Results and Pitfalls in Prenatal Cytogenetic Diagnosis*

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Summary. Since 1969, we have cultured over 200 diagnostic amniotic fluids. Of these, 183 were for cytogenetic diagnosis. The chromosome analysis was successful in 168 cases. The indications and the results of the affected fetuses (followed by therapeutic abortion) are: (1) previous child with Down's syndrome: 62 cases (1:47,XX,+21); (2) advanced maternal age: 54 cases (1:47,XXY; 1:45,X/46,XY mosaicism; 1:47,+18); (3) previous child with multiple anomalies: 12 cases; (4) previous child with 47,XY,+18 or 47,+13: five cases; (5) translocation carrier: two cases; (6) parental mosaicism: three cases; (7) X-linked disorders: six cases (3:XY); (8) others: 24 cases. We have found firstly, that for prenatal sex determination, karyotype analysis of the cultured amniotic fluid cells is the only accurate means and that caution must be taken if sex chromatin and Y-fluorescent body determination from the uncultured amniotic fluid cells is used. Secondly, that diagnosis of chromosomal mosaicism can be problematic as exemplified by our case of 45,X/46,XY mosaicism, where only 45,X cells were recovered from the first culture. Thirdly, that in cases with enlarged satellites, cells of late prophase or early metaphase must be used to eliminate confusion with translocations. We encountered three cases of enlarged satellites—one in the D group and two in the G group —and all three resulted in normal infants. Fourthly, that the karyotype may be altered by contamination and/or treatment or other unknown factors. We have observed two such cases where each mother delivered a normal infant.

The use of amniocentesis for prenatal diagnosis has become a useful adjunct in genetic counselling. The progress in techniques of cultivation of amniotic fluid cells has further enhanced the studies of fetal cells for both chromosomal abnormalities and biochemical defects (Milunsky *et al*, 1970; Nadler, 1972). However, the greatest demand has always been for prenatal cytogenetic diagnosis. Although prenatal cytogenetic diagnosis appears to be simple, as more experience and more information are accumulated, the situation is becoming more complex.

Materials and Methods

Since 1969, we have cultured over 200 diagnostic amniotic fluids. Of these, 93% were for cytogenetic diagnosis. The transabdominal amniocenteses were done between 14 and 20 weeks of gestation, with the majority at about 16 weeks. The ultrasonic localization of the placenta was used before amniocentesis in approximately 40% of the cases. In these cases, no bloody taps were obtained.

Nine per cent of the cases required a repeated amniocentesis because the first culture was not successful. The volume of amniotic fluid ranged from 8 to 25 ml with the majority between 15 and 20 ml. The amniotic fluid cells were cultivated according to the method reported from this laboratory (Lisgar *et al*, 1970). The indications for prenatal cytogenetic diagnosis of the 168 successful cases are listed in Table I.

Sixty-two cases had a previous child with Down's syndrome. Fifty-four were of advanced maternal age;

of these, 37 were over 40 years of age and 17 were between 35 and 40 years of age. Twelve had a previous child with multiple congenital anomalies. Three had a previous child with trisomy 18. Two had a previous child with trisomy 13. Two were for the detection of a translocation. Three fathers had trisomy 21 or 18 mosaicism and each had a previous child with such trisomy. Six had a previous son affected with an X-linked disorder; of these, five had sons with Duchenne muscular dystrophy and one had a son with Wiskott–Aldrich syndrome. The 24 cases listed as others included drug users (LSD and methotrexate), parental exposure to X-radiation, familial history of Down's syndrome, etc.

For prenatal sex determination, identification of X-chromatin body and Y fluorescent body were done according to the method used in this laboratory (Rook *et al*, 1971). An attempt to make a prenatal sex determination was carried out using 20 amniotic fluid specimens obtained from termination of pregnancy cases in the Mount Sinai Hospital clinic.

Results

Chromosome analysis was successful in 168 cases. For completeness, we have also included 20 successful cases previously reported from our laboratory (Gertner *et al*, 1970). The results and the outcome of these 168 cases are shown in Table I.

