Prenatal detection of aneuploidies using fluorescence *in situ* hybridization: A preliminary experience in an Indian set up

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Fluorescence *in situ* hybridization (FISH) is a powerful molecular cytogenetic technique which allows rapid detection of aneuploidies on interphase cells and metaphase spreads. The aim of the present study was to evaluate FISH as a tool in prenatal diagnosis of aneuploidies in high risk pregnancies in an Indian set up. Prenatal diagnosis was carried out in 88 high-risk pregnancies using FISH and cytogenetic analysis. Multicolour commercially available FISH probes specific for chromosomes 13, 18, 21, X and Y were used. Interphase FISH was done on uncultured cells from chorionic villus and amniotic fluid samples. FISH on metaphase spreads was done from cord blood samples. The results of FISH were in conformity with the results of cytogenetic analysis in all the normal and aneuploid cases except in one case of structural chromosomal abnormality. The hybridization efficiency of the 5 probes used for the detection of aneuploidies was 100%. Using these probes FISH assay yielded discrete differences in the signal profiles between cytogenetically normal and abnormal samples. The overall mean interphase disomic signal patterns of chromosomes 13, 18, 21, X and Y were 94·45%; for interphase trisomic signal pattern of chromosome 21 was 97·3%. Interphase FISH is very useful in urgent high risk cases. The use of FISH overcomes the difficulties of conventional banding on metaphase spreads and reduces the time of reporting. However, with the limited number of probes used, the conventional cytogenetic analysis serves as a gold standard at present. It should be employed as an adjunctive tool to conventional cytogenetics.

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1. Introduction

The most common chromosomal abnormalities in newborns are trisomies 21, 18, 13, monosomy X and other sex chromosome aneuploidies (Divane *et al* 1994). These aneuploidies can account for up to 95% of liveborn chromosomal abnormalities (Whiteman and Klinger 1991). Chromosomal abnormalities lead to a significant genetic disease burden on the society. Fluorescence *in situ* hybridization (FISH) introduced more than a decade ago, as a potentially powerful tool in clinical cytogenetics (Cremer *et al* 1986; Julien *et al* 1986) can provide a rapid and relatively reliable detection of aneuploidy of these chromosomes (Jalal *et al* 1998).

Prenatal diagnosis is accomplished by conventional cytogenetic banding of metaphase chromosomes, obtained from fetal trophoblast tissue (from chorionic villus biopsy), amniocytes (from amniotic fluid) or fetal lymphocytes (from cord blood). This technique is accurate and reliable allowing the detection of a variety of numerical

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Abbreviations used: *b*hCG, *b*-human chorionic gonadotropin; CVS, chorionic villus sampling; FISH, fluorescence *in situ* hybridization; MSAFP, maternal serum alpha-feto protein.

and structural aberrations (Ferguson-Smith and Yates 1984; Hook and Cross 1987). The primary disadvantage of the conventional cytogenetics is that the prenatal tissue must be cultured for several days prior to analysis. It takes 10 days–3 weeks to obtain results and has a culture failure rate of about 1% (Thein *et al* 2000). In certain clinical situations the time required to complete the chromosomal analysis might place a significant clinical or emotional burden on the patient and/or health-care provider. In such cases a method which provides rapid and accurate identification of aneuploidies would be a useful adjunctive diagnostic test to conventional cytogenetics (Ward *et al* 1993). FISH has been found to be highly effective for rapidly determining the number of specified chromosomes in interphase cells (Cremer *et al* 1988; Lichter *et al* 1988). FISH technique allows identification of specific nucleic acid sequences from chromosomes, even when the nuclei are in non-dividing interphase stage.

