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Prenatal Detection of Chromosome Aneuploidies in Uncultured Chorionic Villus Samples by FISH

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Summary

We developed a 1-d FISH assay for detection of numerical chromosome abnormalities in uncultured chorionic villus samples (CVS). Probes specific for chromosomes 13, 18, 21, X, and Y were used to determine ploidy by analysis of signal number in hybridized nuclei. Aneuploidy detection using this assay was directly compared with the results obtained by conventional cytogenetic analysis in a consecutive, clinical study of 2,709 CVS and placental samples. The FISH assay yielded discrete differences in the signal profiles between cytogenetically normal and abnormal samples. On the basis of these results, we generated FISH-assay cutoff values that discriminated between karyotypically normal and aneuploid samples. Samples with mosaicism and a single sample with possible heritable small chromosome X probe target were exceptions and showed poor agreement between FISH results and conventional cytogenetics. We conclude that the FISH assay may act as a more accurate and less labor-demanding alternative to "direct" CVS analysis.

Introduction

Currently, the method for earliest detection of fetal chromosome aneuploidies is conventional chromosome analysis of "direct" preparations of the *cytotrophoblast* layer of chorionic villus samples (CVS). A complete karyotype, albeit often of poor quality, can be obtained within 5–16 h (Simoni et al. 1983). However, a higher frequency of incorrect predictions of fetal cytogenetic status has been observed with the direct analysis using *cytotrophoblast* cells than with the long-term culture methods using *mesenchymal* cells from CVS or amniocytes (Simoni and Sirchia 1994). On average, the diagnoses based on the long-term culture methods take 7–14 d to obtain.

A new method for rapid prenatal detection of selected numerical abnormalities has been developed, i.e., FISH with chromosome-specific probes on uncultured fetal cells. FISH assays may be based on *mesenchymal* chorionic villus cells. Thus, a CVS FISH assay has the potential to be more accurate than direct CVS analysis.

We have elsewhere described a FISH assay using uncultured amniotic fluid cells for detection of aneuploidies of chromosomes 13, 18, 21, X, and Y (Klinger et al. 1992; Ward et al. 1993) and a FISH assay using uncultured mesenchymal chorionic villus cells for detection of chromosome 21 aneuploidies (Bryndorf et al. 1994). For the present study, we combined probes and methods from the previous studies and developed a 1-d assay for detection of numerical abnormalities of chromosomes 13, 18, 21, X, and Y in CVSs. A consecutive, clinical study of 2,709 CVS and placental samples processed in parallel by FISH and conventional cytogenetics was conducted in order to compare the results of FISH and conventional cytogenetics and on this basis to generate FISH assay cutoff values for karyotypically normal and aneuploid samples.

Material and Methods

Consecutive CVS and Added Placental Samples

A total of 2,709 samples were evaluated in parallel by cytogenetic analysis and FISH analysis. Consecutive CVSs were derived from ongoing pregnancies (2,669 samples); 1–20 mg of tissue (average 10.1 mg) from each of these samples was used for FISH analysis. In order to increase the number of cases with abnormalities in the study, 40 samples were derived from placental tissue from pregnancies terminated because of previously diagnosed fetal chromosome abnormalities. Nineteen of these 40 pregnancies had previously had a CVS included in the study. CVSs ranged from 7 to 24 (average 10) gestational wk and placental samples ranged from 9 to 20 (average 13) gestational wk. The study design was approved by the regional ethics committee, and all women enrolled provided verbal and written consent.

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Sample Preparation

Single-cell suspensions from mesenchymal chorionic tissue were established by a two-step enzyme treatment as described elsewhere (Bryndorf et al. 1993; Smidt-Jensen et al. 1989). The single-cell suspension was washed twice in Hank's balanced salt solution and then processed by a modification of the method described by Klinger et al. (1992). In brief, the suspension was washed and resuspended in 100 µl phosphate-buffered saline. Twenty-five microliters of this suspension were placed at four positions on precleaned glass slides at 37°C for 15 min. The cells were then processed in situ by the addition of 50 µl preheated 75 mM KCl and incubated at 37°C for 15 min. The hypotonic solution was decanted and replaced by 100 µl of 30% 3:1 fixative (methanol:acetic acid), 70% 75 mM KCl for 5 min at room temperature. The solution was decanted and liberal amounts of 3:1 fixative (methanol:acetic acid) were dropped onto oblique slides. Slides were dried at 60°C for 5 min and dehydrated in ethanol. In order to prevent cross-contamination of samples (see FISH Scoring Results for Sex Chromosomes), slides were placed in individual compartments created by a 24-mm high metal grid during the settling of the cells and the in situ hypotonization. The grid was introduced with sample 1261.

