Maternal fertility, reproductive loss, and defective human embryos

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ABSTRACT It is possible that many abnormal conceptuses are lost at an early stage without the mother's knowledge. To investigate this further the reproductive history of the mothers of defective embryos (neural tube defects, holoprosencephaly, cleft lip, polydactyly, and early embryonic resorption) was compared with that of the mothers of normal embryos. The frequency of prior miscarriages was higher in the case mothers than in normal controls matched for maternal age and gravidity, the difference being significant for all the anomalies except for polydactyly. The case mothers had fewer prior recognised pregnancies than control mothers matched for maternal age. There were more primigravid mothers in abnormal groups, and the difference from controls was significant for neural tube defects. It was assumed that the gravidity of the case mothers may be underestimated, possibly due to increased early abortions which are not recognised clinically. Thus, it seems that both recognised and unrecognised abortions occur more often in the mothers of defective embryos. Since many spontaneous abortuses are morphologically and/or cytogenetically abnormal, some women appear to conceive abnormal embryos repeatedly. Most of these embryos, however, may be screened out prenatally and escape clinical detection. Information on prior reproductive history of the woman should be examined carefully in genetic counselling.

The prevalence of malformations is much higher in prenatal human populations than in newborn infants. Based on the study of a large number of therapeutic abortuses in Japan, Nishimura¹ showed that the prevalences of specific malformations were at least several times higher than those in newborn infants. Such a difference between the two populations has been observed not only for lethal malformations such as neural tube defects but also for less serious anomalies which may not in themselves be lethal, such as oral clefts and limb malformations. Thus many embryos malformed at organogenesis appear to be eliminated prenatally and may not be recognised at birth. This is supported by the fact that nearly half of spontaneous abortuses are morphologically and/or cytogenetically abnormal.^{2–4} Creasy and Alberman⁵ studied a series of 2658 spontaneous abortions and estimated the prevalence of anencephaly and spina bifida to be 5.3 per 1000 at 8 weeks of gestation, 4.1 at 12 weeks, 3.0 at 20 weeks, and 2.8 at 27 weeks. Their findings suggest that 54% of these abnormal fetuses abort spontaneously between 8 and 27 weeks of gestation. Hook⁴ estimated that the proportion of chromosomally affected conceptuses is 5% among all recognisable pregnancies and drops to 4.2% at 8 weeks, 2.4% at 12 weeks, 1.1% at 16 weeks, 0.8% at 20 weeks and 0.6% in livebirths. These findings clearly

show the selective intrauterine death of abnormal embryos and fetuses.

It has recently been reported that a substantially large proportion of human conceptions confirmed immunologically soon after implantation are lost at such an early stage of gestation that the mothers are unaware that conception has occurred.^{6,7} Since the prenatal elimination rate of defective conceptuses is high and since a large proportion of early intrauterine deaths may not be clinically recognised, it seems that many abnormal conceptuses in early pregnancies are eliminated without the knowledge of the mother. This is important in genetic counselling because the occurrence of abnormal conceptions may often escape detection and therefore the familial clustering of malformations can be significantly underestimated.

Using a large collection of human embryos in Japan, we analysed the reproductive histories of the women who conceived a defective embryo. The derived information would be of some significance in estimating the fate of early human conceptuses, especially those of predisposed women.

Methods

The human embryos used in the present study were from the collection of human conceptuses in the

Congenital Anomaly Research Centre of Kyoto University. The embryo collection is composed of about 40 000 human conceptuses, most of which were procured after induced abortion. The details of the embryo collection were described previously.¹⁸ Reproductive history of each mother was taken by attending physicians using a standard interview form before or shortly after the termination of pregnancy. Since the physicians did not examine the embryo, neither the physicians nor the mothers were aware of the abnormalities in the embryo and therefore the reporting bias and maternal memory bias should be minimal.

From the collection, 282 malformed embryos and 424 empty chorionic sacs were selected for the present study. The malformed embryos had a neural tube defect, holoprosencephaly, cleft lip with or without cleft palate, or polydactyly. Among them, 231 cases had an isolated anomaly and the remaining 51 cases had two or three external anomalies. Empty chorionic sacs are intact sacs without proper embryonic tissue and are considered to be products of very early embryonic resorption. All the study cases were the products of induced abortion and the anomalies were found on routine examination in the laboratory. Cases referred after antenatal diagnosis or after physicians' knowledge of anomalies were not included in the present study.