During late 1980s and early 1990s technical issues were the focus of research. Specific probes, determination of cell types suitable for use with FISH, and more effective techniques for cell preparation and signal detection were intensively studied (Philip *et al* 1994). The results of these efforts were technical advances such as commercially available highly specific and reliable probes, direct labelling, and multicolour computerized signal detection systems (Divane *et al* 1994; Jalal *et al* 1998). Such improvements have only increased the demand for immediate answers, and have made clinicians and their patients even more intolerant to delays in receiving results (Evans *et al* 1999). Advances in molecular techniques, including chromosome-specific probes and *in situ* hybridization techniques have generated considerable demand for extremely rapid results, particularly as they can be applied to uncultured cells (Martin *et al* 1996). These have been applied to common trisomies and monosomies with a rapid acceptance in the early 1990s to use FISH in high risk situation. However there has also been pressure towards its utilization in low risk situations (Evans *et al* 1992). Despite these advances, concerns on the sensitivity, specificity and predictive values of the test and lack of uniform laboratory methods produced profound scepticism in the genetics community. The singular benefit of FISH is the rapid detection of aneuploidy by chromosome specific probes applied to interphase cells. A FISH assay can easily be completed in less than 48 h compared to the 10–14 days usually required with conventional cytogenetic analysis. FISH analysis for detection of aneuploidies of chromosome 13, 18, 21, X and Y has been successfully performed with a high degree of concordance with cytogenetic results (Nederlof *et al* 1990; Ward *et al* 1993; Philip *et al* 1994; Eiben *et al* 1999). Not all chromosome abnormalities particularly structural rearrangements can be routinely identified by FISH when compared with conventional cytogenetic analysis (Evans *et al* 1999). Guidelines have been established for the use of FISH in prenatal diagnosis (American College of Medical Genetics 1993). These guidelines stated that FISH for clinical cytogenetic studies should be considered as an investigational technique.

There are extensive data available for prenatal diagnosis of chromosomal abnormalities using FISH from the western countries (Bryndorf *et al* 1996, 1997; Cacheux *et al* 1994; Cooper *et al* 1998; Eiben *et al* 1998; Klinger *et al* 1992; Mercier and Bresson 1995; Jalal *et al* 1998; Verlinsky *et al* 1998; Ward *et al* 1993) but there are no reported studies from an Indian set up. The policy statement of the American College of Medical Genetics (1993) also states that among the foremost unresolved issues in the utilization of FISH for interphase analysis in prenatal cytogenetics is to expand and bring this technology to a large number of prenatal diagnostic centres via independent FISH trials.

The aim of the present study was to evaluate FISH as a tool in prenatal diagnosis of high risk pregnancies in an Indian set up. Prenatal diagnosis was carried out in high-risk pregnancies i.e. advanced maternal age, previous child with chromosomal abnormality, fetal malformation detected through ultrasound examination, abnormal values of biochemical markers in the maternal serum and in parents with chromosomal rearrangements. These high risk patients can benefit from fast results since they suffer from substantial anxieties. It was anticipated that this study would allow cytogenetic evaluation of a significant number of normal as well as abnormal cases as the incidence of chromosomal abnormalities is expected to be relatively high in this group. These cases were analysed using FISH on interphase cells and metaphase spreads and conventional chromosomal analysis in order to test the efficiency and utility of FISH for the detection of common aneuploidies. For prenatal diagnosis, aneuploidies of chromosomes 13, 18, 21, X and Y were screened using FISH. The present study was based on the use of multicolour commercially available probes.

2. Materials and methods

During the years 1997–2000, prenatal diagnosis was performed in 88 high risk pregnancies presenting at the antenatal clinic of the Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi. Indications used to classify the pregnant patients as high-risk pregnancies for prenatal diagnosis were as follows: advanced maternal age (age > 35 years) (n = 22), previous child with chromosomal abnormality (n = 31), fetal abnormality detected through ultrasound

examination (n = 26), abnormal values of maternal serum alpha-feto protein (MSAFP) and *b*-human chorionic gonadotropin (*b*hCG) (n = 3), parental chromosomal abnormality (n = 4) and others (hydatidiform mole) (n = 2).

Invasive prenatal sampling i.e. either chorionic villus sampling (at 11–13 weeks) or amniocentesis (16–18 weeks) or cord blood sampling (after 18 weeks of gestation), were performed by the concerned physicians under ultrasound guidance. Usually either 15–20 ml of amniotic fluid or 10–12 mg of chorionic villus or about 1 ml of cord blood samples was obtained from each patient depending on the gestation stage at the time of sampling. All the samples – amniotic fluid, chorionic villi and cord blood – were then subjected to FISH and cytogenetic analysis.

FISH analysis was performed on interphase cells (from uncultured chorionic villi and amniotic fluid samples) and on metaphase spreads (from cord blood samples) as described. The amniotic fluid and chorionic villus samples were divided into two parts, one part was used for interphase FISH, and the other was used to establish two primary cultures for conventional cytogenetic analysis. Standard culture and harvest methods, described by Rooney and Czepulkowski (1992) were used to obtain chromosome preparations. G-banding on all the samples was performed using the method of Seabright (1971). For cord blood samples interphase FISH was not performed because of small sample volume; slides with chromosome preparations/metaphase spreads made using standard conventional blood lymphocyte culture and harvesting procedures as described by Rooney and Czepulkowski (1992) were used for FISH analysis.

