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Chromosome Abnormalities in 1355 Induced Abortuses

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ABSTRACT

A total of 1536 induced abortuses of less than 12 weeks gestation were cultured and 1355 successfully karyotyped. The study was divided into two periods: in the initial period 1224 abortion specimens were randomly collected, but in the later period 312 specimens of less than 8 weeks gestation or from women over 35 years of age were selectively collected. Eighty-six or 6.3% of the 1355 karyotyped specimens had chromosome abnormalities, and these included 61 trisomics, 9 monosomy X, 7 triploids, 5 double aneuploids, 2 tetraploids, one balanced translocation and one mosaic trisomy 22. Chromosomal abnormality rates by five-year maternal age intervals were 3.4 to 3.6% for women under 34 years of age, 9.3% for women aged 35-39 years and 23.0% for women over 40 years. Factors that may influence the prevalence of abnormal karyotypes in induced abortuses and prenatal selection rates of chromosome abnormalities compatible with livebirth are discussed.

A number of cytogenetic studies in spontaneous abortuses[2,5,8,12,18] have shown that about 50% of spontaneous abortuses in the first trimester of pregnancy are chromosomally abnormal, most having lethal abnormalities. These cytogenetic findings indicate that spontaneous abortion is a mechanism for the natural elimination of abnormal conceptuses, and clinical management of spontaneous abortion has changed from attempted prevention by hormone replacement therapy to nonmedicament therapy with bed rest. Induced abortuses are another source of material for the estimation of the frequency of chromosomally abnormal conceptuses at early embryonic stages. Karyotypic results on more than 7000 induced abortuses have been reported, however, the abnormality rates are very variable, ranging from 0 to 6.4% in the different studies[3,10,11,15,17,19,20]. In our laboratory, karyotypic analysis of induced abortuses has been conducted in parallel with a study of spontaneous abortuses[18]. In the present paper, we report the results of 1355 induced abortuses including 1087 cases reported previously[15] and deal with the effect of both maternal age and of culturing abortion specimens without recovered fetal tissues on the incidence of chromosome abnormalities.

MATERIAL AND METHODS

Specimens consisting of induced abortion of pregnancies terminated before 12 weeks of gestation for sociomedical indication at the Hiroshima University Hospital and several clinics in Hiroshima city were collected. Women who had vaginal bleeding and consequently underwent an induced abortion were excluded from the study. Information on age at menarche, the menstrual cycle, medications and/or abdominal X-ray examinations around the time of conception and atomic bomb exposure were recorded on a standard form by the doctors in charge.

The study was divided into two periods: in the initial period, 1269 abortuses were randomly collected, but in the later period the study was restricted to 316 abortuses that were either less than 8 weeks of gestation or that were from women over 35 years of age.

The abortion specimens were collected in bottles containing physiological saline and delivered to the laboratory on the same day or the day following the operation. They were washed repeatedly in physiological saline and embryonic and extra-embryonic tissues separated from decidua. Fragments from the embryonic tissues when recovered, or fragments from the extra-embryonic tissues were minced with scissors, seeded on glass slides and cultured in Eagle's minimum essential medium supplemented with 20% fetal bovine serum. Cell colonies grown out of the primary cultures were processed in situ to yield chromosome preparations. When a considerable number of mitotic cells were observed, they were treated with colcemid for 4 to 6 hr, hypotonic solution (equal volumes each of 0.075 mol/liter of potassium chloride and 1% sodium citrate) for 30 min, fixed in acetic methanol and air-dried. Slides were stained with Giemsa. Twenty cells from each specimen were microscopically examined and three cells subsequently photographed for karyotypic analysis. Trypin G-banding of Giemsa-stained slides was done as described elsewhere[9] in all cases showing chromosome abnormalities on non-banded Giemsa stained slides.

RESULTS

Embryonic and/or extra-embryonic tissues were recovered from 1536 of the 1585 abortion specimens received during the study period, and 1355 were successfully karyotyped. Of the 1355 karyotyped specimens, 1087 were studied in the initial period of the study and 53 (4.9%) were chromosomally abnormal (Table 1), while 268 were karyotyped in the later period and 33 (12.3%) were chromosomally abnormal. The 1269 abortuses with a normal karyotype included 642 males and 627 females.

