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0014-4754/86/101090-08$1.50 + 0.20/0

First trimester fetal karyotyping using chorionic villi: technical development and diagnostic application

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Key words. Chorionic villi; first trimester fetal karyotyping; direct method.

Introduction

Transabdominal amniocentesis was introduced as a procedure suitable for fetal sex determination during the second trimester of pregnancy[5, 11, 19], but soon it was realized as offering a great potential for diagnostic purposes in cytogenetic and biochemical abnormalities because of the improvements in culture techniques for amniotic fluid cells[4, 12, 13]. However, the advanced time of gestation necessary for amniocentesis, and the cell culture time of 2–4 weeks, mean that selective abortion when an abnormal fetus is diagnosed may have to be performed at a late stage of pregnancy, 18–20 weeks. This frequently causes serious psychological stress to the couple, and the abortive procedures close to the fifth month of gestation are not completely free from medical complications.

In order to reduce the emotional stress and the possibility of clinical complications related to mid-trimester prenatal diagnosis, alternative techniques for fetal diagnosis before the 14th week of gestation have been evaluated in several studies. These techniques included transvaginal amniocentesis between 10 and 13 weeks of pregnancy[18] and analysis of fetal cells in the maternal circulation[1, 17], but at present these methods have no application in the diagnostic activity. Another technique for obtaining cells from the conceptus for cytogenetic, biochemical and

Table 1. A summary of the experimental studies of first trimester trophoblast cultures and cytogenetic analysis

Source	X- and/or Y-chromatin assays	Culture success	Direct chromosome analysis	Karyotype M/F	Observations
Kullander and Sandahl (1973)	–	20/39	–	12/7	Mixed XX and XY mitoses in one case
Hahnemann (1974)	–	34/63	–	16/18	Inclusive of cultures from villi, amnion and decidua
Rhine et al. (1975)	31/36	–	–	–	Disagreement in 5 cases
Chinese Group (1975)	99/100	–	–	–	Disagreement in 6 cases
Rhine et al. (1977)	–	30/34	–	1/16	? (not controlled)
Goldberg et al. (1980)	–	9/12	–	0/9	Disagreement in all the cases
Niazi et al. (1981)	–	9/25	–	5/4	No disagreement
Kazy et al. (1982)	165/165	–	–	–	No disagreement
Coleman and Path (1982)	–	12/12	–	0/11	Disagreement in 3 cases
Simoni et al. (1983)	–	30/32	–	16/14	Mixed XX and XY mitoses in 5 cases
	–	–	20/20	12/8	No disagreement or maternal cell contamination

DNA determinations, using direct or indirect procedures, is the sampling of trophoblast cells.

Fetal sex prediction by X- and Y-chromatin assays and trophoblast cell culture for first trimester fetal karyotyping have been described by several authors (table 1). Studies on villi sampling by transcervical placenta biopsy and culture procedures using trophoblast cells were reported in the early 1970s[7, 10], but although chorionic villi were successfully obtained the trophoblast cells showed a low growth potential under culture conditions, and culture failed in nearly half of the samples. These early attempts to carry out first trimester fetal diagnosis were discontinued because of the greater ease in performing amniocentesis. The success achieved using amniotic fluid cell cultures led rapidly to the development of second trimester diagnosis in many countries. In addition, the identification and selection of pure fetal material after chorionic villi sampling (CVS) was not easy. It was soon realized that the occurrence of maternal cell contamination is a greater problem when using chorionic villi, than in amniotic fluid cell cultures. It followed that these investigations were only of an experimental nature and were not used for diagnostic purposes. In 1975 a further study by a Chinese research group in the Anshan Department of Obstetrics and Gynecology[2] again proposed villi sampling; a blind transcervical aspiration of the fetal material was employed in that study. Because of the difficulties in tissue culture and chromosome analysis using villi the aspiration technique was offered only for fetal sex prediction by X-chromatine determination and the test was proposed as a possibility for an intensive population control program. Fetal sexing was directly performed by Barr-body evaluation in smears set up immediately after sampling and stained by Papanicolau's method. The sex was predicted in 99 cases out of 100 sampled and disagreement occurred in 6 of these. At present the use of the test for fetal sex selection in China is being avoided because of the foreseen dangerous effect that alterations in the sex ratio might produce in familial and social composition.

