

## 형광직접보합법을 이용한 미배양 양수세포에서 산전 이수배수체 확인

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### Prenatal Aneuploidy Detection in Uncultured Amniotic Fluid Interphase Cells by Fluorescence in situ Hybridization (FISH)

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**Objective:** The aim of the present study was to evaluate the clinical efficiency of fluorescent in situ hybridization (FISH) in the prenatal diagnosis of chromosomal aneuploidy.

**Methods:** We reviewed data of 268 cases to identify women undergoing genetic amniocentesis at cytogenetic laboratory, from January 2000 to December 2002. Amniotic fluid was submitted for both rapid FISH on uncultured interphase amniocytes using a commercially available DNA probe for chromosome 13, 18, 21, X, Y and standard karyotyping on cultured metaphase amniocytes. Results from FISH and full karyotype were compared.

**Results:** There were 251 cases (84%) normal and 17 cases (16%) abnormal in FISH results. All 17 cases of trisomy 13, 18, 21 including two cases of mosaicism and sex chromosome aneuploidies which are detected by FISH were confirmed with conventional cytogenetics and there was no false positive result. Twenty two cases had karyotypically proven abnormalities that could not have been detected by the targeted FISH.

**Conclusion:** Interphase FISH analysis of uncultured amniotic fluid cells has been shown to be an effective and reliable technique for rapid fetal aneuploidy screening during pregnancy as an adjunctive test to conventional cytogenetics.

**Key Words:** Prenatal genetic diagnosis, Aneuploidy, FISH

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가 FISH

가 268

10 7 2.

가 1)

20 ml conical tube 10 ml

(probe) (Fluorescence in situ hybridization; FISH) ) flask in situ 1,000 rpm 8 tube 3 ml

FISH 가 25 cm<sup>2</sup> cell culture flask tube 1.5 ml 가

.<sup>1,2</sup> 1980 1990 in situ dish 37 , 5% CO<sub>2</sub>

(signal detection) 가 Flask 3 (inverted microscope, Olympus) 가 In

가 3 situ dish 2 ml 가 3

가 <sup>4,5</sup> 가 colony가 3~4

65% 가 5 가 harvest

13, 18, 21, X Y Harvest flask colcemid 100 µl (10 µg/ml) 가

.<sup>6-8</sup> FISH 가 scraper 2 30 가

가 <sup>9,10</sup> scraper flask

.<sup>11</sup> 0.075 M KCl

13, 18, 21, X Y 20 0.5 ml Canoy's (Me-  
FISH 24 80% thanol : Acetic acid = 3 : 1)

가 <sup>12-15</sup> 30

268 FISH 2 60 1

FISH

In situ colcemid 80 µl 가 ,

50 가 0.0375 M KCl 20 0.5 ml

Canoy's 2 가 3

1.

2000 1 2002 12 1 Gi-  
emsa (G bands by trypsin using Giemsa; GTG)

30 , rubber cement cover glass

5 (Chips, (denaturation) (hybridization) GenDix, Korea) . HYBrite™ Denaturation/Hybridization . HYBrite

1995 International System for human Cytogenetic Nomenclature (ISCN) 가

2) , 75 2 , 37 16

(1) VYSIS 13 (LSI 13 (13q14) SpectrumGreen), 18 (CEP 18 (alpha satellite D18Z1) SpectrumOrange), 21 (LSI 21 (D21S259, D21S341, D21S342) SpectrumOrange), X (CEP X SpectrumOrange/Green (alpha satellite)) Y (CEP Y (satellite III) SpectrumGreen/Orange, LSI SRY (Yp11.3) SpectrumOrange)

2 ml 15 ml conical tube 1,250 rpm

8 0.05% trypsin/EDTA 3 ml

37 20 .

1,250 rpm 8 ,

0.075 M KCl

37 20 .

Canoy's 5 ml (4)

1,250 rpm

8

30 cover glass

2 . 42 50% formamide/2×SSC (pH 7.0)

67 humid chamber 3 , 2×SSC/0.1% NP-40 1 5

25 µl 10 . Antifade (5 µg/ml)-DA-PI (20 µg/ml) counterstaining

(Zeiss) 56 Chips-FISH

heat block 30 (GenDix, Korea) , 50

(2) 37 2×SSC 1

37 pepsin 13 ,

PBS 5 , post fixative formaldehyde (1%) 2000 1 2002 12 가

5 , PBS 5 794 , 268

. 70%, 85%, 100% FISH

1 .

