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Citation	北海道大學理學部紀要, 16(1), 41-46
Issue Date	1966-12
Doc URL	http://hdl.handle.net/2115/27423
Type	bulletin (article)
File Information	16(1)_P41-46.pdf



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Notes on the Chromosomes of Human Abortuses in Early Pregnancy¹⁾

By

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(With 2 Text-figures and 2 Tables)

A broad survey has been conducted by Professor Makino since 1958 on the chromosomes of induced abortuses randomly collected from several private and public hospitals in Sapporo, use being made of current tissue culture techniques, or of direct squash methods. To date, over 400 individuals were chromosomally screened in the Makino laboratory, with the cooperation of clinicians working in various fields (Makino and Sasaki 1961, Makino *et al.* 1962, 1963, Makino 1964, Sasaki *et al.* 1967). The chromosomal survey of this nature is useful for the establishment of base frequencies of chromosome anomalies among abortuses in the general population, as well as essential for providing basic criteria in order to increase our knowledge of human cytogenetics and population studies. As a link of the above project, the present author has been engaging in the chromosome study of a similar scheme. The present paper describes some results obtained in a period from May to October, 1966.

The author wishes to express his sincere gratitude to Professor Sajiro Makino who suggested to undertake this work for his special guidance and improvement of this manuscript. Further he is also much obliged to Dr. Motomichi Sasaki for his direction and invaluable advice, and to Messrs. Jun-ichi Muramoto and Tatsuro Ikeuchi for their friendly assistance given in the course of this work.

Materials and Methods: The embryonic materials used for this study were derived from 31 cases of healthy pregnant women by means of induced abortion, one of whom (no. 9) showed a threatened termination. They were 6 to 15 weeks old starting from the first day of the last menstrual period (refer to Tables 1 and 3).

Parental data were recorded, when possible, with regard to the following items: (1) maternal and paternal age; (2) date of abortion; (3) date of the last menstrual period; (4) numbers of the previous pregnancies including those of induced abortion, spontaneous abortion, stillbirth and livebirth; and (5) exposure to drugs and radiation, fever, virus infection and so on, after the last menstrual period. Gross anatomical and developmental

(1) Contribution No. 764 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 16, 1966.

anomalies were checked in the abortuses when possible. Some mensurations were also made in the embryos concerning crown-to-rump length, limb length, number of fingers, and so on. In the majority of cases, however, those investigations were impossible or incomplete, since abortuses had been broken or crushed in curetting procedures in many cases.

Table 1. Obstetric history relevant to 20 specimens successfully cultured

Case no.	Length of pregnancy (weeks)	Maternal age (years)	No. of previous pregnancies		
			Induced abortion	Spontaneous abortion	Live birth
1	6	22	0	0	0
2	7	27	1	0	0
3	8	23	0	0	0
4	8	30	6	0	1
5	8	37	7	0	0
6	8	23	0	0	0
7	8	27	3	1	1
8	8	21	1	0	0
9	8	35	7	3	2
10	9	31	5	0	1
11	9	26	0	0	0
12	9	41	12	2	3
13	10	30	2	0	1
14	10	28	5	0	1
15	10	30	1	0	1
16	11	21	0	0	0
17	12	22	1	0	0
18	12	21	0	0	0
19	12	24	0	0	0
20	15	22	0	0	0

Foetal specimens of various types were grown with the use of chicken plasma clots in a synthetic medium with 15 per cent calf serum (for detail, see Makino 1964). Cells growing in these primary cultures were exposed to colchicine for one and a half hours in order to accumulate metaphase cells. Then, cells were collected by centrifugation (1,000 r. p. m. for 5 min.) following trypsinization (0.15 per cent) for 15 to 20 minutes at 37°C. After the hypotonic treatment with 0.5 per cent sodium citrate for 25 minutes, at room temperature, cells were fixed with Carnoy's fixative consisting of 1 part of glacial acetic acid and 3 parts of alcohol. They were air-dried on clean slides, and stained with Giemsa. In addition, the sex-chromatin was scanned in each case on the basis of 100 resting nuclei of non-cultured embryonic cells stained with lacto-acetic orcein.

Results and Remarks

Case reports: As given in Tables 1 and 3, the mean maternal age was 26 years and the mean gestational age was 10 weeks, in 31 cases here studied. Out of the 31 mothers, 18 had experienced one or more induced abortions, 3 had one to three spontaneous abortions, and only 10 mothers have liveborn children.

