 22 reported cases of triploidy (Penrose and Delhanty, 1961; Delhanty, Ellis, and Rowley, 1961; Carr, 1963; Thiede and Salm, 1964; Carr, 1965; Szulman, 1965; Schlegel *et al.*, 1966; Shepard *et al.*, 1968) were derived from embryonic tissues. In addition, 5 of the 7 triploid specimens in the present series were derived from cord cultures. It thus appears that triploidy is a common anomaly in conceptuses.

Summary

In a 4-year study on early spontaneous abortion, 127 specimens obtained from a single large maternity hospital were successfully analysed. Nearly 50% of the specimens were found to have a chro-

mosomal abnormality. XO monosomy, trisomy-16, and triploidy were the most common anomalies found. Mosaicism constituted 20% of the abnormal specimens. Deletions, translocations, monosomies, and a ring chromosome were also demonstrated.

The authors wish to acknowledge the help of the hospital pathologists, Drs. C. Curtiss, R. Kelley, and C. Sprague, the nursing staff, and the Research Committee of Kapiolani Maternity and Gynecological Hospital; and to T. Nonomura for technical assistance.

REFERENCES

- Arakaki, D. T., and Waxman, S. H. (1969). Trisomy-16 in a mosaic carrier father and his aborted foetus. *Journal of Medical Genetics*, **6**, 85-88.
- , —, and Nonomura, T. M. (1969). Anomalies of chromosome No. 3 in abortuses. *Journal of Medical Genetics*, **6**, 399-400.
- , —, and Smith, J. B. (1967). Endometrial and fetal cytogenetics. *American Journal of Obstetrics and Gynecology*, **97**, 868-869.
- Carr, D. H. (1963). Chromosome studies in abortuses and stillborn infants. *Lancet*, **2**, 603-606.
- (1965). Chromosome studies in spontaneous abortions. *Obstetrics and Gynecology*, **28**, 308-326.
- (1967). Chromosome anomalies as a cause of spontaneous abortion. *American Journal of Obstetrics and Gynecology*, **97**, 283-293.
- Civantos, F. (1961). Human chromosomal abnormalities. *Bulletin of the Tulane University Medical Faculty*, **20**, 241-253.
- Clendenin, T. M., and Benirschke, K. (1963). Chromosome studies on spontaneous abortions. *Laboratory Investigation*, **12**, 1281-1292.
- Delhanty, J. D. A., Ellis, J. R., and Rowley, P. T. (1961). Triploid cells in a human embryo. *Lancet*, **1**, 1286.
- Geneva Conference (1966). Standardization of procedures for chromosome studies in abortion. *Cytogenetics*, **5**, 361-393.
- Hall, B., and Källén, B. (1964). Chromosome studies in abortuses and stillborn infants. *Lancet*, **1**, 110-111.

- Inhorn, S. L. (1967). Chromosomal studies of spontaneous human abortions. In *Advances in Teratology*, Vol. II, pp. 38–99. Ed. by D. H. W. Woollam. Logos Press, London.
- , Therman, E., and Patau, K. (1964). Cytogenetic studies in spontaneous human abortion (abstract). *American Journal of Clinical Pathology*, **42**, 528.
- Kelly, S., Almy, R., Jakovic, L., and Buckner, L. (1965). Autosomal monosomy in a spontaneous abortion. *Lancet*, **1**, 166–167.
- Kerr, M., and Rashad, M. N. (1966). Chromosome studies on spontaneous abortions. *American Journal of Obstetrics and Gynecology*, **94**, 322–339.
- Lewis, F. J. W., Hyman, J. M., McTaggart, M., and Poulding, R. H. (1963). Trisomy of autosome 16. *Nature*, **199**, 404.
- Miller, O. J. (1964). The sex chromosome anomalies. *American Journal of Obstetrics and Gynecology*, **90**, 1078–1139.
- Penrose, L. S., and Delhanty, J. D. A. (1961). Triploid cell cultures from a macerated foetus. *Lancet*, **1**, 1261–1262.
- Potter, E. L., and Adair, F. L. (1963). Defective babies who die before birth. In *Birth Defects*, pp. 46–48. Ed. by M. Fishbein, Lippincott, Philadelphia.
- Sato, H. (1965). Chromosome studies in abortuses. *Lancet*, **1**, 1280.
- Schlegel, R. J., Neu, R. L., Leão, J. C., Farias, E., Aspillaga, M. M., and Gardner, L. I. (1966). Observations on the chromosomal, cytological and anatomical characteristics of 75 human conceptuses including euploid, triploid XXX, triploid XXY and mosaic triploid XXY/diploid XY cases. *Cytogenetics*, **5**, 430–446.
- Schmidt, B. J., Beçak, W., Beçak, M. L., Soibelman, I., Queiroz, A. da S., Lorga A. P., Secaf, F., Antonio, C. F., and Carvalho, A. de A. (1963). Metaphyseal dysostosis. Review of literature; study of a case with cytogenetic analysis. *Journal of Pediatrics*, **63**, 106–112.
- Shaw, M. W., Cohen, M. M., and Hildebrandt, H. M. (1965). A familial 4/5 reciprocal translocation resulting in partial trisomy B. *American Journal of Human Genetics*, **17**, 54–70.
- Shepard, T. H., Gartler, S. M., Lagerberg, E. V., and Price, B. (1968). Chromosomal aberrations in 2 embryos from the same mother. An embryo with B trisomy and another with triploidy and associated cleft lip. *American Journal of Obstetrics and Gynecology*, **102**, 48–52.
- Szulman, A. E. (1965). Chromosomal aberrations in spontaneous human abortions. *New England Journal of Medicine*, **272**, 811–818.
- Thiede, H. A., and Metcalfe, S. (1966). Chromosome and human pregnancy wastage. *American Journal of Obstetrics and Gynecology*, **96**, 1132–1138.
- , and Salm, S. B. (1964). Chromosome studies of human spontaneous abortions. *American Journal of Obstetrics and Gynecology*, **90**, 205–215.
- Tips, R. L., Smith, G. S., Meyers, D. L., and Perkins, A. L. (1964). Familial mosaicism of a chromosome 16 abnormality associated with multiple congenital anomalies. *American Journal of Mental Deficiency*, **69**, 330–340.
- Waxman, S. H., Arakaki, D. T., and Smith, J. B. (1967). Cytogenetics of fetal abortions. *Pediatrics*, **39**, 425–432.