Rhine and associates[15] reported a sampling procedure to obtain exfolite trophoblast cells in the first trimester by endocervical lavage. In the early phase of the work successful results were obtained with fetal sex prediction using the Y-body fluorescence test. In a later phase the sampled cells were collected for tissue culture and subsequent karyotyping and a male chromosome constitution was diagnosed in one case out of 16 in which suitable mitoses were available for study[16].

A similar sampling procedure was also used by Goldberg et al.[6], but their experience with fetal karyotyping using endocervical lavage was negative. These authors were not able to find XY mitoses in cases in which male fetuses were detected by cytogenetic controls after interruption of the pregnancy.

Fetal sex determination on trophoblast cells was done in another investigation reported by Kazy et al.[9] in which villi samples were obtained in the first trimester of pregnancy by bioptic forceps before inducing abortion. The aim of this work was to establish a safe method for obtaining chorionic biopsy specimens to measure enzyme activity in villi in order to detect some metabolic storage disorders. Fetal sex was diagnosed through the combined X- and Y-chromatin assays by setting up two smears

Table 2. A summary of the results of first trimester fetal karyotyping by direct method at the 1st Clinic of Obstetrics and Gynecology, University of Milan

Indication for CVS	Samples investigated No.	Abnormal karyotypes Unbalanced No.	Balanced No.	Type of abnormality Unbalanced No.	Balanced No.
Chromosomal abnormalities	811	38	16	–29 Trisomies, 1 monosomy X, 8 mosaics, 1 double trisomy	–7 Reciprocal translocations, 8 Robertsonian translocations, 1 inv(X) in a male fetus
Fetal sex determinations	63	3	–	–2 Trisomies, 1 mosaic	–
Enzyme determinations	15	1	–	–1 Trisomy	–
DNA analyses (beta-thalassemia)	27	1	–	–1 Trisomy	–
Total	916*	43	16		

* Diagnostic failure in 5 cases. A 2nd CVS in 2 cases.

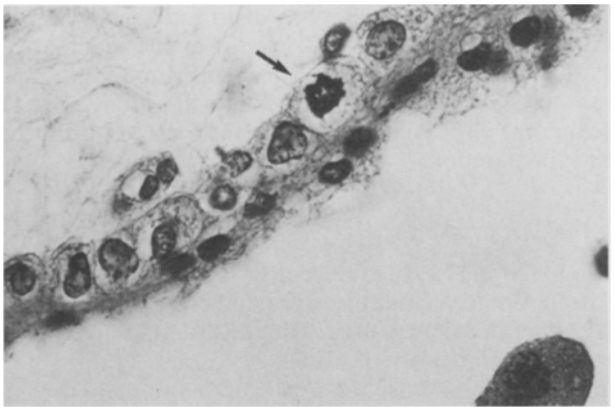

Figure 1. Histological section of a villus showing a mitosis in process (arrow) in the Langhan's layer.

from each villi sample. The aceto-orcein staining technique was used to assay X-chromatin and acridine staining for Y-body visualization. No disagreement was found in this study in 165 cases investigated.

Again, the possibility of obtaining high efficiency in chorionic villi culture for first trimester fetal karyotyping was investigated by Niazi et al.[14] on samples withdrawn by transcervical aspiration using a flexible plastic catheter under ecographic control. These authors applied a technique in which the villi were treated with trypsin to remove the covering layer of syncitiotrophoblasts and cytotrophoblasts and thus promoting the growth of the exposed mesenchymal cells. Moreover, a filtration through a fine wire gauze also allowed them to reduce the presence of maternal cell clusters normally adhering to the surface of the villus.

