

## 클라인펠터 증후군 환자에서 착상전 유전진단의 결과

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### Outcome of Preimplantation Genetic Diagnosis in Patients with Klinefelter Syndrome

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**Objectives:** Klinefelter syndrome is the most common genetic cause of male infertility and presents with 47,XXY mainly or 46,XX/47,XXY mosaicism. It is characterized by hypogonadism and azoospermia due to testicular failure, however, sporadic cases of natural pregnancies have been reported. With the development of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI), sperm can be retrieved successfully and ART is applied in these patients for pregnancy. It has been suggested that the risk of chromosome aneuploidy for both sex chromosome and autosome is increased in the sperms from 47,XXY germ cells. Considering the risk for chromosomal aneuploidy in the offspring, preimplantation genetic diagnosis (PGD) could be applied as a safe and more effective treatment option in Klinefelter syndrome. The aim of this study is to assess the outcome of PGD cycles by using FISH for sex chromosome and autosome in patients with Klinefelter syndrome.

**Materials and Methods:** From Jan. 2001 to Dec. 2003, PGD was attempted in 8 cases of Klinefelter syndrome but TESE was failed to retrieve sperm in the 3 cases, therefore PGD was performed in 8 cycles of 5 cases (four 47,XXY and one 46,XY/47,XXY mosaicism). In one case, ejaculated sperm was used and in 4 cases, TESE sperm was used for ICSI. After fertilization, blastomere biopsy was performed in 6~10 cell stage embryo and the chromosome aneuploidy was diagnosed by using FISH with CEP probes for chromosome X, Y and 17 or 18.

**Results:** A total of 127 oocytes were retrieved and ICSI was performed in 113 mature oocytes. The fertilization rate was  $65.3 \pm 6.0\%$  (mean  $\pm$  SEM) and 76 embryos were obtained. Blastomere biopsy was performed in 61 developing embryos and FISH analysis was successful in 95.1% of the biopsied blastomeres (58/61). The rate of balanced embryos for chromosome X, Y and 17 or 18 was  $39.7 \pm 6.9\%$ . The rate of aneuploidy for sex chromosome (X and Y) was  $45.9 \pm 5.3\%$  and  $43.2 \pm 5.8\%$  for chromosome

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17 or 18, respectively. Embryo transfer was performed in all 8 cycles and mean number of transferred embryos was  $2.5 \pm 0.5$ . In 2 cases, clinical pregnancies were obtained and normal 46,XX and 46,XY karyotypes were confirmed by amniocentesis, respectively. Healthy male and female babies were delivered uneventfully at term.

**Conclusion:** The patients with Klinefelter syndrome can benefit from ART with TESE and ICSI. Considering the risk of aneuploidy for both sex chromosome and autosome in the sperms and embryos of Klinefelter syndrome, PGD could be offered as safe and more effective treatment option.

**Key Words:** PGD, Klinefelter syndrome, Chromosome, TESE, ICSI

(hy-

pogonadism) 가 0.1~

0.2% 3.1% (fluorescent in situ hybr-

<sup>1,2</sup> 47,XXY , dization, FISH)

high grade aneuploidy (48,XXXY; 49,XXXXY )

47,XXY/46,XY , X

,

<sup>3</sup> ,

가 <sup>4</sup> ,

(testicular sperm extraction,

TESE) (intracytoplasmic sp-

erm injection, ICSI) 47,XXY/46,XY 가 3 5

47,XXY (47,XXY 4 47,XXY/46,XY 1 )

<sup>5-10</sup> , 8 1

(47,XXY) (ejaculated sperm)

가 , 4

<sup>11,12</sup> , 29.6±1.2 (mean ± SEM) ,

(preimplantation genetic diagnosis,

PGD) 1

,

2.

가 Gonadotrophin-releasing hormone

(GnRH) agonist (Superfact<sup>®</sup>, Serono, USA) recom-

binant FSH (Puregon<sup>®</sup>, organon, Gonal F<sup>®</sup>, Se-

rono, USA) human menopausal gonadotropin

(Pergonal<sup>®</sup>, Serono, USA)

,

<sup>13-15</sup> , 18 mm 가 3

human chorionic gonadotropin (hCG) 10,000

IU, hCG 36

10% synthetic serum substitute (SSS; Irvine Sci, Sant Ana, CA, USA)가 HTFM Gamete (Vitrolife, Sweden) 37, 5% CO<sub>2</sub>, 95% 3~4

