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### Improvement of Pregnancy Rate in Preimplantation Genetic Diagnosis with FISH Procedure by the Laboratory Optimization and Experiences

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**Objectives:** This study was performed to evaluate the laboratory system for successful PGD using fluorescence in situ hybridization (FISH) and the clinical outcome of PGD cycles in five years experiences.

**Methods:** A total of 181 PGD-FISH cycles of 106 couples were performed, and diagnosed chromosome normality in the preimplantation embryos. The laboratory and clinical data were classified by the following optimization steps, and statistically analyzed. Phase I: Blastomere biopsy with two kinds of pipettes, removal of cytoplasmic proteins without treatment of pepsin and culture of biopsied embryos with single medium; Phase II: Blastomere biopsy with single pipette, removal of cytoplasmic proteins with pepsin and culture of biopsied embryos with single medium; Phase III: Blastomere biopsy with single pipette, removal of cytoplasmic proteins with pepsin and culture of biopsied embryos with sequential media.

**Results:** A total of 3,209 oocytes were collected, and 83.8% (2,212/2,640) of fertilization rate was obtained by ICSI procedure. The successful blastomere biopsies were accomplished in 98.6% (2,043/2,071) of embryos, and the successful diagnosis rate of FISH was 94.7% (1,935/2,043) of blastomeres from overall data. Embryo transfers with normal embryos were conducted in 93.9% (170/181) of started cycles. There was no difference in the successful rate of biopsy and diagnosis among Phase I, II and III. However, the pregnancy rate per embryo transfer of Phase III (38.8%, 26/67) was significantly ( $p < 0.05$ ) higher than those of Phase I (13.9%, 5/36) and Phase II (14.9%, 10/67).

**Conclusions:** The laboratory optimization and experience for the PGD with FISH procedure

can increase the pregnancy rate to 38.8% in the human IVF-ET program. Our facility of PGD with FISH provides the great possibility to get a normal pregnancy for the concerned couples by chromosomal aberrations.

**Key Words:** Preimplantation genetic diagnosis (PGD), Fluorescence in-situ hybridization (FISH), Chromosome, Pregnancy rate

(preimplantation genetic diagnosis, X-

PGD)

Edwards Gardner (1968)가 blastocyst sexing Robertsonian

sexing 20 , McLaren (1985) 가 4-6 가 (genome) 2 DNA 가 (polymerase chain reaction, PCR)

1990 Handyside 가 X 가 가 (probe)가 hybri- dization (fluorescence in-situ hybridization, FISH)

trophectoderm 1. 1997 8 2002 7 106 . 106 181

67 , 37 , 14 Robertsonian , 13 , 28 implantation failure 23 X 10 11 2. GnRH agonist, hMG recombinant human FSH hCG 3. (FISH) 48 Carnoy solution 37 , 5% CO<sub>2</sub>, 95% 6~10 1~2 biopsy FISH signal 1 (Phase I) 1997 7 1999 12 biopsy 2 pipette 3 10 µm pipette (zona drilling) , 30 µm pipette biopsy HTF (Irvine, USA) (Carnoy's solution) , HTF balanced 4 2 (Phase II) 2000 1 2001 7 biopsy 25~30 µm 1 pipette Ca<sup>2+</sup> Mg<sup>2+</sup> 7† T6 biopsy 12 1 Biopsy 1 -hCG 16 , 0.01% pepsin . Biopsy . Biopsy HTF balanced 3 (Phase III) 2001 8 2002 7 biopsy EB-10™ (Vitrolife, Swenden) , 3 G1.2 , biopsy G2.2 (Vitrolife, Sweden) 4~5 balanced 3. (FISH) , 1 Carnoy solution 2 3 Carnoy solution 0.01% pepsin , 1% paraformaldehyde 1 FISH probe 75 hot plate denaturation 5 37 , humidified chamber overnight hybridization . Hybridization 50% formamide /2X SSC, 2X SSC, 2X SSC/0.1% NP-40 buffer 42 probe (Optiphot-2, Nikon) FISH signal 2 가 4. 12 16