No differences have been observed in terms of cell growth between those amniocentesis monitored by ultrasonic localization of placenta and those not monitored by ultrasonography. This is in contrast to the report by Robinson *et al* (1972) where 80% of cultures failed to grow after ultrasonogram in contrast with 17% failure rate when ultrasound was not employed. There has been no known maternal morbidity or mortality directly related to the amniocentesis. There were four premature deliveries and all four died of prematurity. There were two stillbirths at full term. There was one full-term delivery with a birth weight of 1475 g because of placental insufficiency. This infant died shortly after birth. None of these infants had gross abnormalities.

There were 15 unsuccessful cases. All 15 amniotic fluid specimens failed to grow or were contaminated in the culture and all the mothers refused to accept a repeated amniocentesis. Of these, nine gave birth to normal live infants, one had a child with Down's syndrome, two were carrying fetuses which were apparently dead before amniocentesis, one was aborted later as a macerated fetus, and two were lost to follow-up.

TABLE I

Group	Indications	No. of Cases	Karyotype	Outcome of Pregnancy*							
				A	B	C	D	E	F	G	H
1	*Previous child with Down's syndrome*	*62*									
	Maternal age < 35 yr	50	49—normal; 1—46,XY,Ds +	0	0	47	0	0	0	0	3
	Maternal age 35–40 yr	9	8—normal; 1—47,XX, + 21†	1†	0	8	0	0	0	0	0
	Maternal age > 40 yr	3	3—normal	0	0	3	0	0	0	0	0
2	*Advanced maternal age*	*54*									
	Maternal age > 40 yr	37	34—normal; 1—47,XXY†; 1—47,XY, + 18†; 1—46,XY,Gs +	2†	0	29	0	3	1	0	2
	Maternal age 35–40 yr	17	16—normal; 1—45,X/46,XY†	1†	0	15	0	0	0	1	0
3	Previous child with multiple anomalies	12	12—normal	0	0	11	0	1	0	0	0
4	Previous child with trisomy 18	3	3—normal	0	0	2	0	0	0	0	1
	Previous child with trisomy 13	2	1—normal; 1—46,XX,Gs +	0	0	1	0	0	1	0	0
5	*Translocation carrier*	*2*		0	0	2	0	0	0	0	0
	t(DqGq)	1	1—normal								
	t(5p + ;13q −)	1	1—normal								
6	*Paternal trisomy mosaicism*	*3*	2—normal	0	1	2	0	0	0	0	0
	46/47, + 21	2									
	46/47, + 18	1									
7	*X-linked disorders*	*6*									
	Duchenne muscular dystrophy	5	3—46,XX; 2—46,XY†	2†	0	2	0	0	0	0	1
	Wiskott–Aldrich syndrome	1	1—46,XY†	1†	0	0	0	0	0	0	0
8	Others	24	23—normal; 1—46,XY,13p − ‡	0	2	18	1‡	0	0	0	3
	Total	168		7	3	140	1	4	2	1	10

* A = therapeutic abortion; B = induced abortion due to other reason; C = live, normal birth; D = multiple anomalies; E = premature birth and death from prematurity; F = still birth; G = neonatal death; H = lost to follow-up.
† Parents elected to have a therapeutic abortion on the basis of the prenatal cytogenetic diagnosis.
‡ The Dp − was also found in the phenotypically normal mother and other maternal relatives; the baby was born with Noonan's syndrome.

