

# Outcomes of Preimplantation Genetic Diagnosis Cycles by Fluorescent *In situ* Hybridization of Infertile Males with Nonmosaic 47,XYY Syndrome

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## Abstract

**Background:** The 47,XYY syndrome could result in fertility problems. However, seldom studies reported comprehensive researches on the embryonic development and pregnancy outcomes of these patients. This study aimed to evaluate the clinical outcomes of nonmosaic 47,XYY patients performed with fluorescent *in situ* hybridization (FISH) and preimplantation genetic diagnosis (PGD) treatment.

**Methods:** This was a retrospective study. Between January 2012 and May 2017, 51 infertile males with nonmosaic 47,XYY syndrome underwent FISH-PGD were included in the study. According to sex chromosomal FISH results, embryos were classified as normal signal, no nuclei fixed, no signal in fixed nuclei, suspensive signal, and abnormal signal groups, respectively. The incidence of each group, the fixation rate, and hybridization rate were calculated. Embryonic development and pregnancy outcomes were also analyzed. The measurement data were analyzed with Student's *t*-test. The comparison of categorical data was analyzed with the Chi-square test and Fisher's exact test when expected cell count was <5.

**Results:** The 53 PGD cycles with 433 embryos were analyzed. The fixation rate was 89.6%, while the hybridization rate was 96.4%. There were 283 embryos with two sex chromosomal signals with clear diagnosis (65.4%). The numbers of no nuclei fixed, no signal in fixed nuclei, suspensive signal, and abnormal signal groups were 45 (10.4%), 14 (3.2%), 24 (5.5%), and 67 (15.5%), respectively. Embryos with abnormal signals were abandoned. The number of good-quality embryos was 210 (57.4%), including implanted embryos on day 4/day 5 and cryopreserved. The rates of good-quality embryos in the no nuclei fixed (22.2%), no signal in fixed nuclei (28.6%), and suspensive signal groups (33.3%) were comparable ( $P > 0.05$ ), and were significantly lower than the normal signal group (66.4%,  $P < 0.001$ ). The clinical pregnancy rates of fresh and frozen embryos transferred cycles were 70.6% and 85.7%, respectively.

**Conclusions:** Among embryos with a clear diagnosis of sex chromosome, about one-fifth showed abnormal signals. Embryos with two sex chromosomal signals are more likely to develop into good-quality ones. The application of the PGD by FISH may help to improve the clinical outcomes.

**Key words:** 47,XYY Syndrome; Fluorescent *In situ* Hybridization; Infertility; Pregnancy Outcome; Preimplantation Genetic Diagnosis

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## INTRODUCTION

The 47,XYY syndrome is characterized by an extra Y chromosome in a man's cells. It is the most common aberration of sex chromosome after Klinefelter syndrome (47,XXY),<sup>[1,2]</sup> as found in 1 out of 1000 male live births.<sup>[3]</sup> The 47,XYY syndrome could show different influences on men's

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sperm counts, ranging from normal to even azoospermia.<sup>[4]</sup> For those who have difficulty achieving pregnancy, the treatment of *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with medical genetics counseling seems to be a good choice. Meanwhile, some researchers recommended the potentially preimplantation genetic testing to realize the potential risks to their offspring.<sup>[4,5]</sup>

The 47,XYY syndrome could result in high frequency of embryo aneuploidy, while preimplantation genetic diagnosis (PGD) by fluorescent *in situ* hybridization (FISH) is helpful for selecting and transferring chromosomally normal embryos.<sup>[3]</sup> However, seldom studies reported comprehensive researches on the embryonic development and pregnancy outcomes of these patients. Therefore, this study aimed to investigate the results of sex chromosomal of embryos performed with PGD by FISH and analyze the pregnancy outcome of patients with 47,XYY syndrome.