Probes, Hybridization, and Detection

The chromosome 13–, 18–, and 21–specific probes were developed from unique sequence regions (Klinger et al. 1992). The three probes were all three-cosmid contigs containing 80,000–109,000 bp of nonoverlapping DNA. The noncentromeric targets of these autosomal probes allow detection of free trisomies, trisomies involving Robertsonian translocations, and triploids.

The chromosome X probe was composed of a single cosmid, which hybridizes to the paracentromeric region on the X chromosome, and includes both single-copy and repeat elements (Klinger et al. 1992; Ward et al. 1993). The Y probe was derived from the repetitive clone pDP97 (provided by D. Page, Whitehead Institute for Biomedical Research), a subclone of the alpha-satellite repeat present in the cosmid Y97 (Wolfe et al. 1985). The probes were labeled by random priming up to sample 709. Because of inconsistent signal intensity, probe labeling was then optimized by using nick-translation. The autosomal probes and the Y-probe were labeled with biotin-11-dUTP, while the X-probe was directly labeled with either resorufin (up to sample 771) or with Cy-3 (the remaining samples). The first 60 samples were hybridized only with the autosomal probes. From sample 61, every sample was hybridized with all five probes, with the X and Y probes being detected simultaneously. Hybridization under suppression conditions and detection were performed essentially as described by Klinger et al. (1992). In general, an overnight hybridization was performed during week days. For this study, samples analyzed over the weekend were hybridized for 3 d.

The development and validation of the probe set and the hybridization protocol have previously been published (Klinger et al. 1992; Ward et al. 1993). Prior to this study, the probes and hybridization protocol were validated on lymphocyte metaphase spreads and on 50 *un*cultured mesenchymal CVSs to ensure high signal-to-noise ratios and high hybridization/detection efficiencies. Each newly labeled probe set was hybridized to metaphase spreads to monitor chromosome specificity and to *un*cultured mesenchymal chorionic villus cells to assess clarity of signal on the basis of background fluorescence versus signal intensity. Since the first description of these probes, they have been provided on request to investigators worldwide, and they remain available for research purposes (contact K.W.K.).

Quantitative Analysis

Two technicians evaluated 30 and 20 different hybridized nuclei per hybridization, respectively. All nuclei were evaluated under 625× magnification using a 50× oil objective and, if necessary, under 1,250× magnification using a 100× oil objective. The number of nuclei displaying one, two, three, or four hybridization signals were recorded for the autosomal hybridizations. For the sex-chromosome hybridizations, the number of nuclei showing specific combinations of X and Y signals were recorded, e.g., XX, XY, X, XXY, XYY, etc. Results were cumulatively totalled after each subset of 10 nuclei were scored. Overlapping nuclei, and nuclei with high background intensity or low signal intensity, were not scored. Patchy and diffuse signals were included in the evaluation only if they were well separated. Split-spots (i.e., signals in a paired arrangement) were scored as one signal if the distance between the signals was less than the width of one of these signals, otherwise as two signals. Some hybridizations contained <50 nuclei that were scorable according to these criteria. If ≤ 30 nuclei were scorable, the two technicians scored nuclei in the same hybridization area. A hybridization was designated technically unsuccessful if no nuclei were present or every nucleus had faint signals and/or high background. Samples that could be read were considered technically successful. The time necessary to score hybridizations was measured both early and late in the study.

Blind-Study Design

Slides for FISH analysis were coded and scored without knowledge of the indication for the testing and the karyotype of the sample. The samples derived from pregnancies terminated because of fetal chromosome abnormalities were added blindly. On the case report form, the technicians noted whether they believed the sample was from a terminated pregnancy.

No FISH results were communicated to the patients or the referring physicians. However, if FISH scoring results indicated an abnormal karyotype, the sample in question was decoded and the parallel sample was assigned high-priority status in the clinical cytogenetics laboratory.