Of the 168 successful cases, seven mothers (marked in Table I) elected to have a therapeutic abortion on the basis of prenatal cytogenetic diagnosis. One 37-year-old mother who had a previous child with Down's syndrome was found to be carrying another fetus with trisomy 21. The fetus was aborted and was found to have several stigmata of Down's syndrome including mongoloid slant of the eyes, epicanthal folds, redundant skin folds of the back of the neck, and clinodactyly (Gertner et al, 1970). One 47-year-old mother was carrying a fetus with 47,XXY. The extra X chromosome was confirmed by finding one X-chromatin body in the interphase nuclei. The abortus was a phenotypic male (Gertner et al, 1970). One 40-year-old multipara was found to be carrying a 47,XY, + 18 fetus. The extra No. 18 was confirmed by the fluorescent staining technique. The pathological examination of the 20-week-old abortus showed recognizable stigmata of trisomy 18, including low-set, malformed ears, micrognathia, broad neck, bilateral flexion deformity of the thumbs, overlapping index fingers, right clinodactyly, and left polydactyly, the internal anomalies included ventricular and atrial septal defects, bicuspid aortic valve, Meckel's diverticulum, and intestinal malrotation. These findings indicate that the phenotype of trisomy 18 is well established by mid-gestation. The detailed pathological findings are reported by Hsu et al (1973).

The case of 45,X/46,XY mosaicism was from a 36-year-old multipara. She came to see us primarily because she had a previous child with Tay-Sach's disease. Hexosaminidase A assay showed that the fetus was not affected; however, chromosome analysis showed a 45,X constitution in all 20 metaphases examined. This couple elected to have a therapeutic abortion and a phenotypic male fetus was expelled. A second amniotic fluid culture was obtained at the time of abortion. All cells of this culture showed 46,XY. Repeat harvest of the first amniotic fluid sample revealed only cells with 45 chromosomes. No 46,XY cell line was ever found in the culture of the first amniotic fluid sample, but Y-fluorescent bodies were seen in the interphase nuclei. Special staining studies of the 45,X karyotype indicate a probable Y/13 translocation. A detailed description of this case was reported by Kardon et al (1972).

Three mothers who are carriers of X-linked disorders were found to be carrying male fetuses. All three requested therapeutic abortions because of the 50% risk of bearing another affected son.

In the cases listed as 'others', one fetus was found to have a short arm deletion of a No. 13 chromosome by fluorescent staining technique. A similar structural abnormality was also detected in the phenotypically normal mother and several other maternal relatives. This baby was born with clinical Noonan's syndrome.

Three cases with enlarged satellites of one acrocentric chromosome were observed, one in the D group (Fig. 1) and two in the G group (Fig. 2). The enlarged satellites were easily identified in mitotic cells from late prophase or early metaphase. The case with enlarged satellites of a D-group chromosome is from a young mother who had a previous child with Down's syndrome. The chromosomes of the affected child were not studied. A replicate culture of this case was initially misinterpreted as a D/G translocation in another laboratory. The chromosome with enlarged satellites can be mistaken for a translocation if a later metaphase cell is used for analysis, since the chromosomes of a late metaphase cell are rather contracted (Fig. 2). All three pregnancies resulted in normal infants.

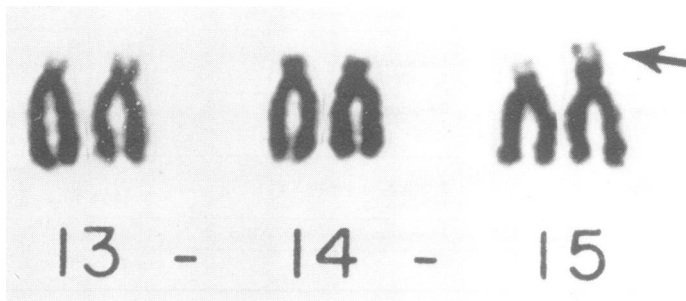We have observed two cases of karyotypes altered by contamination and/or treatment or other