Among the 86 chromosomally abnormal abortuses, 61 were primary autosomal trisomics, 9 were 45,X and 9 were polyploids (Table 2). Trisomies for 18 of the 22 autosomes and for the X were found (Table 3), trisomy 16 being

Table 1. Specimens received

	Initial period	Later period
Total specimens received	1269	316
No identifiable fetus or sac	45	4
Failure in culture	137	44
Karyotype determined	1087	268
Normal karyotype	1034	235
Abnormal karyotype	53 (4.9%)	33 (12.3%)

Table 2. Frequency of different types of chromosome abnormalities

Chromosome abnormalities	No.	Frequency (per 1000)
45,X	9	6.6
Trisomy	61	45.0
Double aneuploidy	5	3.7
Triploidy	7	5.2
Tetraploidy	2	1.5
Translocation	1	0.7
Mosaicism	1	0.7
Total	86	63.5

Table 3. Frequency of different types of trisomies

Trisomy		No.	Frequency (per 1000)
A	2	2	
B	4	1	
	5	2	
C	6	1	
	7	2	
	8	1	
	9	1	
	10	1	
	11	1	
	12	2	
	C*	1	
D	13**	2	1.5
	14	2	
	15	3	
	D*	1	
E	16	14	
	17	1	
	18**	4	3.0
G	21**	8	5.9
	22	9	
47,XXX**		1	0.7
47,XXY**		1	0.7

*Extra-chromosome was not identified.
**Trisomies compatible with livebirth.

the most common, followed by trisomies 21 and 22. Rates (per 1000 induced abortuses) of those chromosome abnormalities compatible with live-birth were as follows: 6.6 for 45,X, 5.9 for trisomy 21, 3.0 for trisomy 18, 1.5 for trisomy 13 and 0.7 for each of 47,XXX and 47,XXY. There were five abortuses with double aneuploidy, one with a 46,X,+16 and four with double trisomy (Table 4). Of nine polyploid abortuses, seven were triploids including two 69,XXY and five 69,XXX, and two were tetraploids consisting of one 92,XXXX and one 93,XXXX,+12. One mosaic was a 46,XX/47,XX,+22 in which chorionic tissues were cultured and as the 46,XX cell line was not examined for its origin, it may reflect maternal cell contamination.

The 1244 abortuses set up in culture in the initial period were classified on the basis of recovered tissues into two groups: complete specimens with an embryo/fetus and incomplete specimens with only extra-embryonic tissues (Table 5). Of 728 complete specimens, 695 were successfully karyotyped and 10 of them (1.4%) were chromosomally abnormal, while of 496 incomplete specimens, 392 were karyotyped and 43 (11.0%) were abnormal. If it is assumed that all of the abortuses set up in culture were successfully karyotyped, the abnormality rate over all would be 5.3%.

Table 4. Double aneuploidy, triploidy, tetraploidy, translocation and mosaicism

Karyotype	No.
Double aneuploidy	
46,X,+16	1
48,XY,+2,+16	1
48,XY,+D,+18	1
48,XY,+16,+20	1
48,XXX,+2	1
Triploidy	
69,XXY	2
69,XXX	5
Tetraploidy	
92,XXXX	1
93,XXXX,+12	1
Translocation	
46,XX,t(1:16)(p22:q24)	1
Mosaicism	
46,XX/47,XX,+22	1

Table 5. Success rates in culturing and chromosome abnormalities for different specimens types

Classification of abortion specimens	Tissue recovered	No. of abortuses set up in culture		Karyotyped No.	Karyotyped (%)	Chromosomally abnormal No.	Chromosomally abnormal	Chromosomally abnormal (%)
Complete specimens	Embryo/fetus	728		695	(95.5)	10		(1.4)
Incomplete specimens	Cord	7		7		0		
	Amnion	20		20		10		
	Yolk sac	11	496	10	392 (79.4)	0	43	(11.0)
	Chorion	458		355		33		
Total		1224		1087	(88.8)	53		(4.9)

Table 6. Maternal age distribution in 1087 karyotyped induced abortuses in the initial period of the study

Maternal age	No. of induced abortuses Observed	Expected[a]
- 24	264	259.8
25 - 29	306[b]	615.2
30 - 34	284[b]	170.7
35 - 39	169[b]	35.9
40 -	64[b]	5.4
Total	1087	1087

[a] The newborns in Japan during 1975-1976 were taken as controls
[b] $p<0.01$

Although an unselected series of the induced abortuses less than 12 weeks gestation were collected in the initial period of the study, the relative frequency of the abortuses from women aged over 35 years was significantly higher than that of newborns in Japan (Table 6). As shown in Table 7, the frequencies of chromosome abnormalities by five-year maternal age intervals were constant in women under 34 years of age, being 3.4 to 3.6%, but increased to 9.3% in the 35-39 years age group and 23.0% in women over 40. A majority of the abnormalities in the advanced maternal age group were autosomal trisomies.