Results

Comparative Analysis

Samples were categorized as normal or abnormal on the basis of karyotype, and these results were compared with those obtained by FISH.

Conventional Chromosome Analysis and Referral Pattern

Two thousand five hundred sixty-seven samples with normal and 142 samples with abnormal karyotypes were analyzed. Of the 142 abnormal samples, 98 (including mosaics) were aneuploid for one of the chromosomes tested, including a Klinefelter specimen processed prior to the inclusion of the X- and Y-probes in the study.

One hundred three of the 142 abnormal samples were CVSs. The remaining abnormal samples were added placental specimens. Of the 103 abnormal CVSs, 59 samples (57%) had abnormalities the FISH assay potentially could have identified, and 44 (43%) had abnormalities the FISH assay was not designed to identify. The identifiable abnormalities included 38 trisomies 13, 18, and 21; 1 triploidy; 11 sex-chromosome aneuploidies; 1 unbalanced translocation (deletion of chromosome 18, see later); and 8 mosaics involving the five tested chromosomes. The abnormalities the FISH assay was not designed to identify included 2 trisomies (of chromosomes 16 and 22); 2 unbalanced translocations; 1 de novo marker; 11 mosaics involving chromosomes not tested for; and 28 balanced translocations and inversions. With the exception of the balanced translocations and inversions, the FISH assay could potentially have identified 79% (59/75) of the abnormalities detected by conventional cytogenetics in the CVSs.

The women were referred to CVS for the following reasons: maternal age $\geq$35 years (69%); chromosome abnormality in the family (19%); maternal anxiety (5%); previous child with either chromosome abnormality, congenital malformation, or mental retardation (4%); and other (4%).

FISH Scoring Results for Autosomal Chromosomes

In samples karyotypically disomic with respect to the tested chromosome, an average of 98% (range 36%–100%) of the scored nuclei on a slide showed two signals, while 0.3% (range 0%–40%) of the nuclei had three hybridization signals (table 1 and fig. 1). By contrast, in samples karyotypically trisomic for the tested chromosome, an average of 15% (range 4%–42%) of the nuclei showed two signals, while 84% (range 52%–96%) had three signals (table 2 and fig. 1). Thirteen (0.2%) of the 7,925 autosomal hybridizations on disomic samples had $\geq$10% nuclei with three signals. Eight of these hybridizations also had $\geq$10% nuclei with four signals. This signal-doubling phenomenon was interpreted to be a result of the presence of tetraploid cells and has previously been detected by FISH in CVS cultures (Lichter et al. 1988).

FISH Scoring Results for Sex Chromosomes

During this study two female samples showed 30% and 42% nuclei with XY signals, respectively, and one male sample showed 42% nuclei with XX signals. We attributed these results to inadvertent mixture of the samples during processing. Therefore, we introduced a grid separating samples during prehybridization processing. Following the introduction of this grid, no admixture of XY nuclei was observed in karyotypically female samples. After the protocol change, female samples had an average of 99% (range 44%–100%; N = 707) of their nuclei showing an XX signal pattern, while male samples had an average of 99% (range 22%–100%; N = 698) of their nuclei demonstrating an XY signal pattern. Ranges do not include a karotypically male sample with 100% XX nuclei and a karotypically female sample with 100% XY nuclei. These two samples were contiguous and were probably mislabeled during sample coding. There were three classes of karyotypically female and male samples that displayed $\geq$10% nuclei with signal patterns other than XX and XY: those with many tetraploid nuclei (N = 13), those which demonstrated weak Y-probe fluorescence (N = 13), and one probable case of confined placental mosaicism (table 3). In total, only 27 (1.9%) of the 1,405 samples with a normal sex chromosome complement had $\geq$10% nuclei with signal patterns other than XX and XY.

A single karotypically female sample processed prior to the protocol change showed 78% nuclei with one X signal of normal intensity and 22% nuclei with one X signal of normal intensity and one X signal of low intensity. Interphase FISH analysis of peripheral blood lymphocytes from both parents showed that all paternal nuclei also had weak X signals.