FIG. 1. Partial karyotype of the D group chromosomes of the case of Dps + , showing the enlarged satellites of one D group chromosome (arrow).
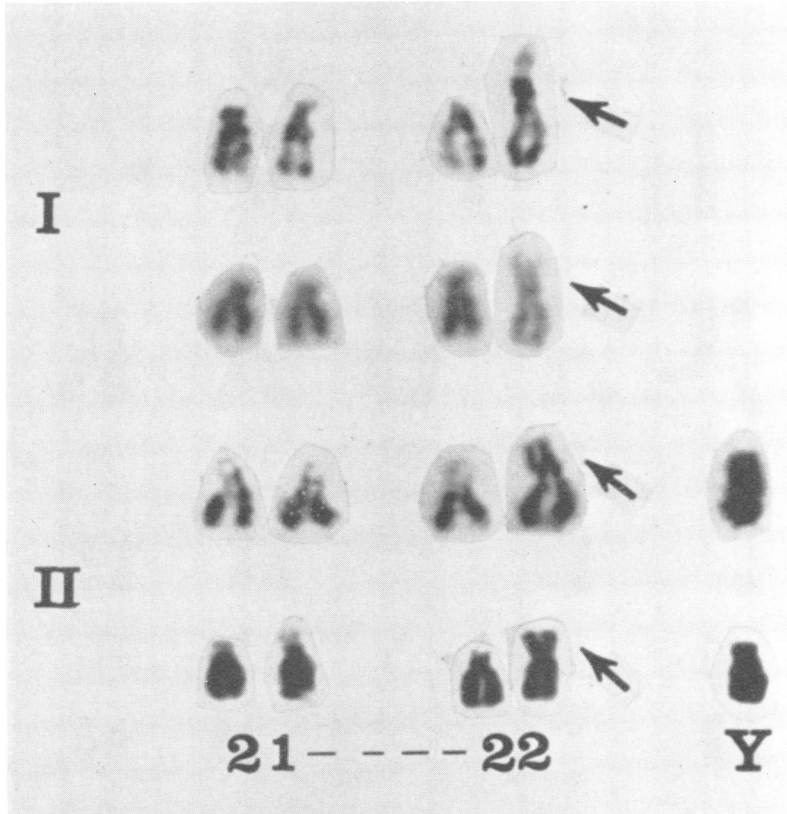
FIG. 2. Partial karyotype of G group chromosomes from the two cases with Gps +. The top three partial karyotypes are taken from three early metaphase cells, showing the enlarged satellites of one G group chromosome (arrows). The partial karyotype on the bottom is from a late metaphase cell. The chromosome with enlarged satellites can easily be mistaken for a G/G translocation chromosome (arrow).

unknown factors. Both amniotic fluids were from young mothers with a previous child with Down's syndrome. In the first case, the initial harvest of the amniotic fluid culture at the end of the third week yielded only two analysable metaphases. Both cells showed a normal 46,XX karyotype. Subsequently the culture was contaminated by a fungus infection and was treated with amphotericin (2·5 γ/ml). After the culture recovered from the infection, a second harvest (19 days after the first harvest) showed eight abnormal cells and two normal cells. The abnormal karyotype contained a small acrocentric chromosome replacing a normal D-group chromosome (Fig. 3). A repeated amniocentesis was suggested to the couple, but was not accepted. This pregnancy resulted in a normal female infant delivered at full term. Unfortunately we have not been able to study the baby because the mother is not co-operative.

In the second case, the first harvest of the culture showed 27 cells with a normal 46,XY constitution and three cells with an abnormal karyotype where one G-group chromosome was missing and one additional D-group chromosome was present (Fig. 4). A repeat harvest two weeks later from the same culture showed 46,XY in all 20 cells examined. This pregnancy also resulted in a normal male infant. Peripheral leucocyte culture from the infant showed a normal male karyotype in all 35 cells examined.

In all our successful cultures, tetraploid cells have rarely exceeded 20%. In one culture, 71% of mitotic cells were found to be tetraploid in the second harvest.