## METHODS

### Ethical approval

The study was approved by the Institutional Review Board of Center for Reproductive Medicine, Shandong University. As a retrospective study and data analysis were performed anonymously, this study was exempt from the informed consent from patients.

### Study population

This is a retrospective study. A total of 51 infertile males with nonmosaic 47,XYY syndrome who underwent FISH-PGD treatment from January 2012 to May 2017 were included in the study. All patients underwent ICSI treatment due to poor sperm quality (sperm concentration <1.0 million/ml).

### Data collection

ICSI was applied to all MII oocytes. After 18–20 h observation, fertilization was determined by the presence of pronuclei. The zygotes were transferred to drops of G-1 (Vitrolife, Sweden) for culture. The embryo cleavage of the two pronuclear oocytes was evaluated 41–44 h (day 2) and 65–68 h (day 3) after ICSI. The embryos were graded as high quality when the number of blastomeres was between 6 and 10; additionally, all of the blastomeres had equal or almost equal size, and the percentage of anucleated fragments was  $\leq 25\%$  on day 3, blastomere biopsies were performed on embryos with  $\geq 6$  cells, and grade better than 2' according to Puissant's criteria on day 3.<sup>[6,7]</sup>

A total of 433 embryos were biopsied 69–72 h after insemination. Biopsied embryos were rinsed repeatedly and placed individually in extended microdroplet culture for potential embryo transfer pending FISH analysis. One blastomere with clear nucleus was biopsied from each embryo (6–10 cells). The isolated blastomeres were individually placed on a glass slide. The fixation and hybridization procedure was conducted as reported previously.<sup>[8,9]</sup> FISH was performed on fixed blastomeres using probes specific for chromosomes X and Y. The samples were assessed at  $\times 1000$

magnifications with fluorescence microscope (OLYMPUS BX53; OLYMPUS CORPORATION; Japan). The image information was recorded and analyzed by FISH analysis software (VideoTesT-FISH 2.1; VideoTesT Ltd.; Russia).

According to the sex chromosomal results, the embryos were classified as normal signal (with clear XX or XY signals diagnosed, Figure 1a), no nuclei fixed [Figure 1b], no signal in fixed nuclei [Figure 1c], suspensive signal (without clear signals diagnosed, Figure 1d), and abnormal signal groups (single, triple, or more signals, Figure 1e), respectively. The incidence of each group, the fixation rate, and hybridization rate were calculated. Embryos with abnormal signals were abandoned on day 4, while the rest were either transferred into the uterine cavity on day 4/day 5 or continued to cultivate the blastocyst for cryopreservation by means of vitrification. Blastocyst scores ( $\geq 4$ ) were used for embryo transfer or cryopreservation.<sup>[10]</sup> The embryonic development and pregnancy outcomes of each group were also analyzed. Moreover, we also evaluated the association between the females' age and the abnormal signal rate.

Clinical pregnancy was defined as the presence of a gestational sac in the uterine cavity at 35 days after embryo transfer, as detected on ultrasonography. Ongoing pregnancy was defined as the presence of a fetus with heart motion at 11–12 weeks of gestation.<sup>[11]</sup> All pregnancy outcomes were obtained through review of medical records.

