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Brief Communication

A Method of Processing First-Trimester Chorionic Villous Biopsies for Cytogenetic Analysis

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SUMMARY

Chorionic villous biopsy is emerging as a technique for obtaining fetal cells for prenatal diagnosis in the first trimester of pregnancy. Chromosome analysis has been performed on small villous biopsies using either direct harvests of uncultured cells or after culturing villous tissue. Here, we describe a method where both techniques can be used simultaneously; from a single villous biopsy, GTG-banded chromosomes of improved morphology are obtained from direct preparations and from cultured villous cells.

INTRODUCTION

Chorionic villous biopsy as a method of first-trimester prenatal diagnosis currently is undergoing active investigation [1]. Early diagnosis allows decisions about termination of pregnancy or treatment of a diseased fetus at 8–12 weeks of gestation, whereas diagnosis by amniocentesis cannot be achieved until mid-to-late second trimester.

A reliable method of culturing human chorionic villi and obtaining metaphase cells for cytogenetic analysis was published by Niazi et al. in 1981 [2]. Recently, Simoni et al. [3] reported a method of obtaining quinacrine-banded karyotypes in 5 hrs from uncultured villi biopsied at 8–12 weeks of gestation.

In an effort to improve the morphology of uncultured villous chromosome preparations suitable for Giemsa banding, we have developed a method of pro-

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cessing small samples of chorionic villi that offers significant advantages for cytogenetic analysis. In addition, the method provides cells for culture that may be used for either cytogenetic or biochemical analysis.

MATERIALS AND METHODS

Samples of human chorionic villi were collected at 7–13 weeks gestation from patients undergoing either elective abortion or prenatal diagnosis in a continuing pregnancy. Using sonographically guided catheters, villous biopsies were obtained by aspiration into 20- or 30-cc syringes containing minimum essential medium (MEM) (pH 7.0–7.4 bicarbonate or Hepes buffer) with 1% penicillin-streptomycin (no. 600-5140, Gibco, Grand Island, N.Y.). From the pregnancies terminated by suction abortion, additional villous specimens plus tissues from the embryo whenever identifiable also were collected.

The samples were transported to the laboratory immediately. Five to 20 mg of chorionic villi were dissected carefully from maternal decidual tissue under an inverted microscope using fine forceps. These villi were rinsed extensively in MEM containing 10% fetal bovine serum (FBS) with a final rinse in calcium- and magnesium-free phosphate-buffered saline. Cleaned villi were transferred to a 22-mm sterile siliconized scintillation vial containing 2 ml of trypsin-EDTA solution (1 ×) (no. 610-5300, Gibco) with 20 μg/ml DNase I (no. D-0876, Sigma, St. Louis, Mo.; reconstituted and stored at −20°C in 0.05 M Tris-HCl, pH 7.5, 50% glycerol). The villi were minced in the vial using fine scissors and gently stirred in the solution for 5 min at 37°C with a 5 × 2-mm Teflon-coated magnetic stirring bar. The cell suspension then was aspirated carefully using a 1-ml plastic serological pipette; the tissue pieces were left on the bottom of the vial. The aspirated suspension was placed into a 15-ml polystyrene conical centrifuge tube containing 3 ml of MEM with 20% FBS and 0.07 μg/ml of Colcemid (no. 120-5211 Gibco) and kept at room temperature.

The process of stirring for 5 min and aspirating the cell suspension was repeated twice more. Each time, 2 ml of trypsin-EDTA + DNase I solution was added to the tissue fragments in the vial. Each cell suspension was added in turn to the 15-ml centrifuge tube containing Colcemid. The three trypsinization steps took altogether 20–25 min. Remaining tissue fragments in the vial were rinsed and suspended in MEM with 10% FBS for establishing cultures later.

Without further delay, a direct harvest was performed on the cell suspension by standard methods, with particular care taken in gently resuspending the pellet to avoid cell loss. Hypotonic treatment consisted of a 20-min exposure to 0.075 M KCl at room temperature. The fixation consisted of two 15-min exposures to Carnoy's fixative solution (three parts methanol:one part glacial acetic acid). Cell suspensions were spread by blowing onto cold wet slides that were transferred rapidly to a 60°C hot plate for 90 seconds. Usually, three slides were made per sample. Giemsa-banding was performed by the method of Sun et al. [4] either immediately or the following day, and the metaphase spreads analyzed.