Samples with sex chromosome abnormalities had an average of 97% (range 94%–100%; N = 8) of their nuclei demonstrating a signal pattern in agreement with their chromosome complement. After the protocol change, 10.5% of the karyotypically male samples demonstrated nuclei with XX signals, while no female sample showed any nuclei with XY signals. Consequently, the 10.5% figure was interpreted as an estimate of the

Table 1

FISH Scoring Results for Autosomal Hybridizations on Cases with Normal Complement of the Tested Chromosome

Karyotypically Disomic with Respect to	Mean % (Range) of Nuclei Showing				No. of Hybridizations (% of Total No. of Hybridizations for Tested Chromosome	
	1 Signal	2 Signals	3 Signals	4 Signals	Technically Successful	Technically *Un*successful
Chromosome 13	2 (0–30)	97 (54–100)	.4 (0–14)	.3 (0–42)	2649 (98)	49 (2)
Chromosome 18	2 (0–25)	98 (36–100)	.3 (0–40)[a]	.2 (0–50)	2650 (98)	42 (2)
Chromosome 21	2 (0–36)	98 (48–100)	.3 (0–18)	.2 (0–42)	2626 (99)	28 (1)
Overall	2 (0–36)	98 (36–100)	.3 (0–40)[a]	.2 (0–50)	7925 (99)	119 (1)

Note.—Scoring results were based on all technically successful hybridizations, including results from the samples that were technically successful but uninformative according to the proposed scoring criteria.

[a] The disomic sample with 40% three-signal nuclei was processed during a holiday and was allowed to hybridize for 5 d. Subsequently, no hybridization was allowed to proceed for >3 d. With the exclusion of this result, an average of .3% (range 0%–14%) three-signal nuclei were scored in karyotypically disomic samples hybridized with the chromosome 18–specific probe.