The frequencies of chromosome abnormalities by gestational age were calculated for the 1087 abortuses karyotyped in the initial period and, as can be seen from Table 8, showed no change in abnormality rate over the gestation times studies.

The results of analysis of menstrual effects on chromosome abnormalities in induced abortuses are presented in Table 9. Chromosome abnormalities were found in 14 (13.2%) of 106 abortuses collected from women with menarche at 16 years or later, however, such a relatively high abnormal rate appeared to be due to a high proportion of women aged over 35 years in this group. No other effect of abnormal menstrual cycles on chromosome abnormalities was noted.

There were 22 abortuses from women who took medications (antibiotics, antipyretics, corticosteriod hormones, insulin and anticancerous drugs) just before or after the last menstruation, and 6 abortuses from women who had diagnostic X rays on their abdomens after the last men-

Table 7. Frequency of chromosome abnormalities in 1355 karyotyped induced abortuses by maternal age group

Maternal age	No. of karyotyped specimens	Chromosome abnormalities			
		Trisomies		Total	
		No.	(%)	No.	(%)
- 24	293	5	(1.7)	10	(3.4)
25 - 29	336	8	(2.4)	12	(3.6)
30 - 34	334	7	(2.1)	12	(3.6)
35 - 39	279	20	(7.2)	26	(9.3)
40 -	113	21	(18.6)	26	(23.0)
Total	1355	61	(4.5)	86	(6.3)

Table 8. Frequency distribution of chromosomally abnormal abortuses by gestational weeks

Gestational weeks	No. of karyotyped specimens	Chromosomally abnormal	
		No.	Percentage of known karyotype
<6	124	6	4.8
6 - 7	535	23	4.3
8 - 9	290	17	5.9
10 -	138	7	5.1
Total	1087	53	4.9

Table 9. Frequency of chromosome abnormalities in induced abortuses from women with abnormal menstruation

Maternal age	Menarcheal age 16 years or later			Menstrual cycle					
				35 days or more			Irregular		
	No. of abort.	Abnormal	(%)	No. of abort.	Abnormal	(%)	No. of abort.	Abnormal	(%)
- 24	8	0		12	1	(8.3)	19	0	
25 - 29	19	0		16	2	(12.5)	33	3	(9.1)
30 - 34	22	2	(9.1)	10	0		32	1	(3.2)
35 - 39	29	3	(10.3)	6	0		19	3	(15.8)
40 -	28	9	(32.1)	4	0		5	2	(40.0)
Total	106	14	(13.2)	48	3	(6.3)	108	9	(8.3)

Table 10. Frequency of chromosome abnormalities in induced abortuses from atomic bomb survivors*

Maternal age	Atomic bomb survivors					Controls		
	Mother	Father	Parents	Total				
	No. of abortuses (abnormal)	No. of abortuses (abnormal)	No. of abortuses (abnormal)	No. of abortuses (abnormal)	Abnormal rate (%)	No. of abortuses (abnormal)	Abnormal rate (%)	Significance (χ^2)
- 24		3 (0)		3 (0)	0	290 (10)	3.4	NS
25 - 29	5 (0)	8 (1)	1 (0)	14 (1)	7.1	322 (11)	3.4	NS
30 - 34	16 (0)	22 (2)	3 (0)	41 (2)	4.9	293 (10)	3.4	NS
35 - 39	15 (3)	14 (2)	3 (0)	32 (5)	15.6	247 (21)	8.5	NS
40 -	8 (4)	11 (3)	8 (1)	27 (8)	29.6	86 (18)	20.9	NS
Total	44 (7)	58 (8)	15 (1)	117 (16)	13.7	1238 (70)	5.7	

NS = not significant
*Either or both parents were exposed to the atomic bomb in Hiroshima

struation. None of them, however, showed a chromosome abnormality. Of 1355 karyotyped abortuses, 117 were products of parents either or both of whom were exposed to the atomic bomb, although the estimated exposure doses were unknown in most cases. Sixteen of them had chromosome abnormalities, all of which were numerical aberrations. There was no statistically significant increase in frequency of chromosome abnormalities in these abortuses in comparison with the controls (Table 10).