A back-up culture was set up from the tissue fragments that were left in the vial after trypsinization. Using MEM with 20% FBS plus antibiotics for cell culture, the fragments were placed either into tissue culture vessels or onto coverslips for later harvesting after trypsinization or by in situ methods.

RESULTS

Villi were readily identified under the dissecting microscope in 35 cases used for direct chromosome analysis. Maternal decidual tissue also was identified and discarded (fig. 1). The trypsinization steps successfully removed the syncytiotrophoblast and cytotrophoblast layers, releasing the cytotrophoblast cells into suspension and leaving a mesenchymal core (fig. 2). Three short exposures to trypsin were found to be less deleterious to chromosome morphology than a single

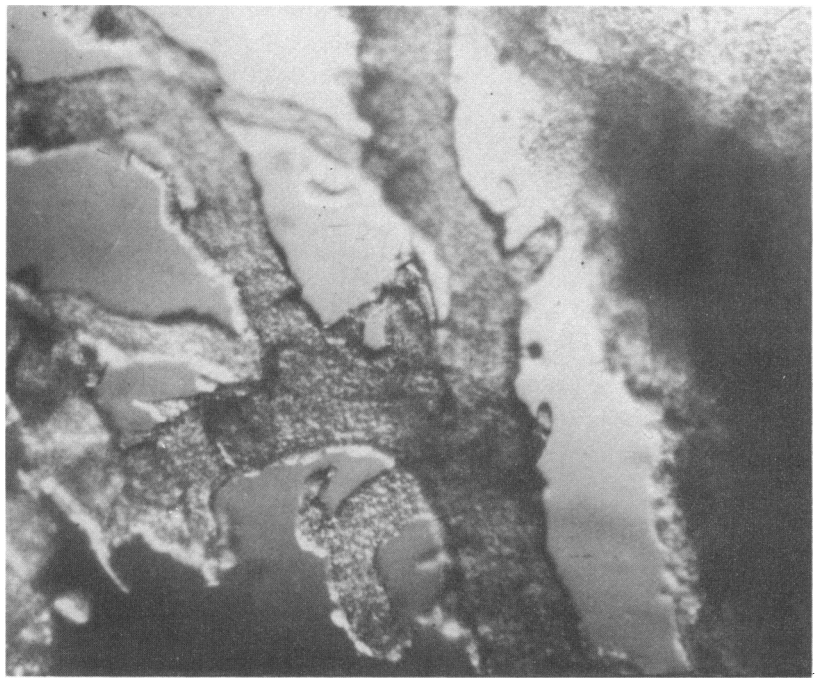

FIG. 1.—Chorionic villi (*left*) and decidua (*right*) from a 9-wk pregnancy (magnification 40×). Note the more defined outline of the villi as opposed to the indistinct edges of the decidua.

prolonged exposure. Results of chromosome analysis obtained from direct processing of the cytotrophoblasts are shown in tables 1 and 2. Four to 50 analyzable metaphase spreads representative of the 250–350-band stage were found in each specimen; karyotypes were 46,XX in 15 cases; 46,XY in 18; 46,XY,6q− in one; and 46,XX/47,XX,+18 in one. In each case, cultured cells were available for further analysis when less than 15 metaphases were analyzable on direct harvest. The time required for culture and cell analysis varied from 1 to 4 weeks. Representative chromosome preparations are shown in figure 3. In 18 of the cases, karyotypes from the embryos were obtained, and these matched the villous karyotypes from both direct and cultured cell harvests.

DISCUSSION

In 1960, Thiede [5] described successful culturing of human chorionic villi from term placentas following trypsinization, and Niazi et al. [2] adapted this for cytogenetic analysis of fibroblasts grown from villi obtained during first-trimester abortions. Trypsin strips the villus of its two outer layers: the syncytiotrophoblast and the cytotrophoblast. Thus exposed, the mesenchymal core yields a monolayer of fibroblastic cells.