**Table 1.** Clinical outcome of preimplantation genetic diagnosis using fluorescence in-situ hybridization

	Number	Mean ± SD
Cycles	181	
Patients	106	
Mean age (range)	32.2 ± 4.8 (21-46)	
Retrieved oocytes	3,209	17.7 ± 10.3
Injected oocytes (%)	2,640 (82.3)	14.6 ± 8.8
Fertilized oocytes (%)	2,212 (83.8)	12.2 ± 7.5
Biopsied embryos	2,071	11.4 ± 5.4
Normal embryos (%)	476 (23.0)	2.6 ± 1.9
Embryo transfer cycles (%)	170 (93.9)	
Transferred embryos	438	2.4 ± 1.4
Positive -hCG cycles (% <sup>*</sup> )	41 (24.1)	
Clinical pregnancies (% <sup>*</sup> )	30 (17.6)	
Clinical abortions (% <sup>*</sup> )	6 (3.5)	
Miscarriage (% <sup>*</sup> )	1 (0.6)	
Deliveries (% <sup>*</sup> )	23 (13.5)	

\* per embryo transfer.

, 476 (2.6±1.9) 가  
 balanced  
 11 170  
 balanced 438  
 5. (2.4±1.4) , 41 (24.1%)  
 positive -hCG  
 ABstat (rel. 30 (17.6%) 24 (14.1%)  
 6.54, Anderson-Bell Co.)<sup>2</sup> test Student's t-test 16 , 6 (3.5%)  
 , p<0.05 10 (Table 1).  
 (n=67)  
 119  
 31.4±3.9 , 1,333  
 309  
 1. (2.6±1.8) 가 balanced (23.2%)  
 106 181 PGD 283 (2.5±1.3)  
 -FISH 32.2±4.8 112 29 (25.9%)  
 (21~46) , 3,209 (17.7±10.3) 가 positive -hCG 19  
 , 2,640 (14.6±8.8) 8  
 , 2,212 (12.2±7.5) , 11  
 (83.8%)가 . 1 16  
 , 2,071 (11.4±5.4) , 24

가 Robertsonian 2  
(n=14) 28 , 1 10 . X  
30.7±  
3.1 , 246 (n=10) 11  
59 (2.1±1.7)  
가 balanced (24.0%) 29.6±4.5 107  
54 (2.1±1.2) 26 45 (4.1±2.9)  
4 (15.4%) positive -hCG 가 (42.1%)  
3 37 (3.4±1.7) 11  
, 2 Robertsonian 1 (9.1%) positive -hCG  
, 1 Positive -hCG가 Duchene muscular  
dystrophy  
23 , 15 (Table 2, 4).  
39.6±4.1 , 249  
64 2.  
(3.0±1.5) 가 (25.7%)  
, 64 21 7 1 26 38 , 2  
(33.3%) positive -hCG 48 71 , 3

**Table 2.** Clinical outcome according to the PGD indications

	Reciprocal translocation	Robertsonian translocation	Aneuploidy screening	Sex chromosome abnormalities
No. of cycles	119	28	23	11
No. of patients	67	14	15	10
Mean age of patients	31.4 ± 3.9 <sup>a</sup>	30.7 ± 3.1 <sup>a</sup>	39.6 ± 4.1 <sup>b</sup>	29.6 ± 4.5 <sup>a</sup>
No. of retrieved oocytes	2,190	434	386	199
Fertilization rate	84.3±15.6	81.1±15.7	84.1±17.1	86.0±10.5
No. of biopsied embryos	1,431	254	271	115
Normal embryos rate (mean ± SD)	23.7±13.2 <sup>c</sup> (2.6 ± 1.8)	24.4±16.2 <sup>c</sup> (2.1 ± 1.7)	26.7±15.7 <sup>c</sup> (3.0 ± 1.1.5)	47.9±32.6 <sup>d</sup> (4.1 ± 2.9)
No. of ET cycles (%)	112 (94.1)	26 (92.9)	21 (91.3)	11 (100)
No. of embryos transferred (mean ± SD)	283 (2.5 ± 1.3) <sup>a</sup>	54 (2.1 ± 1.2) <sup>c</sup>	64 (3.0 ± 1.5) <sup>f</sup>	37 (3.4 ± 1.7) <sup>bf</sup>
No. of (+) -hCG cycles (% <sup>*</sup> )	29 (25.9)	4 (15.4)	7 (33.3)	1 (9.1)
No. of clinical pregnancies (% <sup>*</sup> )	23 (20.6)	3 (11.5)	3 (14.3)	1 (9.1)
No. of clinical abortions (% <sup>*</sup> )	5 (4.5)	0	1 (4.8)	0
No. of miscarriages (% <sup>*</sup> )	1 (0.9)	0	0	0
No. of deliveries (% <sup>*</sup> )	17 (15.2)	3 (11.5)	2 (9.5)	1 (9.1)

<sup>\*</sup> per embryo transfer.