2.1 *Preparation of uncultured cells for interphase FISH*

2.1a *Amniocytes*: For each amniotic fluid sample usually 5–7 ml of clear amniotic fluid sample was centrifuged for 10 min at 1000 rpm. The pellet was resuspended in 4 ml of trypsin-EDTA, gently vortexed, and incubated at 37°C for 20 min. Following centrifugation at 1000 rpm for 10 min. the pellet was resuspended by slowly adding 10 ml of prewarmed (37°C) hypotonic solution (0·8% sodium citrate). The tube was then placed in a 37°C incubator for 20 min followed by centrifugation at 1000 rpm for 10 min. The supernatant was removed and the pellet was resuspended by adding 2 ml of fixative (3 : 1 methanol and acetic acid) and gentle mixing. The suspension was kept in a refrigerator (2–8°C) for at least 1 h before the slides were made. For preparing slides for FISH analysis, 1–2 drops of the above cell suspension was dropped on clean dry slides placed on a slide warmer at 40–42°C.

2.1b *Chorionic villous cells*: Cleaned villi (1–2 pieces) kept in RPMI medium in a 35 mm Petridish, were used for interphase FISH. The medium was slowly replaced by 2 ml of prewarmed (37°C) hypotonic solution (1% sodium citrate) and the villi were incubated at 37°C for 15 min. The hypotonic solution was removed and 2 ml of fixative (3 : 1 methanol : acetic acid) was added, drop by drop, on the villi. The Petridish with villi were placed at – 4°C for 20 min and the fixative was replaced by 2 ml of fresh solution for 5 min at room temperature. The fixative was removed and the villi were air dried for 2 min. The villi were dissociated by adding 60% acetic acid. Slides were prepared starting 2 min after the dissociation solution was added, as described for the amniotic fluid sample in the previous paragraph.

For prenatal diagnosis, 4 slides were prepared for each patient sample. The slides were observed under a phase contrast microscope and the area with highest cell density or metaphase index was marked using a diamond pencil. Specific probes were used for the detection of chromosomal abnormalities in prenatal samples, as described in the following paragraph.

2.2 *Probes*

Commercially available FISH probes specific for aneuploidies common in prenatal samples – 13, 18, 21, X and Y were used for the present study. Probes for chromo-

Table 1. FISH probes used for prenatal diagnosis.

Chromosome	Name	Locus	Label
13	LSl 13	13q14	Spectrum orange
21	LSl	21q22·13-q22·2	Spectrum orange
18*	Chromosome 18	18 alphasatellite D18Z1	Fluorescein
X	CEP X	X alphasatellite	Spectrum orange
Y	CEP Y	Y alphasatellite DYZ3	Spectrum green

All the probes were from Vysis (USA) except *probe for chromosome 18, which was from Oncor (USA).
CEP, chromosome enumeration probe; LSl, locus specific identifier.

somes 13, 21, X and Y were procured from Vysis Inc. (Downers Grove, IL, USA) and that for chromosome 18 was obtained from Oncor Inc. (Gaithersburg, MD, USA). Technical characteristics as supplied by the manufacturers of the probes, are listed in table 1. Four hybridizations were carried out for each sample, one single-colour for each of the autosomal chromosomes (13, 18 and 21) and one dual-colour hybridization for the X- and Y-probes. Denaturation and hybridization of the specimen DNA and the probe was performed according to the manufacturer's instruction. The slides were observed under a fluorescence microscope using appropriate filters. Image analysis and quantitative analysis was done on each slide for interpretation of the results.

2.3 *Quantitative analysis*

For the evaluation of signals, nuclei were observed under X100 oil immersion objective. A minimum of 50 nuclei or 20 metaphase spreads were scored for each probe for prenatal diagnosis in high risk pregnancies. Nuclei free from any attached cytoplasm or cellular membranes showing 1, 2, 3 or 4 signals were selected for scoring. Only those signals, which were well embedded in the nucleus, were included for scoring. Clumped or overlapping nuclei and nuclei with high background intensity or low signal intensity were not scored. Patchy and diffused 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 only if the distance between the signals was less than the width of one of these signals, otherwise they were observed as two signals.