The reproductive histories of the mothers of defective embryos were compared with those of normal embryos procured after induced abortion. For comparison of prior abortions, one normal embryo for each abnormal case was drawn from the collection, matched for maternal age, maternal gravidity (the number of prior pregnancies except induced abortions), gestational age at termination of pregnancy (± 2 days), and the year of procurement. For analysis of the number of prior pregnancies, control cases were matched with each index case for maternal age, gestational age (± 2 days), and the year of procurement. When two or more cases were eligible as

a control, the case was chosen whose termination immediately followed that of the study case.

Statistical analyses were performed using χ^2 test for comparing percentages and Student's *t* test for comparing means.

Results

Table 1 shows the data on maternal age, gravidity, and previous miscarriages in the mothers of defective conceptuses. The average maternal age ranged from 29.2 for neural tube defects to 30.6 for empty chorionic sacs, but these figures did not differ significantly from each other and from the average maternal age (30.2) for the total embryo population under study.⁹

The frequency of previous reproductive loss was compared between the mothers of defective embryos and those of normal embryos matched for maternal age and gravidity. The frequencies of prior spontaneous abortions clinically recognised are shown in table 2. The proportions of the women who had experienced any recognised miscarriages were 35.8% (19/53) for neural tube defects, 30.0% (21/70) for holoprosencephaly, 31.3% (21/67) for cleft lip, 24.3% (17/70) for polydactyly, and 34.5% (122/355) for empty chorionic sacs, and the difference from normal controls was statistically significant for empty chorionic sacs ($\chi^2 = 31.7$, $p < 0.01$) and total defective embryos ($\chi^2 = 35.5$, $p < 0.01$). The average number of previous abortions was 0.51 in the mothers of defective embryos and 0.24 in the normal controls ($p < 0.01$). The average number of abortions was significantly larger than that of controls in the cleft lip and empty chorionic sac groups.

In table 3, the proportions of prior pregnancies which ended in spontaneous abortion are shown. The rate of recognised abortions was fairly constant (12.1–14.3%) in the mothers of normal embryos. In the groups of defective embryos, however, it varied from 17.3% in the mothers of polydactyly embryos to 33.8% in those of cleft lip embryos. The difference

Table 1 Maternal age and reproductive history of the mothers of defective embryos

Anomaly	Number of cases	Mean maternal age (years)	Number of prior pregnancies		Number of prior miscarriages		Percentage of miscarriages in prior pregnancies*
			mean	SE	mean	SE	
Neural tube defects	72	29.2	1.94	0.24	0.47	0.10	24.8 (25/101)
Holoprosencephaly	88	30.2	2.67	0.25	0.46	0.10	22.9 (32/140)
Cleft lip	84	29.9	2.44	0.23	0.65	0.15	33.8 (45/133)
Polydactyly	84	29.6	2.31	0.22	0.31	0.08	17.3 (22/127)
Empty chorionic sac	424	30.6	2.89	0.12	0.54	0.05	25.9 (192/741)
Total†	706	30.2	2.69	0.09	0.51	0.03	26.0 (299/1151)

* Excluding induced abortions.

† Cases with two or more anomalies under study were counted only once.

Table 2 Previous recognised abortions in multigravid mothers of defective and normal embryos

Anomaly in index case	Group	Number of multigravid mothers	Number of previous spontaneous abortions							Significance
			0	1	2	3	4+	Mean	Variance	
Neural tube defects	Cases	53	34	14	4	1	0	0.47	0.52	NS
	Controls*	53	40	13	0	0	0	0.25	0.19	
Holoprosencephaly	Cases	70	49	14	4	2	1	0.46	0.72	NS
	Controls*	70	57	9	2	2	0	0.27	0.43	
Cleft lip	Cases	67	46	11	2	3	5	0.67	1.59	p<0.05
	Controls*	67	53	11	2	0	1	0.28	0.45	
Polydactyly	Cases	70	53	13	3	1	0	0.31	0.39	NS
	Controls*	70	61	6	1	0	2	0.24	0.68	
Empty chorionic sac	Cases	355	233	71	37	10	4	0.54	0.79	p<0.01
	Controls*	355	299	40	12	3	1	0.22	0.32	
Total†	Cases	583	393	118	47	15	10	0.51	0.80	p<0.01
	Controls*	583	482	75	17	5	4	0.24	0.39	

* Normal controls matched for gravidity (excluding induced abortions), maternal age, and embryonic age at termination of pregnancy.