<sup>a,b</sup>p<0.001, <sup>c,d</sup>, <sup>e,f</sup>p<0.05

55 72 10 6  
 30.2±3.1 , 32.6±4.6 , 33.0±5.3 2 5 1  
 3 1 (Table 5).  
 (p<0.05) , biopsy 46,XY,t(Y;15)(q21;p11)  
 (Table 3). 2.3±  
 1.4 , 2.5±1.4 , 2.9±1.3 , 46,XY,t(7;16)(q22;q22)  
 1 5 (13.9%), 2 10 (14.9%), 45,XO  
 3 26 (38.8%) positive -hCG 46,XX,t(11;22)(q23;q11)  
 , 3 1 2  
 (p<0.05) positive -hCG 47,XX,+der(22)t(11;22)(q23;q11)  
 (Table 3). 46,XY,t(7;13)(p21;q21.1)  
 3. 46,XX,t(7;13)  
 (p21;q21.1)  
 46,XX,t(2;3)(q12;q25)  
 12 16 92,XXXXX  
 25 balanced 46,XX  
 가 38  
 (Table 4). , 46,XY

**Table 3.** Clinical outcome of PGD according to the optimization laboratory system

	Phase I	Phase	Phase
No. of cycles	38	71	72
No. of patients	26	48	55
Mean age of patients	30.2 ± 3.1 <sup>a</sup>	32.6 ± 4.6 <sup>b</sup>	33.0 ± 5.3 <sup>b</sup>
No. of retrieved oocytes	594	1,266	1,349
Fertilization rate	81.8 ± 15.3	81.9 ± 17.1	87.0 ± 13.4
No. of biopsied embryos	355	790	926
Normal embryo rate	27.3 ± 18.8	25.1 ± 16.6	25.3 ± 15.6
No. of ET cycles (%)	36 (94.7)	67 (94.4)	67 (93.1)
No. of embryos transferred (mean±SD)	82 (2.3 ± 1.4) <sup>a</sup>	165 (2.5 ± 1.4) <sup>ab</sup>	191 (2.9 ± 1.3) <sup>b</sup>
No. of (+) -hCG cycles (% <sup>*</sup> )	5 (13.9) <sup>a</sup>	10 (14.9) <sup>a</sup>	26 (38.8) <sup>b</sup>
No. of clinical pregnancies (% <sup>*</sup> )	5 (13.9)	8 (12.0)	17 (25.4)
No. of abortion (% <sup>*</sup> )	0	3 (4.5)	3 (4.5)
No. of miscarriage (% <sup>*</sup> )	0	0	1 (1.5)
No. of deliveries (% <sup>*</sup> )	5 (13.9)	5 (7.5)	13 (19.4)

<sup>\*</sup>per embryo transfer.

<sup>a,b</sup>p<0.05.