In the present study, strict and conservative analytical criteria for scoring the FISH signals were adopted from Ward *et al* (1993). On the basis of FISH scoring results the samples were considered to be informative normal and abnormal (however final diagnosis was made only on the basis of karyotyping). Informative normal/disomic samples were defined as samples in which ≥ 80% of all nuclei/metaphase spreads from each autosomal hybridization demonstrated two signals and ≥ 80% of all nuclei/metaphase spreads from the sex chromosomal hybridization demonstrated the XX or XY signal pattern.

Informative abnormal specimen were defined as those in which ≥ 70% of the nuclei/metaphase spreads hybridized with an autosomal probe demonstrated 3 signals or ≥ 70% of the nuclei/metaphase spreads hybridized with the X- and Y-probes demonstrated signal patterns other than XX- and XY-signals.

The criteria for defining a sample as mosaic has not been reported in any literature, known so far. From the laboratory experience in using FISH on different samples (Jobanputra *et al* 1998) for the present study, informative mosaic samples were defined as those in which ≥ 20% of the nuclei/metaphase spreads had a variation in signal number from the majority or showed a signal pattern other than the normal disomic autosomal or normal sex chromosomal (XX and XY) signals.

3. Results

The present study for prenatal detection of chromosomal abnormalities in high risk pregnancies was performed using two approaches – FISH and conventional cytogenetics. Efforts were made to diagnose the aneuploidies first by FISH and then confirm/support the results by conventional cytogenetics. From 1997–2000, prenatal diagnosis was carried out in 88 high risk pregnancies. In this study period 32 (36·36%) amniotic fluid, 41 (46·59%) chorionic villus, and 15 (17·04%) cord blood samples were analysed.

Samples were categorized/reported as normal or abnormal based on karyotype (i.e. final diagnosis was made using karyotype analysis), and these results were compared with those obtained by FISH. Cytogenetic analysis was successful in 80 cases (table 2) and uninfor-

Table 2. Successful FISH, karyotypes and abnormal chromosome complements in each indication group.

Indications	No. of samples	Success FISH	Success karyotype	Abnormal karyotype (No.)	Abnormal* (%)
Previous child with chromosomal abnormality	31	27	26	47,XX,+21(1)	3·84
Advanced maternal age	22	22	22	47,XY,+21(1) 46,XX/47,XX,+18(1)	9·09
Abnormal ultrasound scan	26	23	23	46,XY/46,XY,dup(54)(q33->qter) (1)†	8·69
Abnormal maternal serum screening	3	3	3	46,XX/47,XX,+21(1)	33·33
Parental chromosomal abnormality	4	4	4	–	–
Others	2	2	2	–	–
Total	88	81	80	6	7·5

†Structural chromosomal abnormality, not detected by FISH.
*Percentage of the abnormal fetuses was calculated from the number of cases with successful karyotypes.

mative in 8 cases because of inadequate sample – very less amniotic fluid which did not grow in culture (n = 2); culture contamination in cord blood (n = 3) and chorionic villus sample (n = 1) and blood strained amniotic fluid samples (n = 2) which were difficult to culture. The diagnostic success rate was 90·90%. Of the 80 successful karyotypes there were 6 abnormal cytogenetic results (table 2) giving an aneuploidy rate of 7·5%. Of the rest 74 normal samples, 44 samples had a 46, XX karyotype and 30 samples had a 46, XY karyotype.

Of the 88 samples obtained, FISH was performed in 81 samples (table 2). It was not attempted in 7 samples because of the presence of contaminating blood (n = 3) or insufficient sample volume (n =2) in the amniotic fluid samples or culture contamination in cord blood samples (n = 2). In all the 81 samples tested, analysis was successful and resulted in the generation of an informative report. This included 76 normal (74 normal by FISH and karyotype both + 1 structural chromosomal abnormality not detected by FISH + 1 case with uninformative cytogenetic analysis but successful FISH) and 5 samples with aneuploid chromosome complement (one of the structural chromosomal abnormality was not detected by FISH but was detected by conventional karyotyping). Of these 5 abnormal samples, 3 were pure aneuploid and 2 were mosaic aneuploid for one of the chromosomes tested. FISH analysis was also successful on the uncultured chorionic villus sample in one of the cases where cytogenetic analysis was uninformative. FISH analysis on metaphase spreads was performed only in cord blood samples and in two cases of trisomy 21 (detected on uncultured chorionic villus samples) to see the probe hybridization to metaphase spreads on abnormal samples (figure 1a). The results showed that there was no difference in the hybridization pattern of the probe in the normal and in abnormal samples.