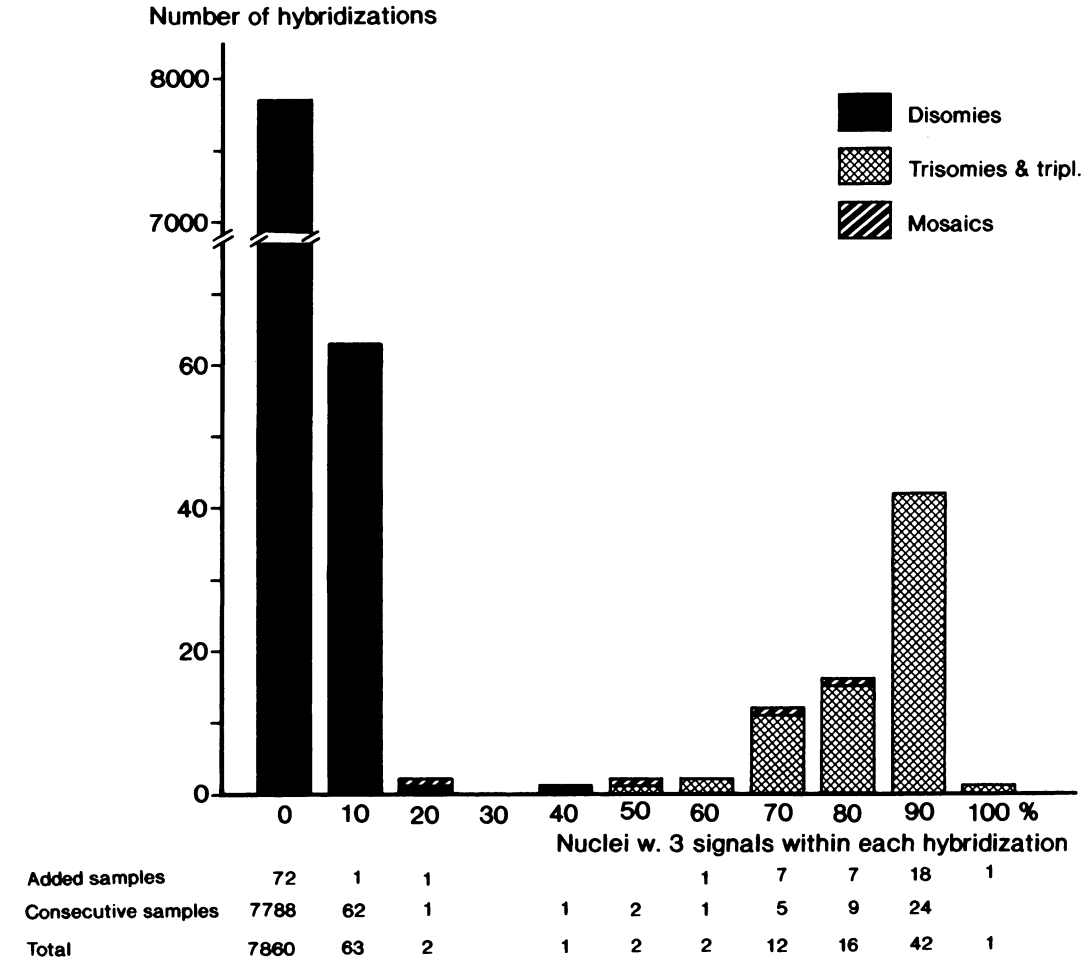

Figure 1 Distribution of "three-signal nuclei" after hybridization with autosomal probes directed at chromosomes 13, 18, and 21. Shown is the number of hybridizations in which a specific percentage of three-signal nuclei was observed. Results were based on all technically successful hybridizations, including results from the samples that were technically successful but uninformative according to the proposed scoring criteria. Samples that were technically unsuccessful were omitted. Samples labeled "mosaics" were specimens with karyotypical mosaicism of the chromosome tested for.

Table 2

FISH Scoring Results for Autosomal Hybridizations on Cases with Trisomic Complement of the Tested Chromosome

Karyotypically Trisomic with Respect to	Mean % (Range) of Nuclei Showing				No. of Hybridizations (% of Total No. of Hybridizations for Tested Chromosome)	
	1 Signal	2 Signals	3 Signals	4 Signals	Technically Successful	Technically *Un*successful
Chromosome 13	2 (0–8)	19 (8–40)	79 (52–92)	.2 (0–2)	10 (100)	0 (0)
Chromosome 18	.6 (0–6)	14 (6–30)	86 (68–94)	.1 (0–2)	14 (100)	0 (0)
Chromosome 21	.3 (0–2)	15 (4–42)	84 (58–96)	.3 (0–4)	48 (91)	5 (9)
Overall	.5 (0–8)	15 (4–42)	84 (52–96)	.2 (0–4)	72 (94)	5 (6)

Note.—Scoring results were based on all technically successful hybridizations, including results from the samples that were technically successful but uninformative according to the proposed scoring criteria. A total of 77 hybridizations were performed on cases with trisomic complement of the tested chromosome; they included (1) 68 trisomies of chromosomes 13, 18, 21 and (2) three triploidies on which a total of nine autosomal hybridizations were performed.

rate of maternal cell contamination. The average rate of nuclei showing XX signals in karyotypically male samples was 0.3% (range 0%–10%).

Mosaics

Samples with karyotypical mosaicism specific for the tested chromosomes showed no direct correlation between degree of mosaicism according to FISH on uncultured cells and conventional cytogenetics on cultured cells (table 4). One case of mosaicism was only discovered because more metaphases were analyzed than routinely used, because of a FISH result indicating an abnormality (table 4, n. b).

Number of Nuclei Scored and FISH Success Rate

A mean of 49.8 nuclei (range 5–50) were scored per hybridization. Attempted hybridizations were technically successful (see Material and Methods) in 96% of the specimens. For the 142 abnormalities, attempted hybridizations were technically successful in 94% of the specimens. A total of 183 hybridizations were technically unsuccessful (49, 43, 33, and 58 hybridizations for chromosomes 13, 18, 21, and XY, respectively). Two unintentional deviations from laboratory protocol accounted for 79 (43%) of these unsuccessful hybridizations.

Time Used for FISH Scoring of Hybridizations

Early in the study an average of 13 min 55 s were used to score all four hybridizations (N = 1,590 hybridizations). Late in the study, an average of 11 min 23 s were used for the same task (N = 539 hybridizations).