**Table 4.** Karyotypes of patients and delivered babies

Patients' karyotype	Babies' karyotype	Pregnancy outcome
<b>Reciprocal translocation</b>		
46,XX,t(2;22)(q14.3;q13)	46,XY,t(2;22)(q14.3;q13)	Delivery
46,XX,t(5;15)(q22;q26)	46,XX	Delivery
46,XX,t(12;19)(q12;p11)	46,XX,t(12;19)(q12;p11)	Delivery
46,XY,t(9;17)(q21;p11.2)	46,XY,t(9;17)(q21;p11.2)	Delivery
46,XY,t(6;7)(q25.2;q21.3)	46,XY	Delivery
46,XX,t(7;18)(q32;q22.1)	46,XX & 46,XY	Delivery
46,XY,t(3;14)(q23;q32.2)	46,XX	Delivery
46,XX,t(2;22)(q14.3;q13)	46,XY	Delivery
46,XY,t(15;17)(q21;p12)	46,XY,t(15;17)(q21;p12) & 46,XX,t(15;17)(q21;p12)	Delivery
46,XY,t(8;14)(q21.3;q31),inv(9)(p11q13)	46,XY,t(8;14)(q21.3;q31),inv(9)(p11q13)	Delivery
46,XX,t(15;20)(q21.2;p12)	46,XX	Delivery
46,XY,t(1;17)(p34;q25)	46,XY	Delivery
46,XX,t(2;13)(q23;q34)	46,XY & 46,XY,t(2;13)(q23;q34)	Miscarriage (at 24 week)
46,XX,t(2;11)(q37.2;p12)	46,XX	Delivery
46,XY,t(7;16)(q22;q22)	46,XX	Delivery
46,XX,t(5;13)(q33;q22)	46,XX	Delivery
46,XX,t(14;18)(q24.2;p11.21)	46,XY,t(14;18)(q24.2;p11.21)	Delivery
46,XX,t(11;18)(p14;q23)	46,XY,t(11;18)(p14;q23)	Delivery
<b>Robertsonian translocation</b>		
45,XX,der(13;14)(q10;q10)	45,XX,der(13;14)(q10;q10)	Delivery
45,XX,der(13;14)(q10;q10)	45,XX,der(13;14)(q10;q10)	Delivery
45,XX,der(13;14)(q10;q10)	46,XX	Delivery
<b>Aneuploidy screening</b>		
46,XX	46,XY	Delivery
46,XX	46,XY	Delivery
<b>Sex chromosome abnormalities and X-linked disease</b>		
46,XX (Duchene muscular dystrophy)	46,XX	Delivery

**Table 5.** Cytogenetic analysis of the abortus from spontaneous abortion after PGD

Patients' karyotype	Karyotype of placenta
46,XY,t(Y;15)(q21;p11)	Not available
46,XY,t(7;16)(q22;q22)	45,XO
46,XX,t(11;22)(q23;q11)	47,XX,+der(22)t(11;22)(q23;q11)
46,XY,t(7;13)(p21;q21.1)	46,XX,t(7;13)(p21;q21.1)
46,XX,t(2;3)(q12;q25)	92,XXXXX
46,XX(Aneuploidy screening)	46,XY

biopsy unbalanced 가 Robersonian  
 11,12  
 PGD 50% balanced 가  
 8,9 PGD 2 6,13  
 biopsy pipette 1 2  
 biopsy 77% 가 unbalanced  
 pipette 1 16  
 biopsy 24  
 10 biopsy balanced 1  
 5 2 balanced  
 biopsy 46,XY,t(7;16)(q22;q22)  
 1  
 2 3 Carnoy 45,XO  
 pepsin  
 signal 3  
 (sequential medium)  
 가 46,XX,t(11;22)(q23;q11)  
 3 1 2 47,XX,+der(22)t(11;22)(q23;q11)  
 가 3:1  
 3:1 가  
 가 14



11q;22q 가 , 가 가  
 trisomy가 3:1 , 4~5 가  
 22 derivative [der(22)] ,  
 tertiary trisomy<sup>15,16</sup> 가  
 blastocyst  
 3:1  
 가 가 blastocyst  
 2:2 ,  
 signal  
 signal 181  
 unbalanced 가 X  
 46,XX,t(2;3)  
 (q12;q25) 1 biospy ( 1 ) , ( 2 ) ( 3 )  
 92,XXXX 1 ) , ( 2 ) ( 3 )  
 3, 18 , (81.8%, 81.9%, 87.0%), biopsy (97.5%,  
 가 4 signal 3 98.5%, 99.2%), (96.2%, 95.4%, 93.6%)  
 signal 4 가 , (30.2±3.1  
 signal 2 vs. 32.6±4.6, 33.0±5.3, p<0.05) 가  
 , (13.9%, 14.9% vs. 38.8%,  
 p<0.05)  
 mosaicism<sup>17,18</sup> ,  
 가 , 10  
 96.9% (31/32)  
 , 가 ,  
 가  
 comparative genomic  
 hybridization  
 5~6  
<sup>19,20</sup> 1. Gardner RL, Edwards RG. Control of the sex



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