3.1 *Number of nuclei scored and FISH success rate*

Of these 81 samples analysed, interphase FISH (on uncultured cells) was performed in a total of 69 samples (chorionic villus and amniotic fluid) and FISH on metaphase spreads was performed in 12 samples (cord blood). A mean of 51 nuclei (range 50 to 63) were scored per hybridization in chorionic villus and amniotic fluid samples. In cord blood samples a mean of 20 metaphase spreads (range 20 to 25) were scored per hybridization. Attempted hybridization were technically successful in 100% of the normal and abnormal specimens.

Of the total 81 samples analysed by FISH, 76 samples were disomic for chromosomes 13, 18 and 21 with a normal sex chromosome constitution, and also confirmed by cytogenetics. These 76 disomic samples included 75 normal samples and one sample with a mosaic structural chromosomal abnormality [46,XY/46,XY,dup(5)(q33 → qter)], which was diagnosed only by conventional cytogenetics (the FISH protocol used in the study was designed to detect only aneuploidies of chromosomes 13, 18, 21, X and Y). Rest of the five samples showed autosomal trisomy using FISH and the results were confirmed by conventional cytogenetics. There were no instances of false – positive (abnormal report, by FISH shown to be normal on cytogenetics) or false – negative (normal result by FISH, diagnosed as aneuploid for the tested chromosomes by cytogenetics) autosomal or sex

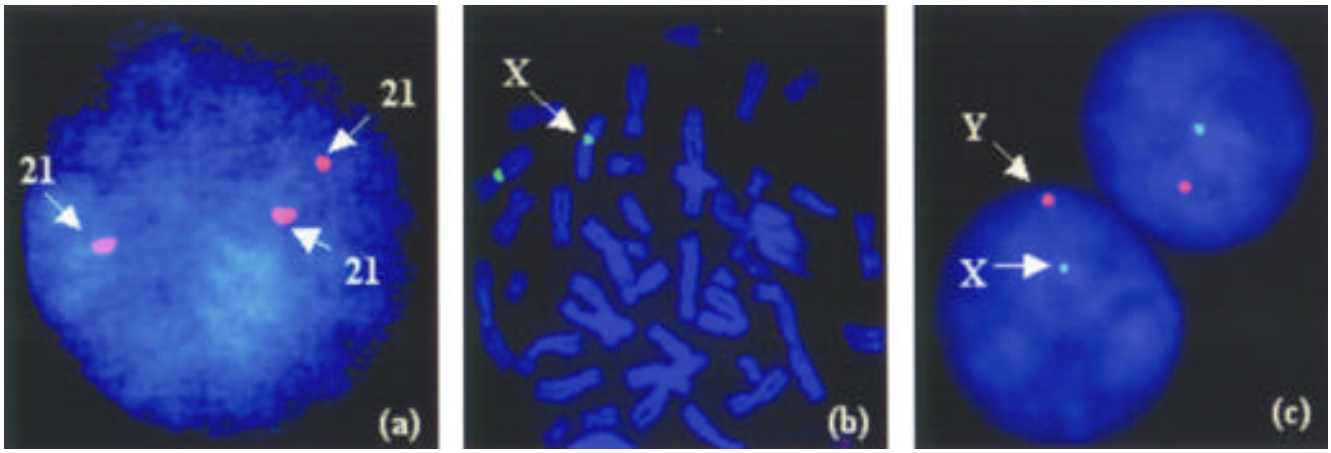

Figure 1. (**a**) Interphase nucleus from uncultured chorionic villus sample hybridized with a probe specific for chromosome 21 showing 3 red signals indicating trisomy 21. (**b**) Metaphase spread from a cord blood sample of a female hybridized with XY probe showing 2 green signals for chromosome X. (**c**) Interphase nuclei from amniotic fluid sample hybridized with XY probe showing one green and red signals each for chromosomes X and Y respectively.

chromosomal results. These data show an aneuploidy detection rate of 100% for the informative specimens. There were no uninformative FISH results.