As to prenatal sex determination, the results of X-chromatin and Y-fluorescent body and the phenotypic sex of each abortus are shown in Table II. The data includes those reported earlier from this
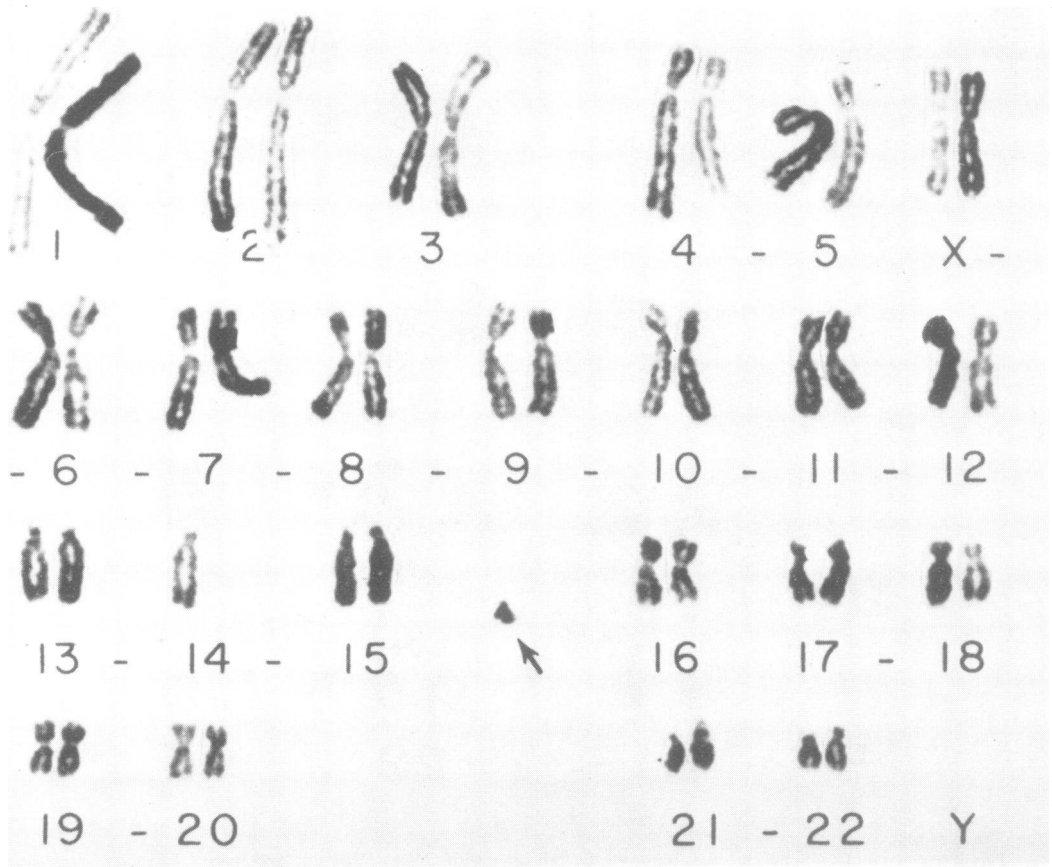
FIG. 3. Karyotype showing 46,XX, − D, + Gq − from a contaminated culture treated with amphotericin.

laboratory (Rook *et al*, 1971). In 20 specimens examined for Y-fluorescent body, there were seven specimens with the fluorescent Y body; there were, however, eight male abortuses. One male was not identified by this method. In 16 specimens examined for X-chromatin, only six specimens were found to be definitely positive; there were nine female abortuses. Most of the uncultured amniotic fluid cells are pyknotic and not suitable for X-chromatin determination according to the selection criteria suggested by Hsu, Klinger, and Weiss (1967). In our very earliest attempts, two females were misdiagnosed as males by X-chromatin studies alone, before the fluorescent Y body was described (Rook *et al*, 1971).

Discussions and Conclusions

We have presented our results and experiences in prenatal cytogenetic diagnosis. In our series, we have observed no maternal morbidity or mortality. There appeared to be no direct relationship between the procedure of amniocentesis and the incidence of four premature deliveries and two stillbirths in our series. Since three premature deliveries and one stillbirth were from the mothers of advanced maternal age (> 40 years; see Table I), the results are not different from what might be expected without amniocentesis. In view of a remote but unlikely possibility that amniocentesis may cause some fetal damage with long-range minor developmental problems, an investigation of this possibility has now been undertaken by our group and several other groups in the United States. We would like to emphasize the following problems or pitfalls in prenatal cytogenetic diagnosis.