† Cases having two or more anomalies under study were counted only once.

NS = Not significant.

Table 3 Proportion of prior pregnancies which ended in spontaneous abortion in mothers of defective and normal embryos

Anomaly in index case	Numbers of mothers	Total number of previous pregnancies*	Previous spontaneous abortions				χ^2	Significance
			Cases		Control†			
			n	%	n	%		
Neural tube defects	72	101	25	24.8	13	12.9	5.10	p<0.05
Holoprosencephaly	88	140	32	22.9	19	13.6	4.64	p<0.05
Cleft lip	84	133	45	33.8	19	14.3	16.49	p<0.01
Polydactyly	84	127	22	17.3	17	13.4	0.48	NS
Empty chorionic sac	424	741	192	25.9	90	12.1	44.67	p<0.01
Total‡	706	1151	299	26.0	154	13.4	56.99	p<0.01

* Excluding induced abortions.

† Normal controls matched for gravidity (excluding induced abortions), maternal age, and embryonic age at termination of pregnancy.

‡ Cases having two or more anomalies under study were counted only once.

NS = Not significant.

from controls was statistically significant for all the anomalies except for polydactyly.

Table 4 shows the numbers of previous recognised pregnancies in the case mothers and normal controls, corrected for maternal age. The average number of prior pregnancies was smaller than that of normal controls for all the anomalies studied, and the difference was statistically significant for neural tube defects ($p<0.05$). Twenty-six per cent (19/72) of the mothers of embryos with neural tube defects were primigravidas, as opposed to only 11.1% (8/72) of the control mothers ($\chi^2=4.56$, $p<0.05$). The proportion of primigravid mothers was 20.5% (18/88) for holoprosencephaly, which was more than twice as high as that of controls (9.1%; 8/88) although the difference did not reach statistical significance

($\chi^2=3.66$, $0.05<p<0.1$).

The proportion of primigravid mothers and the frequency of prior spontaneous abortions were compared between cases with and without associated anomalies (table 5). For each anomaly, the proportion of primigravid mothers was larger in the cases with associated anomalies than in those without, although the differences were not statistically significant. Prior abortions were more frequent in the cases with multiple malformations, and significantly in cases with neural tube defects ($\chi^2=6.10$, $p<0.02$).

Discussion

The present study has shown that the mothers of defective embryos have greater than expected rates of

Table 4 Previous recognised pregnancies in mothers of defective and normal embryos

		Number of previous recognised pregnancies									
Anomaly in index case	Group	Number of mothers	0	1	2	3	4	5+	Mean	Variance	Significance
Neural tube defects	Cases	72	19	23	8	7	3	12	1.94	4.02	p<0.05
	Controls*	72	8	12	18	14	11	9	2.58	3.01	
Holoprosencephaly	Cases	88	18	21	9	8	10	22	2.67	5.56	NS
	Controls*	88	8	22	17	16	9	16	2.84	5.40	
Cleft lip	Cases	84	17	16	16	10	12	13	2.44	4.39	NS
	Controls*	84	20	14	12	13	10	15	2.49	4.71	
Polydactyly	Cases	84	14	21	20	10	6	13	2.31	4.07	NS
	Controls*	84	13	16	12	14	10	19	2.83	4.89	
Empty chorionic sac	Cases	424	69	85	66	58	47	99	2.89	6.30	NS
	Controls*	424	66	61	81	69	51	96	2.95	5.97	
Total†	Cases	706	124	158	109	92	70	153	2.69	5.53	NS
	Controls*	706	106	120	134	118	83	145	2.82	5.36	

* Normal controls matched for maternal age and embryonic age at termination of pregnancy.