3.2 *FISH scoring results for autosomal chromosomes*

In samples karyotypically disomic with respect to the tested chromosomes, an average of 94·05% (range 86%–100%) of the scored nuclei on a slide showed two signals, while 1·43% (range 0%–6%) of the nuclei had three hybridization signals. By contrast, in samples karyotypically trisomic for chromosome 21, an average of 2% of the nuclei showed two signals, while 97% (range 96%–98%) had three signals. In both the disomic and the trisomic samples, 100% of the scored metaphase spreads showed two and three signals (on the respective chromosomes) respectively. 15 (3·70%) of the 243 autosomal hybridizations (3 probes X 81 samples) also had > 10% nuclei with four signals, indicating the presence of tetraploid cells.

3.3 *FISH scoring results for sex chromosomes*

Male samples (n = 30) had an average of 95·20% (range 88%–100%) of their nuclei showing an XY signal pattern while female samples (n = 39) had an average of 94·1% (range 88%–100%) of their nuclei showing an XX signal pattern. In both the male and female samples, where FISH was performed on metaphase spreads (n = 12), 100% of the scored metaphase spreads showed the respective signal patterns (figure 1b,c).

In uncultured cells (interphase nuclei) there were 27 (39·13%) of the 69 samples (39 samples with 46, XX karyotypes and 30 with 46, XY karyotypes) with a normal sex chromosome complement which had > 10% nuclei with signal patterns other than XX and XY. The average rate of nuclei showing XX signals in karyotypically male samples (figure 1c) was 2·33% (range 0%–10%). None of the female samples showed nuclei with XY signals.

3.4 *Mosaic fetuses*

In the samples with karyotypical mosaicism specific for the tested chromosomes the results obtained by FISH (on uncultured cells) and karyotype did not match. In the case with mos46,XX[n = 15]/47,XX, + 21[n = 5] karyotype, 25% of the metaphase spreads showed trisomy for chromosome 21 whereas using interphase FISH 68% of the nuclei showed three signals (trisomy) for chromosome 21. Similarly, in the case with mos46,XX[n = 12]/47,XX, + 18[n = 8] karyotype, 40% of the metaphase spreads showed trisomy for chromosome 18 whereas using interphase FISH 50% of the nuclei showed three signals (trisomy) for chromosome 18.

4. Discussion

The present study was designed for prenatal diagnosis of chromosomal abnormalities in high risk pregnancies using FISH, which is a powerful molecular cytogenetic technique and has demonstrated it's considerable diagnostic potential, in the last five years. Several studies have reported successful application of FISH on interphase cells for rapid prenatal diagnosis. However, as with all emerging techniques, the transition from developmental phase to application as a standard diagnostic procedure requires assurance of reliability, reproducibility and accuracy, which can be addressed by expanding the experience to a large number of independent studies. Though data on such studies are now emerging at the International level, no such studies are available from Indian laboratories. The present study was designed to address the various aspects of use of FISH technique in an Indian set up. This preliminary study provides a baseline data in planning strategies for rapid prenatal diagnoses of chromosomal disorders.

Rapid detection of prenatal aneuploidy using interphase FISH on a large scale were successfully initiated (Klinger *et al* 1992; Ward *et al* 1993). Their studies formed the basis of the clinical protocols for the application of FISH to prenatal diagnosis. However, these two and most of the following studies (Cacheux *et al* 1994; Mericier and Bresson 1995; Bryndorf *et al* 1996, 1997; Cooper *et al* 1998; Eiben *et al* 1998; Jalal *et al* 1998; Verlinsky *et al* 1998) had several obstacles that delayed wide acceptance of FISH as highly reliable method for routine prenatal diagnosis. Some authors used probes prepared by their own laboratories (Bryndorf *et al* 1996, 1997; Klinger *et al* 1992; Ward *et al* 1993). Their probes were indirectly labelled and "home brewed" and are not commercially available (Jalal *et al* 1998). Most cytogenetic laboratories are not qualified and equipped to synthesize DNA probes and to perform necessary quality-control studies. Furthermore, the assay conditions should be modified for each set of probes because the quality and characteristics of the probes are the key factors for successful FISH analysis (Feldman *et al* 2000). Now, with the availability of commercial probes and hybridization chambers there is a need for a standardized technique. It is necessary to validate the usefulness of these new probe sets in order to determine their accuracy.