Table 2. Cytogenetic features of 20 specimens successfully cultured

Case no.	Days <i>in vitro</i>	Tissue source	Sex Chromatin	Chromosome number of distribution					Total no. of cells	Karyotype	No. of cells karyotype
				45	46	47	≥48	≥92			
1	6	lower body	+		17	1		1	19	46-XX	2(46)
2	7	lower body	-	6 ^a	32		1 ^b	3	42	46-XY	2(46), 4(45), 1(56)
3	16	lower body excluding head	+		15				15	46-XX	2(46)
4	9	lower body	+	7 ^c	20				27	46-XX	3(46), 5(45)
5	12	head	+	1	16				17	46-XX	2(46)
6	9	head	-		8				8	46-XY	1(46)
7	5	head	+		5				5	46-XX	1(46)
8	7	head	+		28				28	46-XX	2(46)
9	13	head	+	1	16				17	46-XX	4(46)
10	5	head	+	1	21				22	46-XX	2(46)
11	6	head	-	4 ^d	24				28	46-XY	2(46), 3(45)
12	4	rib with muscle	+		7				7	46-XX	1(46)
13	11	head	+	1	20				21	46-XX	3(46)
14	4	rib with muscle	+	1	9				10	46-XX	3(46)
15	8	rib with muscle	-	1	13				14	46-XY	2(46), 1(45)
16	6	upper limb	-	1	24		1 ^e		26	46-XY	4(46), 1(48)
17	9	rib with muscle	+		8				8	46-XX	1(46)
18	7	lower body	+		20				20	46-XX	2(46)
19	23	head	+		21		1 ^f		22	46-XX	4(46), 1(47)
20	8	head	+		8				8	46-XX	3(46)

a: Missing elements were those in C, E, and G groups in four cells analyzed.

b: 56 chromosomes were present.

c: Missing elements were those in D, E and G groups in five cells analyzed.

d: Missing elements were those in B and E groups in 3 cells analyzed.

e: A 48-chromosome cell with two extra elements in D and E groups.

f: A 47-chromosome cell with two extra elements in B group and one missing from D group.

In five cases (nos. 1, 4, 9, 27 and 29), certain abnormal external features were noted in the embryos. All of these embryos were incomplete, being broken in a more or less degree due probably to handling at the time of operation. Further detailed anatomical and histological studies in those anomalous embryos are now in progress, and the results will be published elsewhere. Only one case of a complete embryo, no. 19, externally normal, was obtained. In nos. 21, 22, 23 and 31, chorionic membranes were subjected to chromosomal study. The remaining 21 specimens were all incomplete ones or parts of body such as limbs and the head. So far as their external features are concerned, they looked normal.

Table 3. Obstetric history relevant to 11 cases of unsuccessful cultures and accounts on sex-chromatin

Case no.	Length of pregnancy (weeks)	Maternal age (years)	Previous pregnancies			Tissue source of culture	Sex-chromatin and its source
			Induced abortion	Spont. abortion	Live birth		
21	7	27	1	0	0	chorion with villi	+ chorion with villi
22	8	23	0	0	0	chorion with villi	+ chorion with villi
23	8	24	4	0	0	chorion with villi	? chorion with villi
24	9	37	2	0	1	head	- head
25	9	22	0	0	0	body	? body
26	10	23	0	0	0	lower limb	+ chorion with villi
27	10	25	6	0	0	head	+ chorion with villi
28	10	31	9	0	1	body	- body
29	11	30	5	0	1	upper limb	+ upper limb
30	12	22	0	0	0	head	+ head
31	15	20	0	0	0	amnion	+ amnion

Cytological findings: As shown in Table 2, the modal chromosomal numbers obtained in 20 cases in which cultures were successful were found at 46 without exception. Karyotype analyses in those modal cells revealed that all of them had a normal chromosome complement of either male or female type, comprising 15 females with the 46-XX karyotype and 5 males with the 46-XY karyotype. Four cases, nos. 2, 4, 11 and 16, showed a considerable number of aneuploid cells. Karyotype analyses in those cells made it clear that they were resulted from a random missing or adding of certain elements, but there was no possible evidence for mosaicism in any one of them. The results of sex-chromatin tests in those subjects were in good agreement with those of the chromosome analyses.

While chromosome studies were not carried out in 11 cases (see Table 3) due to the contamination or the failure of culture, the sex-chromatin study revealed that seven of them were chromatin-positive and two negative, leaving two cases which were not determined on account of some degenerative change of tissue.