In 1983, Simoni et al. described direct cytogenetic prenatal diagnosis using the spontaneously dividing cytotrophoblast cells [3]. Histologically, these cells have a mitotic index of about one in 125 [6]. Ten to 20 mg of villi are exposed

to Colcemid (.04 μg/ml) for 1 hr, and then to hypotonic and fixative solutions, each for 10 min. To release the cytotrophoblasts, the villous fragments are then dissociated using aqueous 60% acetic acid for 3–5 min; slide preparation and quinacrine staining follow [3]. The chromosomes are relatively short but are adequate for detecting abnormal chromosome counts and major translocations. They are not well suited for Giemsa-banding in our experience.

To improve chromosome morphology and devise a direct method that would yield satisfactory Giemsa-banded preparations, we turned to trypsin for cell dissociation. Trypsin had been used with DNase in the preparation of trophoblast suspensions to study chorionic gonadotropin production [7]. We found that trypsinization yields cytotrophoblasts in metaphase that can be used just as readily for immediate karyotyping and, moreover, that the remaining mesenchymal core is available for cell culture.

The cultured cells have several potential uses: (1) they are available when direct methods yield too few cells, (2) they provide good chromosomal morphology that allows the detection of small structural changes or rearrangements, and (3) they may be required for biochemical or other cytogenetic analyses.

At this time, we expend about one-and-one-half times the effort and resources for obtaining a chromosomal diagnosis from chorionic villi as we do from am-

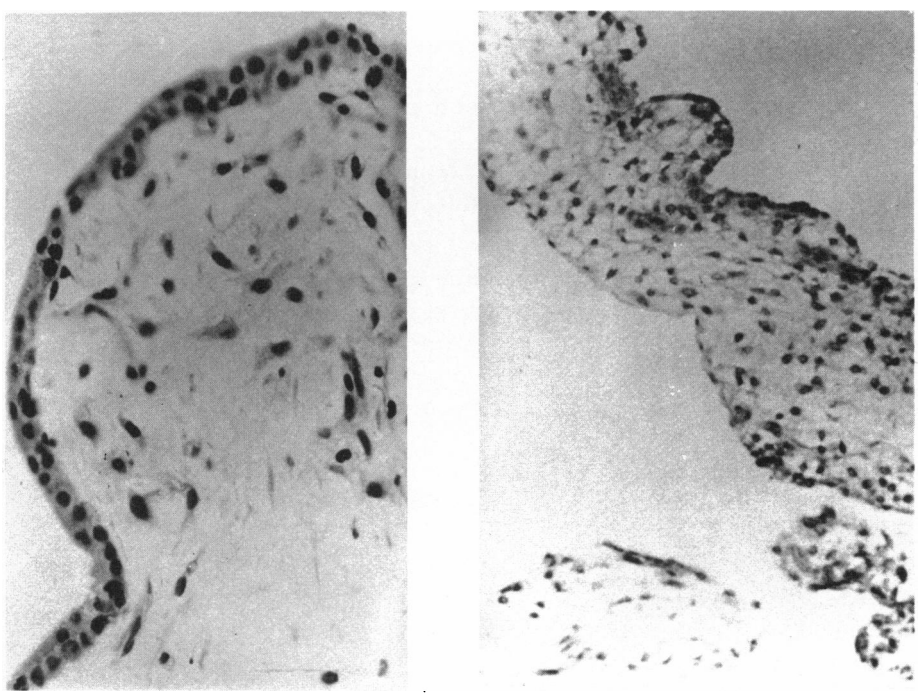

FIG. 2.—Histologic sections of chorionic villi from a 7-wk pregnancy (*left*) before trypsinization, and (*right*) after trypsinization. Trypsinization removes the outer syncytiotrophoblast and cytotrophoblast layers, leaving only the mesenchymal villous core.

TABLE 1

DIRECT CHROMOSOME ANALYSIS OF CHORIONIC VILLI AND COMPARISON WITH KARYOTYPES OF CULTURED EMBRYONIC CELLS FROM TERMINATED PREGNANCIES