In the present study commercially available FISH probes (from Vysis, Inc, USA) were used. The criteria for interpretation of the results was defined (as described

in §2) to allow very few uninformative, false-positive and false-negative cases. All available fetal cell types were included and FISH was performed on both uncultured/ interphase nuclei and on metaphase spreads. Also important is the fact that FISH findings were reported to the physicians as preliminary results as soon as they were available, and the final results were reported with conventional cytogenetic analysis. In the present study, modified routine FISH technology with a potential of 100% accuracy, was integrated in a clinical prenatal diagnosis program.

The assay confirmed the cytogenetic findings, for the probes used, on interphase nuclei and in metaphase spreads in all the cases analysed, except one case of structural chromosome abnormality (duplication 5q) which this FISH protocol was not designed to detect. In one case of culture contamination, FISH analysis was useful in excluding the aberrations of specific chromosomes 13, 18, 21, X and Y on the uncultured/interphase nuclei. Two mosaic cases were identified by interphase FISH and also confirmed by cytogenetic analysis.

The study was also designed to compare the FISH assay with conventional cytogenetic analysis and on this basis to evaluate the clinical utility of the assay for prenatal diagnosis of aneuploidies. 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 (figure 1a,b). All the disomic hybridization had ≤ 2% nuclei with three signals and all trisomic hybridization had ≥ 97% nuclei with three signals. Karyotypically male and female samples had, an average 94% nuclei with a signal pattern consistent with their sex.

Although the reporting criteria defined by Ward *et al* (1993) was chosen for the present series, and it was adequate for reliable detection of aneuploidy, the results from the present study suggest that a more strict reporting criteria could be adopted in future. To make prospective assignment of genotypes based on the FISH-assay described in the present study, the cut-off point could be optimized to be as high as 90% for both the disomic as well as trisomic prenatal samples. Unfortunately, the number of abnormal samples in the study were small, allowing only the establishment of the criteria/cut-off points and not a subsequent assessment of the FISH assay performed based on these criteria. Accurate determination of sensitivity, specificity and uninformative rate must await a further study in which these criteria can be applied.

The sensitivity, specificity and predictive values for the routine FISH analysis as a method to detect aneuploidies of chromosome 13, 18, 21, X and Y in this relatively small study were all 100%. However, even with 100% accuracy of the test, routine FISH analysis will miss about 25–30% of the detectable cytogenetic abnormalities (Feldman *et al* 2000).

The high cut-off points optimized from the present study were due to very reliable probes and optimization of several steps in preparation of cells, hybridization and detection. The relatively small number of cells needed for diagnosis (practically 50), gave a much better option to be more selective in cell quality and was a major factor in the efficiency and accuracy of the tests.

In the present study, mosaic samples showed no direct relationship between the degree of mosaicism as judged by FISH and by conventional cytogenetics. It has been proposed that there might be variations in the range of nuclei exhibiting the signal which can be attributed to parameters such as sample quality, slide preparations, and hybridization or detection efficiency rather than to low level of mosaicism (Spathas *et al* 1994). This probability, however can never be excluded. The other reason for this variability could be that using interphase FISH more number of nuclei/nondividing cells are available for analysis wheareas using karyotyping/ conventional cytogenetics analysis is restricted to only the dividing cell population which is arrested at the metaphase stage. The average range of maternal cell contamination per sample was 2% (range 0%–10%), as judged by the number of nuclei with XX signals in karyotypically male samples. This does not seem adequate to cause misdiagnosis with the FISH assay.

Results from this study on different samples, clearly show that application of FISH to interphase nuclei is of tremendous value to prenatal diagnostic cytogenetics. The major advantages and disadvantages of FISH have been reported earlier (Jobanputra *et al* 1998). The FISH assay used for the present study is technically robust and can readily be performed in two days in the laboratory.

The present study concludes that molecular cytogenetic technique of FISH is very useful in urgent cases of prenatal diagnosis where it can be used on uncultured amniocytes and chorionic villus samples for rapid and accurate detection of common aneuploidies. Well-designed multicentre prospective trials are required to expand the experience with FISH, from the present study to a larger number of prenatal diagnostic centres, with regulatory guidelines established by the recognized authorities in the Indian Medical Council/governmental agencies. From this study the prospects of interphase FISH for routine prenatal diagnosis seem both exciting and bright. With more laboratories attempting prenatal diagnosis using FISH, the cost of the commercially available probes will also come down. As this work continues to be integrated with patient care and management of high risk pregnancies in future we will be able to offer rapid and accurate prenatal diagnosis to more number of pregnancies and have healthy, normal babies.

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