(3) 가

0.5 µl, H<sub>2</sub>O 0.5 µl, 7 10

3.5 µl 5 µl , FISH 24

가 가 cover glass 17~18 가 268 145 (54%) 가 ,

15~16 19~20 가 53 11 4% (Table 2).  
 20% (Table 1). FISH 17  
 FISH (6%) , 14 (82%)가 21 (7 ), 18 (6  
 ), 13 (1 ) ,  
 가 135 50% ,  
 가 3 (18%)가 47,XXY (2 ) 47,XYY (1 )  
 가 87 32%, 가 33 12%, (Table 3).

**Table 1.** Gestational age at amniocentesis

Gestational age	No. of cases (%)
11~12	2 (0.74)
13~14	3 (1.12)
15~16	53 (19.78)
17~18	145 (54.10)
19~20	53 (19.78)
21~22	6 (2.24)
23~24	5 (1.87)
25~26	0 (0.00)
27~28	1 (0.37)
Total	268 (100.00)

FISH 13, 18, 21, X Y

**Table 2.** Indications for prenatal interphase FISH studies

Indications	No. of cases (%)
Positive maternal serum screening	135 (50.37)
High Down risk	87 (32.46)
Previous anomaly	33 (12.31)
Abnormal ultrasound findings	11 (4.11)
Others	2 (0.75)
Total	268 (100.00)

**Table 3.** Numerical chromosomal anomalies detected by FISH

Numerical chromosomal anomalies	Indications	No. of cases	Total (%)
Autosome			14 (82.35)
trisomy 21	high Down risk	4	
	positive maternal serum screening	2	
trisomy 18	abnormal ultrasonography	3	
	high Down risk	1	
	positive maternal serum screening	1	
trisomy 13	positive maternal serum screening	1	
Mosaicism *			
trisomy 21	abnormal ultrasonography	1	
trisomy 18	abnormal ultrasonography	1	
Sex chromosome			3 (17.65)
47,XXY	positive maternal serum screening	1	
	high Down risk	1	
47,XYY	positive maternal serum screening	1	
Total			17 (100.00)

\*; detected as trisomy by FISH and confirmed as mosaicism by conventional karyotyping.

**Table 4.** Chromosomal anomalies and normal variants not detected by FISH in the uncultured amniotic fluid cells

Indications	Karyotype	FISH result	No. of cases	Total (%)
Positive maternal serum screening				14 (63.64)
	46,XX,inv(9)	normal female	8	
	46,XY,inv(9)	normal male	1	
	46,XX,der(19)t(12;19)	normal female	1	
	46,XY,inv(3)	normal male	1	
	46,XY,t(6;12)	normal male	1	
	46,XX,t(1;2)	normal female	1	
	46,XY,t(4;6)	normal male	1	
Previous anomaly				4 (18.18)
	46,XX,inv(9)	normal female	1	
	47,XX,+mar	normal female	1	
	46,XX,t(16;17)	normal female	1	
	46,XY,t(1;11)	normal male	1	
High Down risk				4 (18.18)
	46,XY,inv(9)	normal male	2	
	46,XY,inv(2)	normal male	1	
	46,XX,22s+	normal female	1	
Total			22	22 (100.00)

22 가

(Table 4). 1 marker

trisomy 18, FISH trisomy 21  
 +21/46,XX 47,XX,+18/46,XX

가 가

Figure 1 (A) FISH LSI 21

trisomy 21, Figure 1 (B)

Figure 2 (A)

FISH 24 48

FISH, Figure 2 (B)