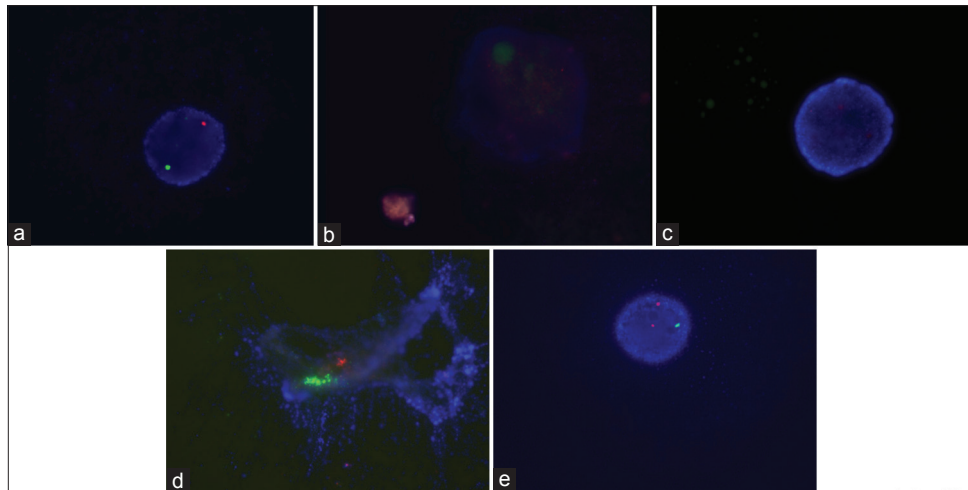
### Statistical analysis

Data analysis was performed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were shown as a mean  $\pm$  standard deviation (SD) and analyzed with Student's *t*-test. The comparison of categorical data was analyzed with the Chi-square test and Fisher's exact test when expected cell count was  $< 5$ . A two-sided  $P < 0.05$  was considered statistically significant.

## RESULTS

The mean age of the females and males was  $28.9 \pm 4.2$  years and  $28.8 \pm 3.9$  years, respectively. There were 53 PGD cycles with 433 embryos analyzed in total. Moreover, there were 283 embryos with two sex chromosomal signals (normal signal group) with clear diagnosis (65.4%). The number of no nuclei fixed, no signal in fixed nuclei, suspensive signal, and abnormal signal groups was 45 (10.4%), 14 (3.2%), 24 (5.5%), and 67 (15.5%), respectively. When we confined the embryos to which with a clear diagnosis of the sex chromosomal signals, the percentage went up to approximately one-fifth of 350 embryos (19.1%, 67/350). The fixation rate of the FISH-PGD was 89.6% (388/433), while the hybridization rate was 96.4% (374/388).

After diagnosis and cultivation, embryos with abnormal signals were abandoned. The number of good-quality embryos was 210 (57.4%, 210/366) including implanted embryos on day 4/day 5 and cryopreserved ones. The rates of good-quality embryos in the no nuclei fixed (22.2%),



**Figure 1:** The embryos were classified as normal signal (a), no nuclei fixed (b), no signal in fixed nuclei (c), suspensive signal (d), and abnormal signal groups (e).

no signal in fixed nuclei (28.6%), and suspensive signal groups (33.3%) were comparable ( $\chi^2 = 1.029$ ,  $P > 0.05$ ) and were significantly lower than the normal signal group (66.4%,  $\chi^2 = 42.650$ ,  $P < 0.001$ ).

In total, 42 cycles got clinical pregnancy among 55 transfer cycles with embryos available (76.4%). Moreover, there were 34 fresh embryo transfer cycles with 24 clinical pregnancies (70.6%) while 21 frozen cycles with 18 clinical pregnancies (85.7%). Thirty births were obtained, with a total of 41 infants born. There were also eight ongoing pregnancies. Miscarriages occurred in four cycles, while biochemical pregnancy happened in three cycles. Seven cycles did not obtain clinical pregnancy, and one of them failed to perform embryo transfer due to bad embryos after FISH procedure. Another female has not undergone transfer because she was inadequate for fresh embryo transfer. Women  $\geq 35$  years old obtained significantly higher abnormal signal rate (10/26, 38.5%) than those  $< 35$  years (57/407, 13.9%,  $\chi^2 = 6.911$ ,  $P = 0.018$ ).

