Introduction

Waiting for the results of prenatal genetic analyses can sometimes place a difficult burden on the `expectant´ parents, especially if the pregnancy is considered to be high-risk for a chromosome abnormality and requires diagnosis as fast as possible.
After amniocentesis, results of the conventional chromosomal analysis will be available after approx. 1 – 3 weeks. Waiting for cytogenetic reports can be very stressful for pregnant women under these circumstances.

Advances in molecular techniques, including the method *fluorescence in-situ hybridization*, facilitate a substantial speedup in detecting aneuploidies in interphase cells. For this analysis, cells do not require growth and seperation in an incubator.

Using uncultured amniotic cells, chromosome-specific probes for chromosomes 13, 18, 21, X and Y are applied. Up to 90% of the clinically significant chromosomal abnormalities observed in the second trimester can be detected (1,2,).

Method

The FISH analysis utilizes fluorescent DNA probes, that attach themselves (hybridize) to specific chromosomes in the fetal cells. These colored probes are added to a portion of the cells obtained from the amniocentesis. After the FISH probes are mixed with cells, they hybridize to their corresponding chromosomes.

For example, when a chromsome 21 specific FISH probe hybridizes to the corresponding region on chromosome 21, a red-colored signal within the fetal cell can be detected with special filters under a light microscope. In a normal cell usually two signals are seen. In cells with trisomy 21, three signals for the specific probe can be detected (see picture).

Results are reported based on the percentage of spots (hybridization signals) for each probe. FISH analyses are considered to be succesfully processed if all recommendations according to the guidelines of the German Society for Human Genetics are fulfilled (3).

Limits of the Method

The interphase FISH analysis detects numerical abnormalities of the chromosomes 13, 18, 21, X and Y. It is not designed to obtain information about aneuploidies of other autosomes. In addition, chromosomal rearrangements or structural aberrations can not be detected.

A maternal contamination of the amniotic fluid probe can falsify the result and could obscure an aneuploidy present in fetal cells. Therefore, to avoid false results bloody probes should not be analysed by interphase FISH.

→ **It is important to know that due to the described limitations of the method prenatal interphase FISH does not replace conventional chromosome analysis.**
The result should always be confirmed by a conventional cytogenetic analysis if other clinical data give no indication for the presence of a pathological situation. FISH is not intended for use as a stand-alone test on which to base clinical decisions.

Indication

Specific indications for this diagnostic tool are for example:

- abnormal ultrasound findings
- abnormal maternal serum screening

Requirement

At least 18 ml clear amniotic fluid is necessary. Samples with visible contamination of blood will not be analyzed by interphase FISH.
15 ml of the sample used for conventional chromosome analysis. At least 3 ml are set up for interphase FISH. In case of an inadequate amount of amniotic fluid, the analysis with the higher diagnostic priority is performed: the conventional chromosome analysis.

Interphase FISH results are reported within approx. 24 to 48 hours upon arrival of the specimen in the laboratory.

References

1. Klinger, K. et al. Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). *Am J Hum Genet* 51:55-65, 1992

2. Philip, J. et al. Prenatal aneuploidy detection in interphase cells by fluorescence in situ hybridization. *Prenat Diag* 14:1203-1215, 1994

3. German Society for Human Genetics and German Association for Medical Genetics. *Guidelines for rapid prenatal aneuploidy screening*. *MedGen* 1998;10:319.

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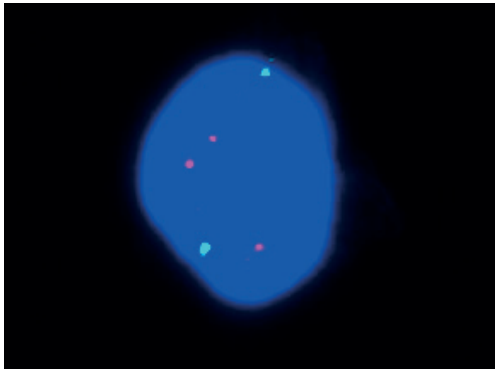
RAPID PRENATAL DIAGNOSIS OF ANEUPLOIDIES IN UNCULTURED AMNIOCYTES BY *FLUORESCENCE IN-SITU HYBRIDIZATION (FISH)*

- Interphase FISH -



Analysis of uncultured amniocyte, hybridzized with probes for chromosome 13 and 21.
Green signals: “LSI 13” (RB1); red signals: “LSI 21” (D21 S259/D21S341/D21S342). Picture shows trisomy 21.

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