Case	mg*	Cells studied	Karyotype	Embryo karyotype†
1	10	15	46,XX	46,XX
2	15	10	46,XY	46,XY
3	10	15	46,XX	46,XX
4	15	15	46,XX	N.A.‡
5	10	10	46,XY	46,XY
6	10	15	46,XY	46,XY
7	10	11	46,XY	46,XY
8	10	12	46,XX	46,XX
9	5	10	46,XY	N.A.
10	10	15	46,XX	46,XX
11	15	15	46,XY	46,XY
12	8	15	46,XY	46,XY
13	10	4	46,XX	46,XX
14	10	34	46,XX	46,XX
15	10	15	46,XX	N.A.
16	8	15	46,XY	46,XY
17	15	7	46,XX	N.A.
18	10	15	46,XY	46,XY
19	8	9	46,XY	46,XY
20	10	50	46,XY	46,XY
21	15	5	46,XY	46,XY
22	20	16	46,XX	46,XX

* Approximate wet wt. of biopsied villi.
† Cells cultured from tissues of the embryo.
‡ N.A. = not available.

niocytes. Further experience is expected to make the cost of these two types of studies comparable.

We have made several observations pertinent to the success of this method. The processing of villous samples for direct preparations should begin as soon

TABLE 2

DIRECT CHROMOSOME ANALYSIS OF CHORIONIC VILLI FROM PRENATAL DIAGNOSTIC CASES

Case	mg*	Cells studied	Karyotype
23	15	20	46,XY
24	20	20	46,XY
25	8	6	46,XX
26	15	15	46,XX/47,XX,+18†
27	25	27	46,XX
28	15	20	46,XY,6q−
29	15	11	46,XX
30	25	20	46,XX,inv(9qh)
31	15	17	46,XY
32	25	20	46,XY
33	15	12	46,XY
34	15	9	46,XX
35	15	15	46,XY

* Approximate wet wt. of biopsied villi.
† Both cell lines were recovered after abortion from cultured placental tissue.