1. Diagnosis of chromosomal mosaicism can be problematic as emphasized by our case of 45,X/46,XY mosaicism; where only 45,X cells were recovered
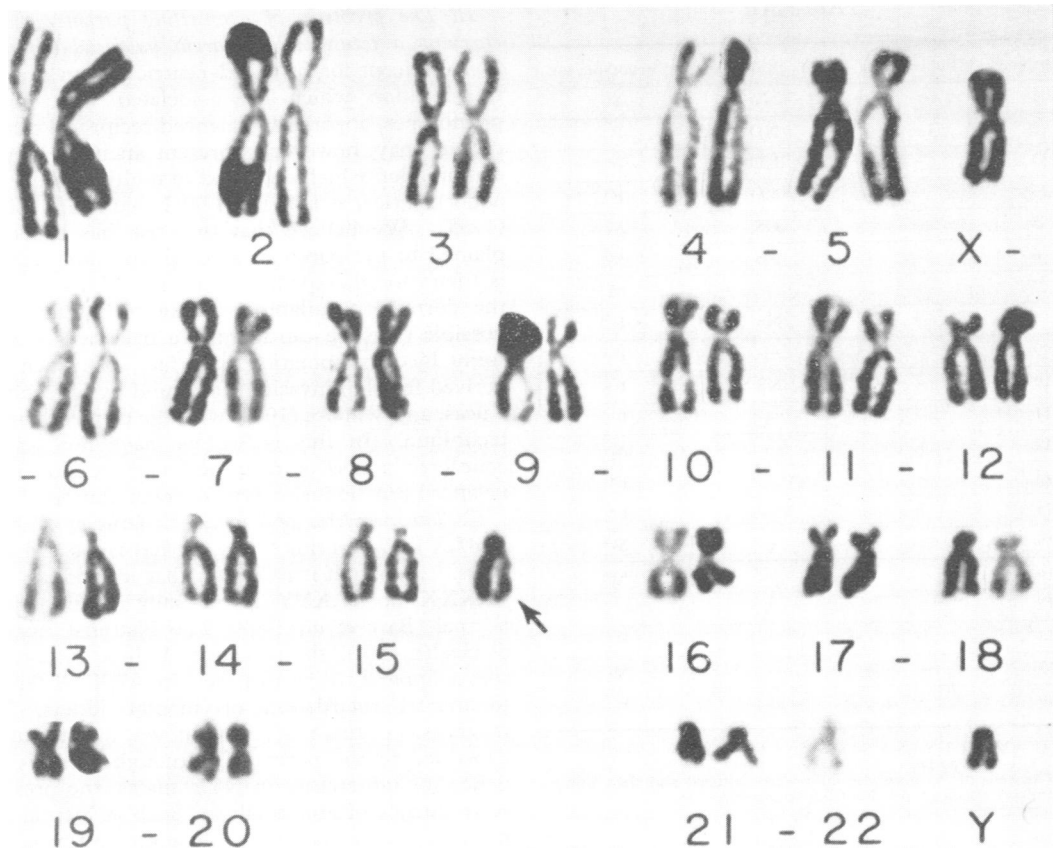
FIG. 4. Karyotype showing 46,XY, + D, – G from one of two harvests of a culture which only showed normal male cells in the other harvest.

from the first culture and only 46,XY cells were recovered from the second culture. The mosaicism would have been missed if only the normal cell line had been found initially. It is possible that these two cultures represent clonal outgrowths from single cells. It is important to point out here that multiple flasks must be used for cultivation of amniotic fluid cells so as to increase the chance of detecting mosaicism.

The possibility of maternal cell contamination can be excluded in the case above since we are dealing with a male fetus. However, the likelihood of this added confusion would be possible if we are dealing with a female fetus. According to Nadler's experience (1972) 0·5% of the cases may be contaminated with maternal cells.