Using cells from chorionic tissues we had a preliminary experience in fetal karyotype study in which two different techniques were compared[20]. The first was a traditional procedure using culture before chromosome harvesting, and the method previously reported by Niazi et al.[14] was applied in this investigation with some minor modifications. On the contrary, the second was a direct technique by which spontaneous mitoses were freed from the cytotrophoblast layer (fig. 1) using a short treatment with aqueous 60% acetic acid solution. This procedure was an adaptation to human placental villi of the method described by Evans et al.[3] to obtain chromosome preparations from mid-term mouse embryos. As shown in figures 2 and 3, the quality of metaphases obtainable following the direct preparation is suitable for diagnostic purposes. Moreover, a simple incubation of the villi for a short time (24–48 h), avoiding setting up conventional cultures, may also be useful in applying techniques for which exposure to agents is required. Thus, we were able to obtain RBA banded mitoses and sister chromatid exchanges visualization after bromodeoxyuridine (BrdU) incorporation (figs 4 and 5). While maternal cell contamination occurred after culture, no maternal metaphases were observed in pregnancies with male fetuses when the direct method was applied. This procedure allowed us to have fetal karyotype determination in a very short time of villous sampling even in cases in which a minimal amount of fetal material (about 1 mg of weight) was obtained. For these reasons we chose the direct chromosome analysis for the diagnostic activity in the first trimester of pregnancy[22].

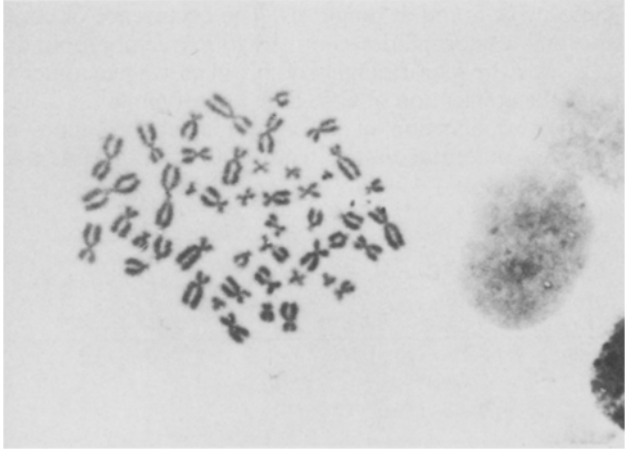

Figure 2. A metaphase obtained by the direct method and stained with Giemsa.

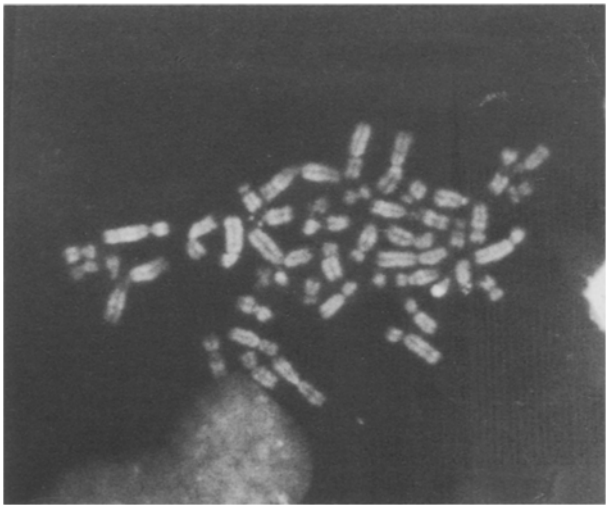

Figure 3. QFQ-banded metaphase obtained by direct chromosome analysis of chorionic villi.

Fetal karyotyping by direct method

The technical experience acquired in first trimester fetal karyotyping by direct chromosome analysis allowed us to realize that the optimal procedure for chromosome preparations is reached by the following modification of the original method. Immediately after sampling the presence of villi is controlled under an inverted microscope to evaluate the success of the attempt and to quantify the amount of the villi. Fragments showing typical villous morphology are separated from maternal fragments of decidua, and about 20 mg of villi are washed in Hanks saline balanced solution and transferred in a 30-mm Petri dish containing 3 ml of RPM I with 5% fetal calf serum and antibiotics. Villi are incubated at 37 °C in a 5% CO_2 incubator for 48 h. Colcemid is added 1 h before chromosome preparation at 0.04 µg/ml final concentration. Then the medium is removed by a Pasteur pipette and replaced with 3 ml of 1% sodium citrate solution for hypotonic treatment (10 min). The hypotonic solution is then