Blinding of Study

CVSs were all delivered to the laboratory within hours of sampling, while added placental samples generally were in transit for several days. This caused the techni-

Table 3

Borderline Sex-Chromosome Signal Patterns for Samples with a Normal Sex-Chromosome Complement

Signal Patterns	Sex[a]	Mean % (Range) of Nuclei Showing									No. of Hybridizations
		XX Signals	XY Signals	X Signals	XXY Signals	XYY Signals	XXX Signals	XXXX Signals	XXYY Signals	XYYY Signals	
Double signals[b]	Females	64 (44–90)	...	1 (0–2)	...	...	3 (0–8)	29 (8–48)	...	...	7
	Males	1 (0–4)	71 (46–86)	...	4 (0–12)	...	...	...	25 (12–48)	...	6
Weak Y-probe fluorescence	Males	...	85 (74–90)	15 (10–24)	0 (0–2)	...	...	...	...	...	13
Mixed signal pattern[c]	Male	...	22	...	...	30	...	...	2	46	1

Note.—Signal patterns for karyotypically female and male cases that displayed ≥10% nuclei with signal patterns other than XX and XY were included in this table. Samples processed prior to the introduction of a grid separating samples during prehybridization processing were excluded.

[a] Sex according to conventional cytogenetics.

[b] The double signals were probably due to the presence of tetraploid nuclei.

[c] The mixed signal pattern in a karyotypically male sample was probably due to confined placental mosaicism (the fetus has been delivered, but a blood sample cannot be obtained).

Table 4

FISH Scoring Results for Mosaics

Sample Code	Tissue	Karyotype	Metaphases with Abnormal Karyotype[a] (%)	Nuclei with Abnormal FISH Signal Pattern (%)
E0221-94	Placental	46,XX/47,XX,+13	10	84
C1750-94	CVS	46,XY/47,XY,+18	32	70
C2894-93	CVS	46,XX/47,XX,+21[b]	7	54
E3862-94	Placental	46,XY/47,XY,+21	28	20
C0054-94	CVS	45,X/46,XY	51	52
C0807-94	CVS	45,X/46,XY	12	2
C2893-93	CVS	45,X/46,XY	32	10
C1242-95	CVS	45,X/46,XX	40	14
C2051-94	CVS	45,X/46,XX	38	46
C3996-94	CVS	45,X/46,XX	82	66
E3484-94	Placental	45,X/46,XX/47,XXX	45,X: 20; 47,XXX: 28	One X-signal: 0; Three X-signals: 24

NOTE.—All hybridizations on mosaics were technically successful.

[a] For the conventional chromosome analysis, an average of 37 (range 25–50) metaphases were evaluated.

[b] No abnormal metaphases were encountered in the routine conventional cytogenetic analysis of 10 metaphases cells from a minimum of two independent cultures. Because of the abnormal FISH result, 18 further metaphases were analyzed, and 3 of these metaphases were abnormal.

cians to believe a sample was of placental origin if the cells looked degraded.

Of the 40 samples from terminated pregnancies which were added to the trial, the technicians correctly recognized 15 as being placental. Four times, the technicians incorrectly believed a sample from an ongoing pregnancy to be a placental sample from a terminated pregnancy.

A Case with Deletion of Chromosome 18

A single sample showed a signal pattern consistent with monosomy 18; 96% and 4% of the nuclei showed one and two signals, respectively. Conventional cytogenetic analysis of prometaphase chromosomes showed a normal female karyotype. However, FISH with the chromosome 18–specific probe on metaphase spreads revealed a deletion on one of the chromosomes 18.

Discussion

We have developed a 1-d FISH assay for detection of numerical chromosome abnormalities in samples of uncultured mesenchymal chorionic villus cells. The purpose of the present study was to compare this FISH assay with conventional cytogenetic analysis and on this basis to generate FISH-assay cutoff values for samples with normal and abnormal chromosome complement.

The assay yielded discrete differences in the signal profiles between cytogenetically normal and abnormal samples. Karyotypically disomies and trisomies were easily and distinctly differentiated by using the number of nuclei with three signals as criteria (fig. 1 and tables 1 and 2). All disomic hybridizations had $\leq 40\%$ nuclei with three signals, and all trisomic hybridizations had $\geq 52\%$ nuclei with three signals.