Because of the fact that the mother of no. 9 had three threatened abortions and two livebirths in the past, and one of her children suffered from measles during her last pregnancy, the chromosomes of two parents were examined in their

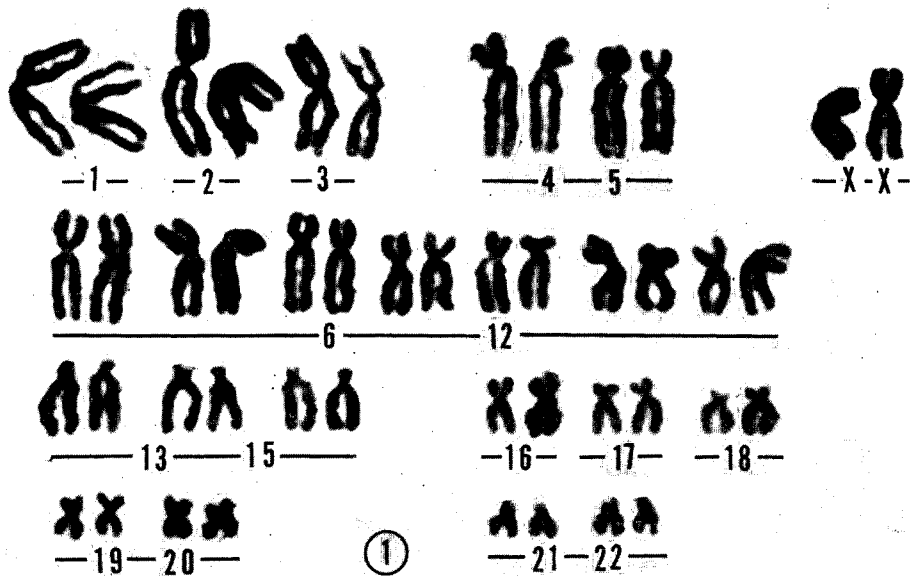


Fig. 1. Female karyotype (46-XX) from no. 9, normal in constitution.

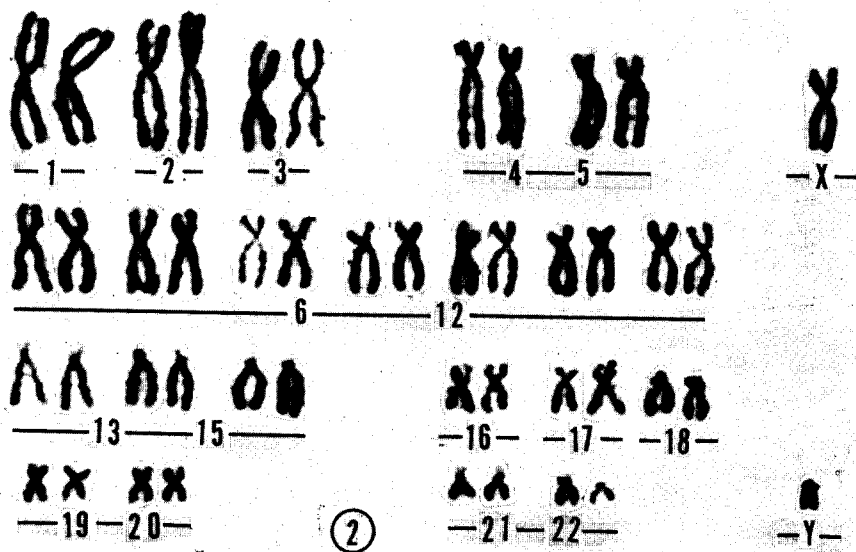


Fig. 2. Male karyotype (46-XY) from no. 6, normal in constitution.

cultured leucocytes. The results indicated that they possessed a normal chromosome constitution. It is especially noticeable that a high incidence of chromosome breakage was noted in the mother: 22 per cent of metaphase cells had at least one chromatid break in them.

Summary

Embryonic specimens for this study were derived from 31 healthy pregnant women by means of induced abortion. One of them showed a threatened termination. They were 6 to 15 weeks old starting from the first day of the last menstrual period. Except one case, the remaining 30 cases were incomplete in external features, probably caused by improper handling at operation. In five cases, certain abnormal external features were noted. The mean maternal age was 26 years and the mean gestational age was 10 weeks.

The modal chromosomal number in 20 cases in which cultures were successful was found at 46 without exception, involving 15 females with the 46-XX complement and 5 males with the 46-XY.

The mother of no. 9 had three threatened abortions and one of her children suffered from measles during her last pregnancy. Her cultured leucocytes showed a high incidence of chromosome breakage in 22 per cent of metaphase cells.

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