(ICSI), 16~18

3. Percoll gradient centrifugation (Tunica vaginalis) (tubica albuginea) (seminiferous tubule)

0.4% BSA가 Ham's F10 medium (Sigma) ICSI 37, 5% CO<sub>2</sub>

4. 48 6~10, 1~2 EB10 G-PGD (Vitrolife) 20 μm pipette acidified Tyrode pipette

2 가 G2 10% SSS가 HTFM SEM) G1/G2 (Vitrolife), 61 0.6% BAS가 0.5% trisodium citrate 5 Slide glass, 58 FISH 가 95.1% FISH 가 39.7±6.9% (mean ± SEM) 8 가, 2.5±0.5

70, 85, 100% ethyl alcohol 2

5. FISH FISH probe X,Y 17 18 CEP (chromosome enumeration probe) FISH Probe 12 mm cover glass (Fisher Scientific), Slide glass rubber cement . 75 hot plate 5 denaturation humidified chamber 6~16 hy-bridization . Hybridization 50% formamide/ 2X SSC, 2X SSC, 2X SSC/0.1% NP-40 buffer 10, 10, 5, probe 125 ng/ml DAPI가 antifade mounting solution (Vysis) mounting (Optiphot-2, Nikon) FISH signal signal signal 가 signal 2 signal, 2 signal . 4

Table 1 127 76 (2 pronucleus) 65.3±6.0% (mean ± SEM) , 61 FISH 가 95.1% FISH 가 39.7±6.9% (mean ± SEM) 8 가, 2.5±0.5



**Table 2.** Chromosome abnormalities of embryos diagnosed by FISH in patients with Klinefelter syndrome

Case	cycle	karyotype	Origin of sperm	Abnormal FISH results
1	1	47,XXY	Fresh TESE	Monosomy 18, XO
2	1	47,XXY	Fresh TESE	Monosomy18 / monosomy 18,XO
2	2	47,XXY	Fresh TESE	Monosomy 18 / multiploidy
3	1	46,XY/47,XXY	Fresh TESE	Trisomy 17,Y / monosomy 17 / XXY / diploidy
3	2	46,XY/47,XXY	Fresh TESE	Monosomy 18 / aneusomy 18,XO / trisomy 18,XXX / trisomy 18,XYX
4	1	47,XXY	Thawing TESE	Chaotic / XYY / monosomy 18
5	1	47,XXY	Ejaculated sperm	Trisomy 18,XXX / XYY / aneusomy 18,XO
5	2	47,XXY	Ejaculated sperm	XXY / trisomy 18,XXY / monosomy 18,XO

가 0.9~2.5%,<sup>23</sup> 47,XXY 2.5~21.7%  
 47,XXY 가 ,<sup>24,25</sup> Estop<sup>26</sup>  
 16 , 17% 61%  
 1 non-disjunction  
 extra X가 hyperploidy rate 6.75~25%  
 (primordial germ cell)<sup>22,27</sup>  
 가 (degeneration), disomy 21 가 trisomy 21  
 Sertoli cell germ cell communication de-  
 fect<sup>17</sup> 47,XXY 가 ,<sup>28</sup> spermatid  
 가 , 가  
 , 가 ,<sup>4,18</sup> 46, 가 ,  
 XY/47,XXY 가 가  
 가 ,<sup>5,15</sup>  
 ,<sup>19</sup> 47,XXY 가  
 ,<sup>6,7</sup> ,<sup>29</sup>  
 가 21~54.5% 가  
 가 ,<sup>8,20</sup> 가  
 가 46,XY germ cell ,  
 , 47,XXY germ cell 가  
 가 ,<sup>21,22</sup>  
 24,XY, 24,XX hyperplod ,  
 1.5%

<sup>30</sup>, Silber <sup>12</sup> . FISH XY 17 18  
 PGD 18 21  
 가 ,<sup>15</sup>  
 (41.8% vs. 22.0%), 가  
 가 (26.5% vs.  
 53.0%) chaotic mosaicism 가 ,  
 centrosome , 가 ,  
 가 , 47,XXY 가 ,  
 가 , 가  
<sup>20</sup>,  
<sup>13-15</sup> Bielanska <sup>31</sup> 46,XXY/  
 47,XXY , hyperploidy가  
 3.9% X Y  
 가 chaotic embryo가  
 , chaotic  
 가 , centriole ,  
 centriole , chaotic  
 mitosis 가 ,  
<sup>32</sup>  
 39.7±6.9%  
 , Staessen <sup>15</sup>  
 77.2%  
 54%, X, Y, 18  
 53%  
 . Kahraman <sup>14</sup>  
 가 39.1% ,  
 55.5% haploidy, triploidy, 13, 18  
 trisomy monosomy .  
 (XXY, XXX, XYY ) 45.9%, 17  
 18 43.2%  
 가 , multiploidy chaotic aneu-  
 ploidy .

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