## DISCUSSION

Men with a 47,XYY karyotype are generally fertile.<sup>[12]</sup> However, the association between 47,XYY and fertility problems has been reported by several studies with an increased incidence of chromosomally abnormal spermatozoa of men with 47,XXY syndrome.<sup>[13,14]</sup> The extra Y chromosome showed an increased risk to deliver to the offspring due to the high prevalence of hyperhaploid sperm.<sup>[15]</sup> A recent study demonstrated a higher frequency of aneuploid sperm of 37.2–37.8%, with approximately one-half of the abnormalities caused by sex chromosomal aneuploidy. Moreover, they found that the frequency of embryos with aneuploidy was 32%.<sup>[5]</sup> What's more, Zouli *et al.*<sup>[3]</sup> reported that the frequency of aneuploidy in the embryos was high (about 83%), and they thought that it might be the cause of the recurrent first trimester miscarriages. However, the sample was very small as they

got only six embryos analyzed. More cases should be provided to illustrate this issue.

Despite the XYY karyotype, spermatogenesis in affected men results mainly in 23,X and 23,Y sperm as the supernumerary Y is eliminated during meiosis. Since many 47,XYY men have normal semen parameters, the severe oligozoospermia observed in these men might indicate more perturbations during meiotic pairing, subsequent loss of germ cells, and the production of aneuploid sperm. Moreover, there also have been reported cases of infertile men with severe oligoasthenoteratozoospermia or even azoospermia. Patients with low semen parameters may require further assisted reproductive techniques to achieve pregnancy. Genetic evaluation is recommended before proceeding.<sup>[4]</sup> Therefore, it is necessary to validate the prevalence of the sex chromosome abnormalities in XYY men. Unfortunately, we did not have the opportunity to study sperm for aneuploidy in the present case. Then, we focused on the sex chromosomal results. In the current study, we sought to assess the prevalence of the normal and abnormal embryos performed with PGD by FISH and whether this approach could do good to the clinical outcomes of the couples.

There was a slightly high rate of abnormal embryos in this study. About 15.5% of all detected embryos showed abnormal signals. When we confined the embryos to which with clear diagnosis, the percentage went up to approximately one-fifth of 350 embryos (19.1%). This rate was even higher than that of Klinefelter patients. Ni *et al.*<sup>[16]</sup> reported that there were 90 embryos with two sex chromosomal signals among 103 embryos with clear diagnosis (87.4%) in the ICSI-PGD cycles, which meant that about 12.6% of the embryos realized abnormal. We considered this might due to that the XYY patients have an increased incidence of diploid spermatozoa than the XXY patients.<sup>[17]</sup> Attention should be paid to the patients with 47,XYY syndrome in the clinic when performing with IVF/ICSI as they might have more

abnormal embryos. Therefore, the PGD treatment seems to be a good approach for these patients.

FISH was among the first and has been the most widely used technology to screen for aneuploidy in human embryos. Initially, FISH was employed because it can be rapidly performed on single cells at interphase. Researchers demonstrated that blastomeres from day 3 embryos with better morphological quality had higher nucleus spreading rate and higher full signal rate during FISH.<sup>[7]</sup> However, some researchers hold the view that cleavage-stage FISH technology is poorly predictive of aneuploidy in morphologically normal blastocysts. Our results showed that embryos with two sex chromosomal signals with clear diagnosis were more likely to develop into good-quality embryos than those in the no nuclei fixed, no signal in fixed nuclei, and suspensive signal groups.

The clinical pregnancy rate of either fresh or frozen embryo transferred cycles was high in the current study (70.6% and 85.7%, respectively). We thought that several reasons might lead to this result. First, we demonstrated that women who were  $\geq 35$  years old obtained significantly higher abnormal signal rate (10/26, 38.5%) than those  $< 35$  years (57/407, 13.9%). However, the majority of these females in the current study were quite young with the mean age of  $28.9 \pm 4.2$  years, and there were only four women elder than 35 years. This might reduce the incidence of adverse pregnancy outcomes. Second, embryos on day 4/day 5 or with cryopreservation were transferred in this research. There was moderate quality evidence for clinical pregnancy that fresh blastocyst stage transfer was associated with higher rates than fresh cleavage stage transfer.<sup>[18]</sup> What's more, all the patients were performed with PGD treatment, and this might improve the pregnancy outcomes. Therefore, we obtained higher clinical pregnancy rate than studies reported before.

