

A patient with non-mosaic 47, XXY karyotype fathering a normal healthy infant using intracytoplasmic sperm injection (ICSI) - a case report

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Summary

Background: Klinefelter's Syndrome (KS) is rarely reported in China, which is associated with insufficient sperm production and infertility in male patients. Despite the fact that infertility treatments, such as intracytoplasmic sperm injection (ICSI) and preimplantation genetic diagnosis (PGD), have been widely used in KS population, almost all these patients have to use donor semen in China to establish successful pregnancies nowadays. **Case Report:** The authors report a case of KS in a patient with unremarkable characteristics except for oligospermia. The patient presented to this center with infertility and the chromosome analysis demonstrated a non-mosaic 47, XXY karyotype. Further testing showed no deletions in the *SRY*, *AZF-a*, *AZF-b*, and *AZF-c* genes. Finally, the patient successfully impregnated the partner, and then the partner delivered a healthy male neonate. The patient became fertile through ICSI, together with cryopreservation of a small number of spermatozoa after the first blastocyst transfer. **Conclusion:** This report further confirms that KS men can father their own healthy children. While adequate sperm cryopreservation and blastocyst transfers are strongly recommended. Nevertheless, it is necessary for such couples to be offered extensive genetic counseling before pregnancy and prenatally.

Key words: Klinefelter's Syndrome; ICSI; Semen preservation; Blastocyst transfer.

Introduction

Klinefelter's Syndrome (KS), originally described in 1942 by Klinefelter *et al.* [1], is characterized by hypogonadism, azoospermia, or oligospermia [2]. It is a male chromosomal disorder, with a prevalence of approximately 1:600 live-born males. The etiology is that at least one extra X chromosome is added to the normal 46, XY male karyotype [3]. The incidence of the classic type of 47, XXY is up to 80% in KS cases. The characteristics depend on the number of cells with an additional X chromosome. It is found that the more the number of the germ cells with X chromosomes, the greater the degree of feminization, and the severer the fertility-associated problems, ranging from azoospermia to various grades of testicular insufficiency [4].

With the rapid development of assisted reproductive technology, intracytoplasmic sperm injection (ICSI) makes bearing offspring possible in males who could produce one sperm. In recent years, the advances of sperm freezing technology for a small amount of sperm [5] have helped avoid the damage caused by testicular sperm aspiration (TESA) and testicular biopsy, further expanding the scope of ICSI treatment. Although studies have reported normal offspring of KS patients using ICSI [6, 7], KS patients with fertility problems face an enormous challenge in China, due to low

incidence of KS in China and few successful cases in history. And, almost all of these patients adopt donor semen. Therefore, it is of significant that, in this paper, the authors report the first case of a patient in China with non-mosaic 47, XXY KS, who acquired successful ICSI, together with cryopreservation of a small number of human spermatozoa, accompanied by blastocyst transfer, instead of using TESA.

Case Report

A 28-year-old man, 1.74 m in height and 80 kg in weight, who rejected donor semen at another reproductive center, and had no family history of medical diseases, presented to the department of assisted reproduction of the Ninth People's Hospital of Shanghai Jiaotong University School of Medicine in 2013. Medical examination revealed normal pubic hair distribution and limb muscles, and no gynecomastia or feminization; however, his facial hair was scarce, and he lacked laryngeal prominence. He denied any history of drinking ethanol or smoking. The volumes of the right and left testis were 3.9 ml and 3.1 ml, respectively. The penile length was 6 cm. Three semen analyses with an average volume of 1.2 ml showed severe oligospermia, according to the fifth edition of the standard semen analysis by the World Health Organization [8]. Chromosome analysis showed a 47, XXY karyotype in 20 peripheral blood

lymphocytic cells and a G-band karyotype was identified. Semen analysis of DNA ploidy and a cell check indicated that the proportion of 2N cells in the semen was 95.8%. Further testing showed no deletions in the *SRY*, *AZF-a*, *AZF-b*, and *AZF-c* genes. Clinical diagnosis primarily focused on the hypoplasia of the seminiferous tubules of the testis and KS. The patient was offered ICSI using his own spermatozoa. Sufficient semen was frozen prior to the controlled ovarian stimulation of his wife to avoid egg aging. The patient's first partner was a 27-year-old healthy woman with normal ovulatory cycles. Ovarian stimulation was applied only one time, with a combination of clomiphene citrate and human menopausal gonadotropin (hMG). Stimulation was terminated by the subcutaneous injection of gonadotrophin-releasing hormone agonist for oocyte maturation. Oocytes were collected after 34.5 hours. Seventeen oocytes were collected for fertilization that was subsequently carried out by ICSI. Embryos were examined for the number or regularity of blastocysts and the degree of fragmentation, which was graded according to the criteria of Cummins *et al.* [9]. All genetically normal embryos of sufficient morphological quality were cryopreserved within three days of oocyte retrieval. The non-top-quality embryos were placed for further extended culture until the blastocyst stage and good-morphology blastocysts were frozen.