After a protocol modification, karyotypically male and female samples had, on average, 99% nuclei with a signal pattern consistent with their sex, and all sex chromosome abnormalities had $\geq 94\%$ nuclei showing a signal pattern in agreement with their karyotype. Two samples were exceptions. One karyotypically female sample showed 78% nuclei with one X signal. On the basis of FISH analysis of paternal cells, this discrepant FISH result probably was due to a heritable small chromosome X probe target. While heteromorphism of the sex chromosome centromeres is uncommon, autosomal centromeric heteromorphisms are well described, and variability of probe target has been reported previously for a chromosome 13 and 21 repetitive probe (Bartsch and Schwinger 1991; Mizunoe and Young 1992; Verma and Luke 1992; Weier and Gray 1992; Seres Santamaria et al. 1993; Pellestor et al. 1994; Verlinsky et al. 1995). Another sample showed a mixture of XY, XYY, XYYY, and XXYY signals, while conventional chromosome analysis showed the karyotype to be 46,XY. This result was most likely due to confined placental mosaicism (Simoni and Sirchia 1994) and selective in vitro growth and subsequent chromosome analysis of a clone with a normal karyotype.

In order to make prospective assignments of genotype based on FISH results, we used our data set to generate FISH-assay cutoff values for karyotypically normal and aneuploid samples. Unfortunately, the number of abnor-

malities in the study was small, allowing only the establishment of criteria and not a subsequent assessment of the FISH assay's performance based on these criteria. A statistical establishment of an appropriate single cutoff criterion for the FISH assay was not indicated, since scoring results for samples with normal and abnormal karyotypes were distinctly differentiated and nonoverlapping with the above-mentioned exception of mosaic samples and the sample with a possible heritable small X-probe target. Instead, the scoring criteria were defined conservatively and cautiously. This resulted in the concept of uninformative diagnoses for samples with borderline signal patterns.

Informative samples were defined as samples in which ≥ 45 nuclei were scored per hybridization. *Informative abnormal* specimens were defined as those in which $\geq 60\%$ of nuclei hybridized with an autosomal probe demonstrated three signals or $\geq 60\%$ of nuclei hybridized with the X and Y probes demonstrated signal patterns other than XX and XY signals. In order not to discount important clinical information, the entire specimen was considered informative abnormal if one or more hybridizations fulfilled these criteria for abnormality even when other hybridizations on the same sample were uninformative. *Informative disomic* samples were defined as samples in which $\leq 20\%$ of all nuclei from each autosomal hybridization demonstrated three signals, and $\leq 20\%$ of all nuclei from the sex chromosome hybridization demonstrated other signal patterns than XX and XY signals. If hybridization with any probe was technically unsuccessful or did not meet the above criteria, the entire sample was designated *uninformative*. These criteria differ from the criteria described for uncultured amniotic fluid cells (Klinger et al. 1992; Ward et al. 1993).

In this study, there was a nonoverlapping signal distribution between karyotypically normal and trisomic samples, allowing discrimination of normal and aneuploid samples. Analysis of this prospective data set permitted us to establish the diagnostic criteria described above. Accurate determination of sensitivity, specificity, and, e.g., uninformative rate must await a further study in which these criteria are applied to a newly generated prospective data set. However, if the diagnostic parameters described above were applied to the data reported herein, the assay would have the following performance characteristics: the specificity and sensitivity for informative cases would be 99.9% (2,437/2,439) and 95% (80/84), respectively (according to our diagnostic criteria, 84 of the 97 karyotypically confirmed abnormalities would be informative and 13 would be uninformative). Two false-positive results would be FISH diagnoses of sex-chromosome aberrations for the karyotypically normal samples with possible (1) small chromosome X probe target and (2) confined placental mosaicism. Four false-negative results would be informative disomic FISH diagnoses for karyotypically mosaic samples (table 4). The rate of informative cases totally would be 93% (2,523/2,709), while the detection rate for the numerical abnormalities of the tested chromosomes would be 82% (80/97) (97 samples, including mosaics, were karyotypically aneuploid for the tested chromosomes, excluding a Klinefelter specimen processed prior to the inclusion of the X and Y probes in the study).

In order to establish whether technician time could be reduced without affecting the assay performance, we evaluated the impact of reading fewer nuclei per hybridization. This was possible because scoring results were cumulatively totalled after each subset of 10 nuclei were scored. However, applying the above-stated diagnostic criteria and using the results obtained scoring fewer nuclei, rising numbers of false FISH results were generated. Similarly, to reduce the cost of weekend and holiday personnel we attempted hybridization over a 5-d holiday (table 1, n. a). However, this created a false level of three-signal nuclei in a disomic sample. Accordingly, we do not recommend extended hybridization periods.