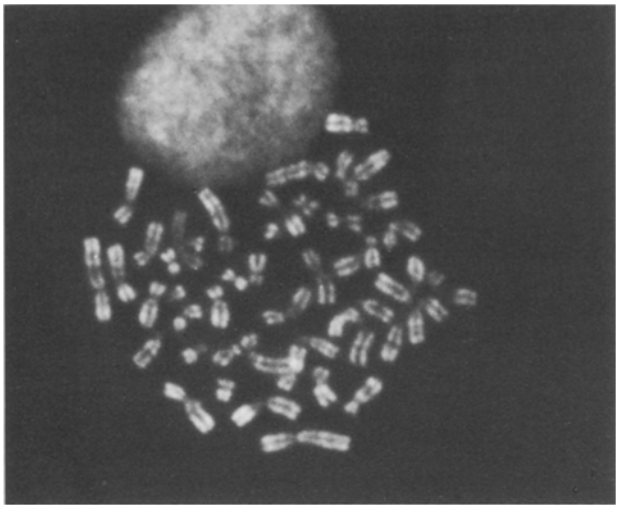

Figure 4. RBA- banded metaphase obtained after 24-h incubation of the villus at 37 °C in 5% CO_2 and 6 h exposure to BrdU.

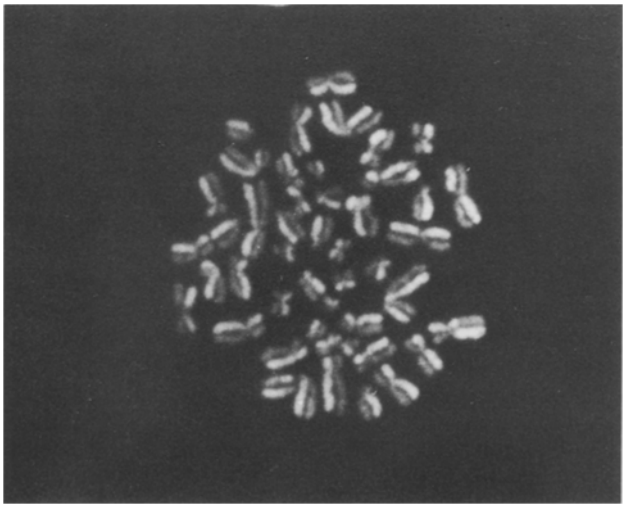

Figure 5. Sister chromatid exchanges; visualization after 48-h incubation of the villus at 37 °C in CO_2 with BrdU.

removed and replaced with 3 ml of ethanol-acetic acid (1:4) fixative. It is renewed twice, each for at least 20 min. After a complete removal of the fixative by a Pasteur pipette, the cell dissociation of the trophoblast tissue is obtained by a short treatment with 60% aqueous acetic acid solution (about 0.2 ml for each 10 mg of villi) for 10 min. The action of the acetic acid must be controlled under the inverted microscope until dissociation of the cytotrophoblast layer is nearly complete. The cell suspension so obtained is then evenly distributed onto the surface of warmed slides (40 °C) by moving the bent tip of a Pasteur pipette, or by a mechanical distribution with the machine GIRO 3283 (Mare-Milano, Italy) in order to reduce the physical damage of the metaphases during the slide preparation.

Cytogenetic findings

From February 1983 to December 1985 a total number of 916 chorionic villi samples were studied at the Cytogenetic Laboratory of the 1st Clinic of Obstetrics and Gynecology of University of Milan. The results of this study are summarized in table 2. Fetal karyotyping was carried out according to the direct procedure after a short term incubation of the sample and without conventional culture. Diagnostic failure occurred in 5 cases (0.5%) including 4 cases in which an insufficient number of metaphases was found and one case with microbial contamination of the sample. 811 samples were investigated for chromosomal abnormality, 63 for fetal sex determination, 15 for inborn errors of metabolism, and 27 for beta-thalassemia. There were 59 (6.4%) abnormal karyotypes, 16 of which were balanced (1.7%), and 43 were unbalanced (4.7%).

In the 811 cases for which fetal karyotyping was the primary indication for CVS, the total number of unbalanced fetal karyotypes was 38 (4.7% of the total). As already pointed out[21] the presence of trisomic fetuses in categories in which the primary indication was other than fetal chromosomal disorders indicates that no biochemical or molecular analysis should be performed without a chromosome study. Table 3 details the 811 cases for which fetal karyotyping was the primary indication for CVS. Maternal age was the major indication in this group (674 cases). The total number abnormal karyotype in this risk group was 33, of which 1 (0.14% of the total) were balanced and 32 (4.7% of the total) unbalanced.