In the subsequent frozen embryo transfer (FET) cycle, two day-three embryos (grade 1 and 8-cell and grade 2 and 9-cell) were transferred, resulting in a pregnancy. However, spontaneous abortion occurred in three months. A routine progesterone-primed ovarian stimulation (PPOS) protocol [10] was advised for the second partner. All ICSI programs were the same as those in the first attempt. Thirteen ova were obtained for subsequent ICSI fertilization. There was only one cleavage-stage grade 1 and 7-cell embryo, and the remaining lower quality embryos were allowed to develop into two blastocysts. In the following FET cycle, the authors took into account the spontaneous abortion that occurred after the transfer of a two cleavage-stage embryo in the first partner. And finally, one blastomere was successfully transferred. A full-term healthy boy with a normal karyotype (46, XY) without PGS was born. The birth height and weight were 50 cm and 3,265 grams, respectively. During the pregnancy, non-invasive prenatal testing showed 46, XN, low-risk. However, PGD was performed in the remaining four embryos with insufficient quality. The results were as follows: -2 (x1), -5 (x1), -13 (X1, mosaic), -16 (x1), multiple chromosomal abnormalities; 45, XN, -4 (x1); 46, XN; 47, XN, -5q (Q33.1- QTER, ~30M, X1), +16 (x3).

Discussion

The findings in present case are significant for China, given the low incidence of KS and the fact that it is difficult for KS patients to father biological children even with the advent of ART approaches. In recent years, significant progresses have been achieved in the elucidation of the pathophysiology and the treatment of KS, including advance-

ments in testicular microsurgery, sperm freezing technologies for a small number of sperms, ICSI, and X-inactivation [11]. As a result, patients with KS can now become biological fathers. The premise of these techniques is that there is focal spermatogenesis in the patient's testis. In addition, focal spermatogenesis and severe oligo-zoospermia have been reported in non-mosaic 47, XXY individuals. The development of a single-sperm freezing technique in this center has helped patients avoid the risk of multiple testicular punctures. Furthermore, studies have shown that there is no significant difference of the clinical pregnancy and live birth rates using fresh and frozen semen in KS patients [12]. In this case, ICSI, together with this technology, allows the patient to obtain a good outcome.

Although fathering biological children for KS patients has become possible, a concern for many patients is the potential inheritance of genetic abnormalities in offspring born through ICSI. Since ICSI itself can increase the risk of genetic abnormalities in offspring. Fortunately, several studies have suggested that ICSI does not increase the risk of birth defects [13]. Moreover, with the advent of PGD, physicians can decide whether PGD needs to be performed in such cases or not. The experience of combining ICSI with PGD demonstrates that there has been a significant drop in the rate of normal karyotype embryos in the cases with KS. Therefore, the identification of abnormalities in morphologically good-quality embryos prevents inheritance of abnormalities, thus improving clinical pregnancy outcomes. However, other authors have argued that PGD might affect the potential development of the embryos which are generated after ICSI [14], possibly affecting the implantation rate. The present patient achieves a good pregnancy outcome without PGD. Initially, PGD is not approved in this center, and the authors doubt that PGD could accurately detect blastocyst chromosomes as limited cells are examined in this technique. The authors believe that there is an advantage in transferring blastocysts that it performs like natural process. Nevertheless, the successful cases are limited in the literature, including published journals, case reports and studies of small sample, due to low incidence of KS, thus, more cases are required to confirm this.

In the present case, karyotype examination showed that only 20 nuclear types could be identified in the peripheral blood. While it was reported that at least 50 nuclear types can be detected without missing a chimeric type diagnosis [15]. Generally, it is assumed that larger the number of the cells that are detected is, the more accurate the detection rate will be. According to the couple's blastocyst embryo chromosomes that were of inferior quality, the authors speculate that there may be two different germ cell lines in KS patients: a cell line derived from spermatogonia with normal chromosomes and the other line displaying aneuploidy. Therefore, such patients may easily have a 47, XXY karyotype or may be chimeric. Although the karyotype detected from peripheral blood lymphocytes remains

the gold standard for diagnosing KS and no mosaicism in the present patient, the effect of mosaicism of the germ cell line could not be excluded. Thus, this patient may not be representative of the KS population as a whole.

Conclusions

KS patients are no longer absolutely infertile and are capable of conceiving healthy children, which should be considered as a breakthrough. Generating sufficient frozen sperm by using a sperm freezing technology for ICSI is highly recommended. The authors also suggest that all cleavage-embryos should be cultured to the blastocyst stage, and thereafter, allowed to progress according to their natural course [16]. Although healthy newborns from KS patients have been reported, prenatal diagnosis should be suggested for such couples. However, new therapeutic approaches for KS remain to be further explored in the near future.

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