† Cases having two or more anomalies under study were counted only once.

NS = Not significant.

Table 5 Proportion of primigravidas and prior reproductive loss in mothers of defective embryos with and without associated malformations

Type of anomaly	Number of cases	Average maternal age (years)	Number of primigravidas (%)	Abortions/prior pregnancies (%)
Neural tube defects				
Isolated	54	29.5	13 (24.1)	14/77 (18.2)
Multiple	18	28.4	6 (33.3)	11/24 (45.8)†
Holoprosencephaly				
Isolated	59	30.2	10 (16.9)	24/109 (22.0)
Multiple	29	30.3	8 (27.6)	8/31 (25.8)
Cleft lip				
Isolated	58	30.7	9 (15.6)	33/99 (33.3)
Multiple	26	28.2	8 (30.8)	12/34 (35.3)
Polydactyly				
Isolated	60	28.6	9 (15.0)	16/97 (16.5)
Multiple	24	30.4	5 (20.8)	6/30 (20.0)
Total				
Isolated	231	29.4	41 (17.7)	87/382 (22.8)
Multiple*	51	29.1	13 (25.5)	15/48 (31.3)

* Cases with two or more anomalies under study were counted only once.

† Significantly different from the cases without associated malformations (p<0.02).

spontaneous abortion in previous pregnancies and fewer than expected previous pregnancies. Since maternal age was matched between the case and control groups in the analysis of previous pregnancies, the chance of fertilisation could not differ significantly between the two groups. This means that the number of prior pregnancies might be underestimated in the mothers of abnormal embryos, possibly due to

increased subclinical loss of conceptuses in these women. Thus, both recognised and unrecognised reproductive losses appear to be more common in the women who conceived a defective embryo. Since a large proportion of spontaneously aborted embryos are abnormal,²⁻⁴ it seems that some mothers of malformed embryos may have conceived abnormal conceptuses repeatedly and experienced recurrent

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abortions which had not always been recognised clinically. It has been pointed out that spontaneous abortion is an important screening process that reduces the birth of malformed infants.¹⁰ In the present series, however, none of the women reported that she had had a malformed abortus before. This is probably because aborted embryos and fetuses are often seriously macerated and anomalies in early abortuses can be easily overlooked. Furthermore, some embryonic deaths may occur before organogenesis begins.¹¹

Previous studies have shown the increased risk of spontaneous abortion in the mothers of infants with neural tube defects. The proportion of mothers of infants with these defects who reported any prior abortions was shown to be higher than that of control mothers.¹²⁻¹⁴ Record and McKeown¹⁵ found that the mothers of infants with central nervous system defects had an abortion rate of 11.2%, compared with 7.5% in a control group. In South Wales, 17% of pregnancies prior to the birth of a child with a neural tube defect ended in abortion, significantly higher than 11.6% in the control population.¹⁶ Such studies in postnatal populations are usually based on retrospective interviews and they are sometimes criticised because of the possible overreporting by the mothers of defective infants. In the present study, such a reporting bias and maternal memory bias can be neglected, since neither the mothers nor the attending physicians were aware of the anomaly in the embryo, and since the interval between conception and termination of pregnancy was relatively short (up to 8 weeks) when the memory of the mother should be fresh and accurate.

It is interesting to note the previous finding that the rate of reproductive loss was lower in the families of cleft lip patients than the corresponding figure in the general population or in the families without cleft lip patients.¹⁷⁻¹⁹ In the data from Indiana and Montreal, none of the reported incidences of fetal mortality in sibships of cleft lip patients exceeded 11%, which is lower than the accepted spontaneous abortion rate in the human.^{20 21} These data seem incompatible with our present result that the mothers of cleft lip embryos have an increased risk of spontaneous abortion. One possible explanation for such inconsistency is that the apparently reduced rate of reproductive loss in postnatal studies may be due to the selection of those families in which cleft lip embryos escaped spontaneous abortion and survived to birth. Secondly, compensatory reproduction may have occurred in the couples who gave birth to a malformed baby. Furthermore, maternal recall bias may have affected the reported incidence of spontaneous abortion, since in some postnatal cases family histories may have been taken several years after the birth of probands. Further epidemiological studies will be required to

unravel the complex relation between the occurrence of oral clefts and reproductive loss in the families. No previous data regarding maternal reproductive loss are available for holoprosencephaly, polydactyly or empty chorionic sacs.