In 10 cases a discrepancy was found between metaphases from placental cells studied by direct analysis and those obtained from amniotic fluid cell cultures or at cytogenetic control after termination of pregnancy, as shown in table 4. Of these, 9 were mosaics at direct chromosome preparation, while in one case an homogenous abnormal cell population showing a 21q21q Robertsonian translocation was found at diagnosis. The occurrence of chromosome abnormalities confined to extraembryonic tissues was the major diagnostic problem we encountered with the application of CVS fetal karyotyping.[22].

While the detection of chromosomal abnormalities in cultured placental tissues from both spontaneous and

Table 3. Cases in which fetal karyotyping was a primary indication for CVS

Group at risk	Fetuses investigated	Karyotype Normal			Abnormal Balanced			Unbalanced			Diagnostic failures
		M	F	T	M	F	T	M	F	T	
Maternal age	674	346	294	640	1	–	1	13	19	32	2*
Previous child/fetus with chromosome abnormality	80	34	43	77	–	–	–	1	2	3	–
Chromosome abnormality in the parents	33	5	10	15	5	10	15	–	3	3	–
Other**	24	11	13	24	–	–	–	–	–	–	–
Totals	811	396	360	756	6	10	16	14	24	38	2*

* Repeated CVS; ** exposure to mutagenic agents.

Table 4. Discordant karyotypes in embryonic and extraembryonic tissues in 10 cases

Direct preparation at CVS	Direct preparation after termination	Cultured villi after termination	Cultured amniocytes	Fetal fibroblasts	Outcome
46,XX/47,XX, + 3	46,XX	46,XX	–	46,XX	Induced abortion
46,XY/47,XXY	–	–	46,XY	–	Normal
46,XY/45,X	46,XY	46,XY	–	46,XY	Induced abortion
46,XY,−21, + t(21q21q)	46,XY/,−21, + t(21q21q)/ 46,XY,21p−	46,XY,21p−	–	46,XY,21p−	Induced abortion
46,XY/47,XY, + 3	46,XY/47,XY, + 3	46,XY	–	46,XY	Induced abortion
46,XX/45,X/46,X,i (Xq)	46,XX/45,X/46,X,i (Xq)	46,XX	–	46,XX	Induced abortion
46,XX/47,XX, + mar	–	–	46,XX	–	Normal
46,XX/47,XX, + mar	–	–	46,XX	–	Continuing pregnancy
46,XX,inv(8)/47,XX,inv(8), + 3	–	–	46,XX,inv(8)	–	Continuing pregnancy
46,XY/47,XY, + 13	–	–	46,XY	–	Continuing pregnancy

induced abortions[23, 24] is usually considered representative of the chromosome constitution of the conceptus, our findings cast doubts on this widespread assumption. The discordance betweeen extraembryonic material and the embryo proper was investigated by Kalousek and Dill[8], who found mosaics confined to chorionic tissue culture in matched chorion, amnion, and cord blood samples of human conceptuses. These authors suggested that a random nondisjunctional event occurring at a very early stage after fertilization may produce mosaicism in the placenta or in the fetus, but not necessarily in both.

A serious problem is posed by chromosomal abnormalities, whether detected as a mosaic or in an entire sample on direct preparations of chorionic cells, which karyotypic analysis of the aborted fetus fails to confirm. The evidence available thus far is insufficient to allow any firm conclusion about the clinical effect that the presence of chromosomal abnormalities in the placental tissue may play on the development of the pregnancy or even on the fetal phenotype. We think that additional investigations in the second trimester should be offered either when a mosaic is found in the chorionic tissue or when a rare type of autosomal trisomy, compatible only with minimal embryonic development, is found in a pregnancy with apparently normal growth of the conceptus. In these cases, if the abnormal fetal karyotype is not confirmed in the second trimester and the fetus is developing normally as revealed by ultrasound monitoring, a normal chromosome constitution may be predicted with a high degree of confidence.

Although we feel that the risk of true diagnostic errors remains low, our experience indicates the necessity of examining placental and fetal tissues as carefully as possible in all cases with abnormal karyotype.

Acknowledgments. The authors wish to thank Dr Brambati for performing obstetrical work in developing first trimester fetal karyotyping and Prof. Fraccaro for his constructive criticism and encouragment. We are also grateful to Dr Danesino for performing biochemical determinations. The study was partially supported by a grant from Associazione Italiana per lo Studio delle Malformazioni.

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