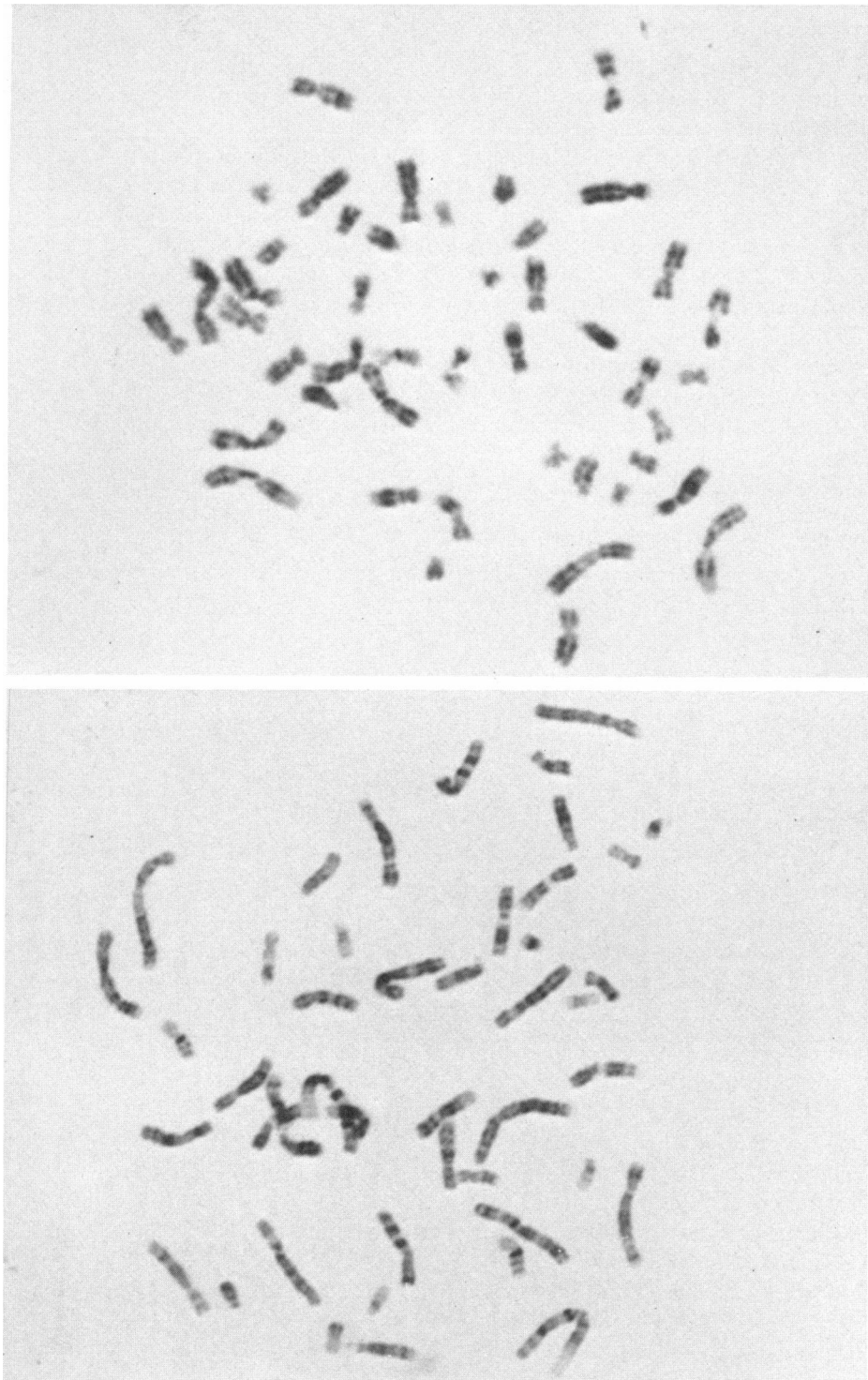

FIG. 3.—GTG-banded chromosomes from chorionic villi: (*A*, *top*) direct preparation and (*B*, *bottom*) preparation from cultured cells.

as possible (we began within 20 min) to maximize the number of recovered metaphases. Also, exposure of villi to phosphate-buffered saline for more than a few minutes led to failures both in culture and in direct chromosome preparations.

Despite the possibility of introducing microbial contamination during dissection, this has not occurred in our experience thus far with the backup cultures. Antibiotics are present in the culture medium, but an antifungal agent was not used.

Prolonged exposure of the cytotrophoblast cells to trypsin resulted in a ragged appearance to the chromosomes. This was observed even after 10 min of continuous exposure to trypsin-EDTA at 37°C, and became very marked after 25 min of continuous exposure. Serial 5-min exposures resolved this problem.

Fewer metaphase spreads were observed without DNase. DNase aids in releasing cells from a sticky substance that forms about the core fragments if trypsin-EDTA is used alone. This substance fluoresces when stained with ethidium bromide, suggesting that it contains DNA from lysed syncytiotrophoblast nuclei.

When villous samples were under 5 mg, we did not recover an adequate number of metaphases for direct cytogenetic diagnosis. Such tiny samples are used for short-term culture.

No evidence of maternal-cell contamination was seen in the 18 direct preparations yielding 46,XY cells. However, this is a limited series, and we see the need for careful studies on a larger series that will determine the frequency of maternal contamination both in direct as well as in cultured villous chromosome preparations. Presumably, careful initial villous dissection, removing any visible decidual tissue, will be a key factor in controlling maternal-cell contamination.

The significance of mosaicism and polyploidy needs to be established in both direct preparations and harvests of cultured cells from villous biopsies. Amniocytes demonstrate a high frequency of tetraploidy that is of no recognized significance. Simoni et al. found polyploidy in 13.2% of cultured villous metaphases and in less than 1% of metaphases from direct preparations [3]. Of interest, however, prior to the series reported here, we encountered a villous specimen that on direct chromosome analysis yielded 24 tetraploid cells of a total of 26 cells (two diploid), whereas only normal diploid cells were found in the cultured embryo. This occurred while we were developing our current method, but the harvest did not differ in any obviously significant way. The incidence of mosaicism in chorionic villous biopsy preparations is unknown. In the largest series reported to date, no cases of mosaicism occurred in 98 villous biopsy samples [8]. However, in one of our prenatal cases (no. 26), mosaicism was encountered. Furthermore, discrepancies between chorion and fetus have been described by Kalousek and Dill using second- and third-trimester placentas [9].

The processing of chorionic villous samples for cytogenetic analysis described in this report has already proven useful in ongoing pregnancies. It allows not only the option of providing a cytogenetic diagnosis to the patient several hours after the procedure, but it also makes available cells in culture for use whenever required. In the 13 prenatal diagnostic cases, a preliminary result was reported to the patient 4–6 hrs after biopsy. In four of the 13 cases, cultured cells were utilized to complete the study. In the remaining nine, final results were reported to the patient on the day following her biopsy procedure.

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