2. In cases with enlarged satellites, cells of late prophase or early metaphase must be used for ana-

lysis in order to eliminate confusion with translocations. A replicate culture of one of the three cases described was initially misinterpreted as a D/G translocation by another laboratory. In addition, the enlarged satellites can be further identified by the fluorescent staining technique (Wahlström, 1972).

3. Karyotypes of the cultured amniotic fluid cells may be altered by contamination and/or treatment or other unknown factors. The change of the karyotype in the first case reported here may have been attributed to the fungus infection and/or treatment with amphotericin. The finding of 6% aneuploid cells in the second case indicates other unknown factors which may have caused alteration of the normal karyotype. However, we must keep proper control of the culture conditions and be aware of any mild microbial contamination.

TABLE II

Case No.	Y fluorescence	Sex chromatin	Phenotypic sex
1	+	ND	M
2	−	ND	F
3	−	ND	F
4	−	ND	F
5	−	NS	M
6	+	NS	M
7	+	−	M
8	−	NS	F
9	−	+	F
10	−	NS	F
11	−	+	F
12	+	−	M
13	−	+	F*
14	?	−	F*
15	+	−	M*
16	+	−	M*
17	−	+	F*
18	−	+	F
19	+	−	M
20	−	+	F

ND = not done; NS = not satisfactory; M = male; F = female; + = positive; − = negative.
  * The cells of the amniotic fluid were cultured and the chromosome analysis corresponded with the phenotypic sex.

4. As to prenatal sex determination, we have found that karyotype analysis of the cultured amniotic fluid cells is the only accurate means. Failure to detect X-chromatin or Y-fluorescent body in uncultured amniotic fluid cells does not necessarily indicate the absence of the corresponding chromosomes, since it is known that a small Y may fail to fluoresce (Borgaonkar and Hollander, 1971) and most of the uncultured amniotic fluid cells are pyknotic and therefore not suitable for X-chromatin determination (Rook *et al*, 1971). Therefore, karyotype analysis for prenatal sex determination is the only accurate method.

5. Other possible problems

A. Finding of tetraploid cells. In our series, we have rarely found more than 20% tetraploid cells: however, we have found one case in which the second harvest showed 71% tetraploids. Since the case reported by Kohn and Robinson (1970), we have not been puzzled by this problem.

B. The problems of counselling parents who are carrying a fetus with apparent balanced translocation. Except for balanced centric fusion types of translocation which are associated with normal phenotypes, apparently balanced reciprocal translocations may, however, represent aneusomy by recombination which may lead to multiple congenital abnormalities, as suggested by Lejeune and Berger (1965). We believe that this risk has to be explained to the parents, although the final decision is theirs for therapeutic abortion. However, with the currently available banding techniques, it is possible that one can identify a true case of aneusomy by recombination. In fact, one such case derived from an invasion was found by Hirschhorn, Lucas, and Wallace (1972) with the trypsin-banding technique. In this series, we have not yet encountered a case of reciprocal translocation nor a balanced centric fusion type of translocation.

C. The problems of carrying a fetus of 47,XXX or 47,XYY. In this series, we have not had such cases. Although it is known that individuals with 47,XXX or 47,XYY are usually phenotypically normal (Barr *et al*, 1969; The National Institute of Health, 1970), the presence of three X chromosomes apparently predisposes the 47,XXX females to mental retardation or mental illness. The magnitude of this risk is not known, but it must be explained to the parents. Although there is evidence for tall stature in XYY males, the presence or frequency of abnormalities, such as mental subnormality or mental illness and aggressive behaviour has not been substantiated. The whole picture must be interpreted to the parents with great caution.

We have discussed some of the problems and pitfalls of prenatal cytogenetic diagnosis. Although it appears that the situation is becoming more complicated as experience grows, with more information accumulated in this field and the recent development of various improved methods of chromosome identification, we may learn to solve these problems.

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