There were several limitations/weaknesses in the current study. First, we had no control group of patients with normal karyotype because seldom would agree to perform PGD treatment as they seemed to be normal. Second, we did not study the autosomal conditions which might underestimate the abnormal rate of the embryos. Third, as the embryos diagnosed as abnormal on day 4 were all abandoned, we failed to obtain the embryonic development results of these embryos. Therefore, further study will be performed to assess whether men with 47,XYY syndrome would have autosomal abnormalities other than the sex chromosome abnormalities and the association between the PGD results with microarrays and the embryonic development.

We did a comprehensive study on the clinical outcomes of patients with nonmosaic 47,XYY syndrome who underwent FISH-PGD treatment. Moreover, in this research, we focused on the sex chromosomal signals of the embryos and their development outcomes. We considered that the application of the PGD by FISH could show optimistic results in the clinical outcomes. However, whether PGD is essential for

these patients still need consideration since the economic condition may be a challenge. If PGD is not performed, active prenatal examination will be demanded.

## Statement

Our hospital is qualified to conduct PGD, which is performed in strict accordance with relevant laws and regulation as well as professional standard and expert consensus. The Ethics Committee of Center for Reproductive Medicine, Shandong University has approved the standardized procedure for PGD. The embryos to be implanted are selected under the indication of PGD to reduce the risk of miscarriage and birth defect. Patients who meet the indication are provided with the choice for PGD and those opt for PGD should sign informed consent after fully informing of the advantages and disadvantages. Therefore, we consider it is in the best interests of patients under the PGD criterion, and the PGD is our preferred treatment since the patients may have their healthy infants while reducing social burden of birth defect.

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## Conflicts of interest

There are no conflicts of interest.

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# 非嵌合型47, XYY综合征不育男性采用荧光原位杂交技术行胚胎植入前诊断助孕治疗的结局

## 摘要

**研究背景:** 47, XYY综合征可以造成生育困难。然而,很少有研究深入、完整地报道这类患者的胚胎发育情况及妊娠结局。

本研究旨在评估非嵌合型47, XYY综合征不育男性采用荧光原位杂交技术行胚胎植入前诊断助孕治疗的结局

**方法:** 本研究为回顾性研究,从2012年1月至2017年5月间,共纳入51例行FISH-PGD治疗的非嵌合型47, XYY综合征不育男性。依据性染色体的FISH检测结果,将胚胎分为正常信号组、无核组、无信号组、未确定组以及异常信号组。统计FISH的固定率及杂交率,统计各组占比。同时,分析胚胎发育情况及临床妊娠结局。

**结果:** 本研究共分析53个PGD周期的433个胚胎。固定率为89.6%,杂交率为96.4%。共有283个胚胎明确出现2个性染色体信号(占65.4%)。无核组、无信号组、未确定组以及异常信号组的胚胎数分别为45(10.4%)、14(3.2%)、24(5.5%)及67(15.5%)。放弃异常信号的胚胎,将剩余胚胎继续培养。总体优质胚胎数为210(57.4%),包括移植胚胎及冷冻保存的胚胎。无核组、无信号组、未确定组的优胚率无明显差异,分别为22.2%、28.6%及33.3% ( $P>0.05$ ),均显著低于正常信号组(66.4%,  $P<0.001$ )。鲜胚移植周期和冻胚移植周期的临床妊娠率分别为70.6%及85.7%。

**结论:** 在所有性染色体明确诊断的胚胎中,有近五分之一的胚胎出现异常信号。出现正常性染色体信号的胚胎发育为优质胚胎的几率更大。应用FISH-PGD技术可以改善非嵌合型47, XYY综合征患者的生育结局。