가

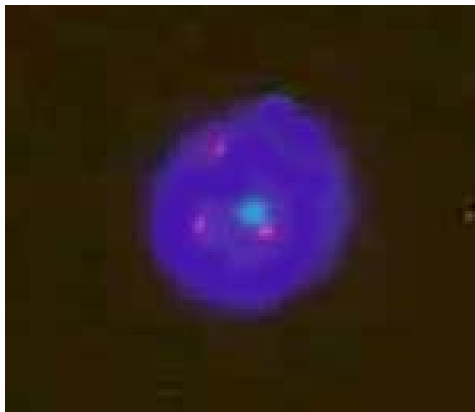
17-19

13, 18, 21, X Y

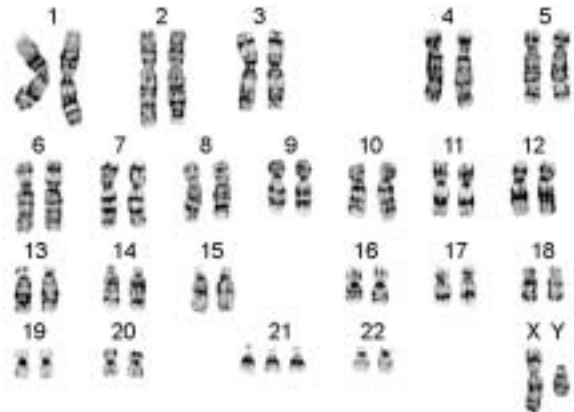
가

가

가

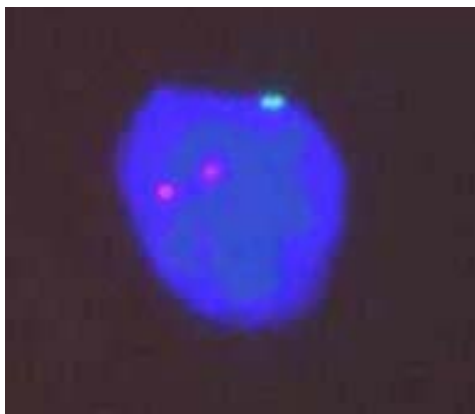


A

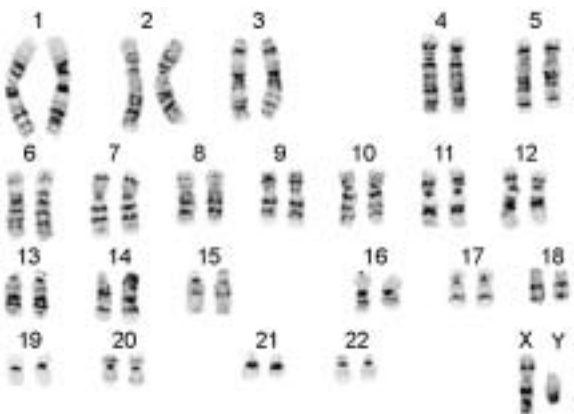


B

**Figure 1.** (A) Interphase nucleus of the uncultured amniotic fluid cell hybridized with a specific probe for chromosome 21 showing 3 red signals indicating trisomy 21. Green signal is chromosome X. (B) Result of chromosomal analysis for (A): 47,XY,+21.

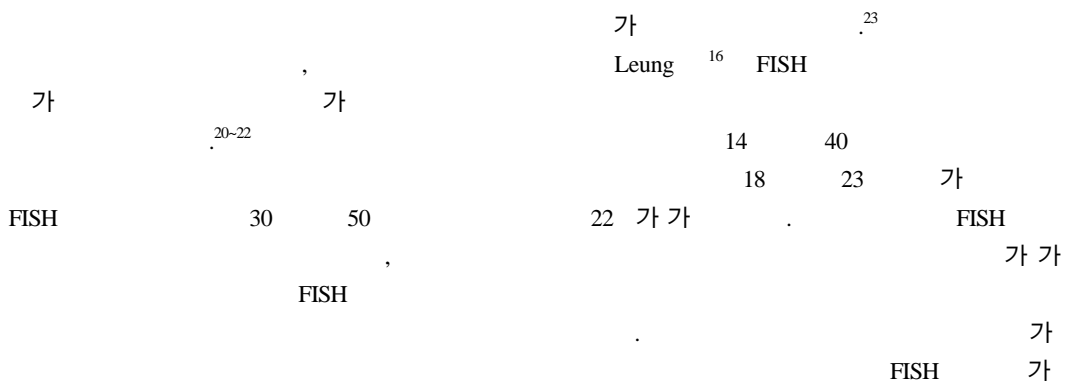


A



B

**Figure 2.** (A) Interphase nucleus of the uncultured amniocyte after FISH shows two red (chromosome 21) signals and one green (chromosome X) signal. (B) Result of chromosomal analysis for (A): 46,XY,t(1;3).



가  
 FISH  
 52% (26/50)  
 , 22% (11/50)가  
 17~18  
 (54%) FISH 가  
 50% 가  
 4%  
 가  
 FISH  
 가 100%  
 가<sup>24-26</sup>  
 FISH  
 22 FISH  
 marker 47 가  
 1 가  
 marker 가  
 NOR satellite stalk  
 marker  
 acrocentric FISH  
 가 FISH  
 false-positive  
 false negative  
 .<sup>21</sup> tri-  
 somy 18 trisomy 21 1  
 FISH  
 FISH 45,X marker  
 가 SRY  
 FISH

FISH  
 (Preimplantation Genetic Diagnosis; PGD)  
 가<sup>27,28</sup>  
 FISH  
 Cri-du-chat DiGeorge  
 가  
 FISH  
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