DISCUSSION

The frequency of chromosome abnormalities in induced abortuses directly reflects an abnormality rate of human conceptuses at the corresponding gestational age. In the present study, 4.9% of induced abortuses collected unselectively from women who terminated their pregnancies before 12 weeks of gestation were chromosomally abnormal. However, since a proportion of women over 35 years of age was significantly higher in induced abortions than in newborns and the proportion of abnormal abortuses increases with advancing maternal age, an abnormality rate in general pregnancies would be lower than 4.9%. Yamamoto and Watanabe[20] reported an abnormality rate of 6.4% in 1250 induced abortuses, the highest incidence in the literature, in which 20% of abortuses studies were products of women over 35 years of age, being 5 times of that in newborns.

The frequencies of chromosome abnormalities by five-years maternal age intervals ranged between 3.4 and 3.6% in women under 34 years of age, but increased with advancing maternal age to 9.3% in women aged 35-39 years and 23.0% in women over 40. Hassold and Chiu[6] showed that chromosome abnormality rates in spontaneous abortuses increased with advancing maternal age; being 50% in women under 34 years of age, 60% in women aged 35-39 and 70% in women over 40. Therefore, if it is assumed that more than 90% of chromosomally abnormal conceptuses result in spontaneous abortion, the spontaneous abortion rate is 7% for women under 34 years of age, 15% for women aged 35-39, and 30% for women over 40.

In the present study, an embryo/fetus was recovered in 60% of the induced abortion specimens collected unselectively, and 1.4% of the embryos were chromosomally abnormal. This abnormality rate was consistent with the result of Kajii et al[10] that 1.6% of induced abortuses with recovered fetal tissues had chromosome abnormalities. Tonomura et al[19] and Sasaki et al[17] reported abnormal rates of induced abortuses with an embryo/fetus to be 1.8% and 1.1%, respectively. These results in induced abortion studies indicate that, in the instances where the existence of an embryo/fetus is confirmed by ultrasonography during the first trimester of pregnancy, a chromosome abnormality rate of the conceptuses is less than 2%. On the other hand, of the induced abortuses without recovered fetal tissues which included both blighted ova and abortuses with a loss of an embryo/fetus through the curettage and/or aspiration, 11% had chromosome abnormalities. Spontaneous abortion studies[8,18] have shown that approximately 50% of spontaneous abortuses are blighted ova either with no embryo or

with a stunted embryo and 60% of them are chromosomally abnormal. Therefore, it is emphasized that the inclusion of incomplete specimens in abortion studies is important in learning the incidence of chromosome abnormalities during the early gestational stage.

Chromosome abnormalities compatible with livebirth such as 45,X, trisomies 13, 18 and 21, 47,XXX, 47,XXY and balanced translocations were found in 26 out of 1355 induced abortuses in the present study. Of these abnormalities, 45,X and autosomal trisomies are frequently also found in spontaneous abortuses (semilethal abnormalities), but 47,XXX, 47,XXY and balanced translocations are relatively rare. On the basis of the present study and the newborn studies[4,7,13,14] in the literature, prenatal selection rates of the semilethal abnormalities were calculated to be 99% for 45,X, 96% for trisomy 13, 97% for trisomy 21. These figures are quite similar to those obtained from spontaneous abortion studies[16].

Prolonged and irregular menstrual cycles had no effect on chromosome abnormalities in induced abortuses, suggesting no correlation of prolonged follicular phase to intrafollicular overripeness of the ova, one of the suggested causes of chromosome abnormalities. Radiation is a well-known cause of chromosome abnormalities, and structural aberrations are frequently found in cultured peripheral blood lymphocytes of atomic bomb survivors[1]. 117 induced abortuses in the present series were obtained from parent(s) who were exposed to the atomic bomb in Hiroshima. However, all of the chromosome abnormalities found among these abortuses were numerical aberrations and there was no statistically significant difference in abnormality rates between atomic bomb survivors and the controls. Therefore, we could not further discuss the influence of radiation from the atomic bomb on the chromosomes of germ cells.

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