Our present data have shown that prior spontaneous abortions were more common in the mothers of embryos with multiple anomalies than in those of embryos with an isolated anomaly. According to Dronamraju and Bixler,²⁰ the incidence of fetal mortality was found to be significantly greater in sibships of probands with bilateral cleft lip than in those of probands with unilateral cleft lip. These data suggest that the severity of malformations is positively associated with fetal mortality in the mother or other family members. Thus, they seem to support a multifactorial two threshold concept with a lower threshold beyond which the malformation occurs and a higher one beyond which the embryo dies.²²

As for the effects of pregnancy order, the increased risk in births of children with neural tube defects to primiparous women has been reported by several investigators.²³⁻²⁵ Czeizel and Révész,²³ in their large Hungarian study, showed the highest rate of neural tube defects in primiparas and especially in older primiparas. The risk of bearing a child with neural tube defect has been shown to be highest in first borns and decreases with birth order.²⁶ These findings are consistent with our own finding that the mothers of abnormal embryos, especially those with neural tube defects, were more often primigravidas than the mothers of normal controls.

Our study suggests that the number of prior conceptions may be underestimated in the mothers of defective embryos, and this is probably also the case in postnatal studies. The elevated risk of births of infants with neural tube defects to primiparous women may suggest that some cases are conceived after unrecognised abortions. According to the data from Birmingham, England, an increased risk to firstborn was noted for anencephaly, spina bifida, hydrocephalus, and congenital dislocation of the hip.²⁷ In this study, there was no evidence of an association between birth rank and the occurrence of oral clefts. No data have been reported concerning the effects of birth order on the occurrence holoprosencephaly, polydactyly and empty chorionic sacs.

The significance of our finding is twofold. First, abortion prone women and women with subnormal fertility have an increased risk of having a malformed embryo. Second, some mothers appear to conceive defective conceptuses repeatedly but many of these escape clinical detection. Such "silent" recurrent cases may be related to some predisposition of the mother which can be genetic, environmental, or both.

Environmental factors may include social, nutritional and other maternal factors. Lower socioeconomic status and undernutrition have been implicated in the occurrence of neural tube defects.²⁸⁻³³ In Japan, however, socioeconomic status appears quite uniform and nutrition problems are rare these days. Some other factors which are genetic or biological in origin may possibly be related to the predisposition of the mothers. The effects of maternal age per se or those of the chromosome abnormalities for which the risk increases with advancing maternal age can be excluded because the average maternal age was not significantly different between the case and control groups. Balanced translocations are the most well documented chromosome abnormalities in couples with multiple miscarriages. These anomalies have been found in 2-8% of the couples experiencing multiple abortions.³⁴⁻³⁷ The cases in the present study were not examined cytogenetically, and the role of chromosome aberrations in the causation of recurrent embryonic abnormalities should be studied in future. It is noteworthy that in the present study, the mothers of embryos with syndromic neural tube defects had significantly more previous miscarriages than the mothers of those with isolated neural tube defects. Malformation complexes or syndromes are often associated with chromosome abnormalities, but the implication of our finding awaits further investigation.

Clarke *et al.*³⁸ suggested that neural tube defects may arise from an interaction between an embryo and a trophoblastic cell rest remaining from a previous pregnancy. They postulated that such a cell rest is more likely to occur after a spontaneous abortion than after a normal delivery. Our data showing that both recognised and unrecognised abortions are relatively common in the mothers of embryos with neural tube defects do not contradict this hypothesis.

It is well known that many malformations occur sporadically without an apparent family history. This occurrence, however, must be underestimated because a substantially large number of abnormal embryos are screened out prenatally and often escape clinical detection. In genetic counselling, information on the fertility and prior reproductive loss of the mother should be examined carefully to provide a basis for prognosis of her pregnancy and a criterion for selecting patients for antenatal diagnosis.

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