Mosaic samples showed no direct relationship between degree of mosaicism as judged by FISH and by conventional cytogenetics (table 4). Since the FISH assay is based on uncultured cells, this assay may better reflect the clonal distribution in a sample than conventional cytogenetics, which may be biased by clonal selection in the necessary tissue culture. In fact, FISH analysis suggested the presence of an abnormality that would not have been detected by using standard cytogenetic criteria. Because of the FISH data, additional metaphases were analyzed, ultimately confirming the presence of a mosaic abnormality (table 4, n. b).

The average rate of maternal cell contamination per sample was 0.3% (range 0%–10%), as judged by the number of nuclei with XX signals in karyotypically male samples. This was not enough to cause misdiagnosis with the FISH assay used but could possibly cause problems with diagnosis based on gene amplification using the PCR.

The blinding of the trial was not ideal. Fifteen of 40 times, the laboratory technicians correctly recognized that a placental sample was added to the trial. The technicians did not know which abnormality to expect, but their awareness was increased when they believed they were analyzing an added sample. However, as is demonstrated by figure 1, scoring results were not notably different for the added abnormal samples compared with the abnormal consecutive samples. It would have been beneficial for some of the added samples to have had a normal karyotype, but attempts to obtain karyotypically normal placental tissue for this purpose were unsuccessful.

Herein we report the first large-scale series of consecu-

tive CVS specimens analyzed for aneuploidy by using interphase FISH. While several studies using chromosome-specific probes on uncultured chorionic villus cells have been presented, the sample sizes were small (10–60 samples) (Evans et al. 1992; Lebo et al. 1992; Rao et al. 1993; Bryndorf et al. 1994). Evans et al. (1992) used the same probe set as in our study; however, they reported a relatively large number of FISH assay failures. In a study of 49 uncultured chorionic villus cell samples, 4 failed to hybridize with one of the five probes, and 1 sample failed to hybridize with all five probes.

We have developed an assay for prenatal detection of numerical abnormalities of chromosomes 13, 18, 21, X, and Y. The assay is technically robust and can readily be performed in 1 d in the laboratory. The assay requires ~1 h direct labor time per sample, including ~12 min for microscope analysis. This compares favorably with cytogenetic analysis. While direct CVS analysis can also be performed in 1 d, because it analyzes the cytotrophoblast cells, there is a 1%–2% false-positive rate and a 0.04% false-negative rate (Simoni and Sirchia 1994). In contrast, the FISH assay analyzes the mesenchymal core cells, which has been shown to be a more accurate representative of the fetal karyotype. Thus, the FISH assay may act as a more accurate and less labor-demanding alternative to direct CVS analysis.

The results reported herein and the cutoff values for assignment of normal and abnormal ploidy status depend on a variety of parameters, including the specific probes and sample processing techniques used for the analysis. A variety of other FISH probes are available, including probes from commercial sources. These other probes may yield different results. Interlaboratory differences in technique and scoring may also lead to performance variations. Therefore, the diagnostic criteria described herein cannot be directly extrapolated to other laboratories.

It still remains to be determined how and when to use the assay in pregnancy management. Should the results of the FISH assay be used as sole basis for irreversible therapeutic action or only as an effective means of prioritizing samples for conventional cytogenetics? We believe that scientific and cultural experiences will play a role in answering this question. At the present time, it is not considered the standard of care in the United States to substitute interphase FISH assays for conventional cytogenetics (American College of Medical Genetics 1993; Schwartz 1993). Employed as an adjunctive tool to conventional cytogenetics, the most effective way of using the FISH assay may be to offer the assay to pregnant women with an elevated risk of fetal aneuploidy (Strovel et al. 1992), e.g., to women >40 years of age who choose to have CVS performed. In the future, first-trimester pregnancies with elevated fetal aneuploidy risk may also be identified by early ultrasound and/or maternal serum screening programs (Wald et al. 1995). Rapid notification about a positive test result may expedite counseling and patient scheduling, while facilitating pregnancy management decisions.

FISH analysis of CVS is extremely rapid and is an effective enhancement to conventional cytogenetics. We believe that, over time, the clinical role of FISH technology will evolve as physicians evaluate its utility in their clinical practice.

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