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Prenatal cytogenetic diagnosis

Techniques have been proposed recently which use fluorescent in situ hybridisation (FISH) or quantitative polymerase chain reaction (PCR) to identify certain of the more common aneuploidies in samples of amniotic fluid obtained for prenatal diagnosis.[1–4] The rationale for this recommendation is that routine cytogenetic studies require 14 days for completion, resulting in a long period of anxiety for the mother, especially when she is at high risk. The proponents argue that these newer techniques can provide at least some information to the patient in a few days. However, even the most ardent advocates admit that these techniques will not identify all chromosome abnormalities routinely identified by classical cytogenetic studies, that they require significant additional expense, and that they do not approach the success and accuracy of classical studies. Because of this, classical cytogenetic studies must still be performed.[5–8] In addition, these techniques require a relatively large portion of the amniotic fluid sample, thereby decreasing the amount available for classical cytogenetic studies. This increases the time needed to complete the traditional testing and also increases the chance for a failure. Even given the above shortcomings, many have advocated liberal use of FISH or PCR, or both, in order to alleviate maternal anxiety.

As elegant as these techniques may be, their technical shortcomings and significant cost should limit their application to specific and uncommon clinical situations. Rather than spend an additional $200 to $300 to provide partial results, prenatal cytogenetic laboratories should improve their efficiency in order routinely to provide cytogenetic results in an average of seven to nine days. This could be accomplished by replacing the traditional flask or subculture method with the in situ culture and harvesting method. Once this technique is mastered, a modification of it should ultimately allow cytogenetic results to be obtained in three to four days in most high risk cases without any increased cost—a worthwhile goal in the current medical economic climate. If classical cytogenetic results could be provided in the above time frames, the rationale and demand for FISH or PCR would no longer apply in most of the cases in which they are currently used.

The in situ method, described by Cox *et al*[9] and Peakman *et al*,[10] enables a more rapid processing of samples at a significantly reduced cost. It also provides a more accurate method for assessing clinical significance of chromosomal mosaicism (if present). The in situ method relies on initiating cultures by applying a small volume of cell suspension to a number of coverslips within a culture vessel. These coverslips carry the cell population that will ultimately be analysed without any further culture manipulation. The cells attach for 12–24 hours before the coverslip is flooded with more medium. The cultures are monitored for growth at four to five days. Those demonstrating small colonies with actively dividing cells are processed "in situ" and stained for cytogenetic analysis. The metaphases are found within and around the colonies. If a cell has a cytogenetic aberration, other cells from the colony are also examined. The ability to examine cells from multiple distinct primary cultures increases the accuracy of the analysis. The ease with which the coverslips can be harvested, stained, and analysed decreases the amount of time spent handling the cultures and at the microscope.

We recently reported experience with a modification of the in situ method, which allows for karyotype analysis to be performed within three to four days of sampling.[8] This modification involves three minor changes: (*a*) culture conditions, most importantly, media and media supplements, are optimised; (*b*) cultures are initiated at a slightly higher cell density; and (*c*) the technologist is retrained to harvest coverslips at an earlier time. With these modifications, over 80% of the cases yield analysable metaphases within 72 to 96 hours. The overall completion time is about 1.5 days less than the routine case completion time. The rapid karyotype method is able to identify about 80% of the chromosome abnormalities in an unselected population within 96 hours, while FISH identifies less than 60% of the chromosome abnormalities within 48 hours. Figure 1 shows the reasons for this difference in sensitivity.

The decreased sensitivity of FISH methodology is due to the following: (*a*) low volume samples or samples with obvious blood contamination cannot be used; (*b*) because FISH and PCR can only identify numerical abnormalities for certain chromosomes, these techniques, by definition,

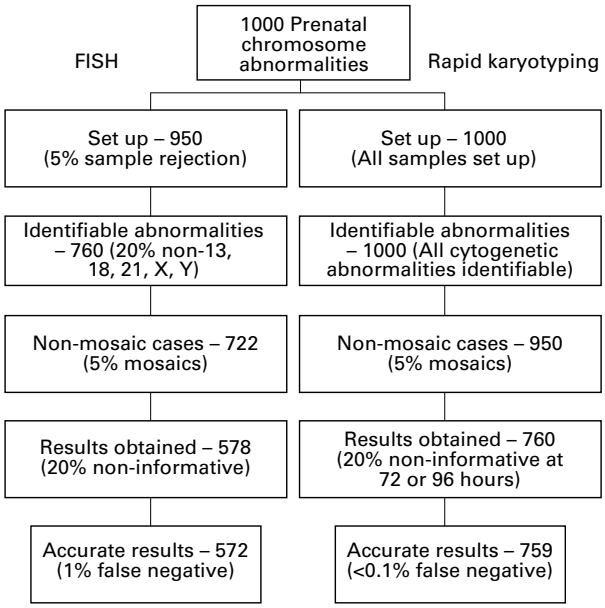

Figure 1 Identification of chromosome abnormalities.

cannot identify 20% or more of the chromosome abnormalities identified by classical cytogenetic studies; (*c*) a higher false negative rate occurs with FISH than with classical cytogenetics (about 1% in a large prospective study). Neither technique identifies mosaic conditions. Both fail to provide results in about 20% of the abnormal cases: FISH because the percentage of cells with abnormal signals fall into the uninformative range, and rapid karyotyping because of a failure to obtain dividing cells for analysis at 72 or 96 hours. If the FISH testing fails, or yields inconclusive results, a large portion of the amniotic fluid sample has been wasted. However, with rapid karyotyping, the eventual success of the testing is not at risk because no part of the sample is diverted from classical cytogenetic studies; if coverslips are not ready for harvest at 72 or 96 hours, they are held for routine harvest for five or six days.

In summary, we agree that if routine cytogenetic studies on amniotic fluid samples are not being completed for 12 to 14 days, something needs to be done to alleviate parental anxiety. Cytogenetic laboratories can improve the turnaround time for these routine samples to seven to nine days, which should alleviate most of the parental anxiety. In addition, with a slight modification of the technique, classical cytogenetic results can be obtained in 72 to 96 hours in high risk cases, further decreasing the need for additional, expensive testing except in the most unusual of cases. In most cases this will avoid the need for additional tests (FISH, PCR), which provide only partial answers, at a lower success rate, which take away sample volume from the basic testing protocol, and add significantly to the cost.

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