

·Case Report·

Birth after intracytoplasmic sperm injection of ejaculated spermatozoa from a man with mosaic Klinefelter's syndrome

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Abstract

Aim: To report a birth after intracytoplasmic sperm injection (ICSI) of ejaculated spermatozoa from a man with mosaic Klinefelter's syndrome detected by fluorescence *in situ* hybridization (FISH) analysis. **Methods:** A 35-year-old man with a normal appearance consulted our hospital because of sterility over a 5-year period. Chromosome analysis showed low-incidence mosaic Klinefelter's syndrome. Using FISH, 96 % hyperploidy of the lymphocytes was found. We examined the sex chromosome of the ejaculated spermatozoa. Using FISH, we examined 200 ejaculated spermatozoa and no hyperploidy was found. **Results:** The 33-year-old female partner of the male patient underwent an uncomplicated controlled ovarian hyperstimulation sequence using a combined recombinant-follicle stimulating hormone (rec-FSH) + human menopausal gonadotrophin (hMG) protocol, following late luteal phase pituitary down regulation. This culminated in the retrieval of seven oocytes, six of which were fertilized with ICSI. One ICSI attempt led to clinical pregnancy with a healthy baby girl. **Conclusion:** We report a male patient with low-incidence mosaic Klinefelter's syndrome whose ejaculated spermatozoa were identified as being haploid by FISH before ICSI, leading to the successful pregnancy of his wife and the birth of a healthy baby girl. (*Asian J Androl* 2005 Jun; 7:217–220)

Keywords: Klinefelter's syndrome; intracytoplasmic sperm injection; fluorescence *in situ* hybridization

1 Introduction

With an incidence of about one in 600 newborn boys, Klinefelter's syndrome is one of the most common sex chromosomal abnormalities in humans and usually is a form of hypergonadotropic hypogonadism and infertility

resulting from a supernumerary X chromosome (47, XXY). Some men with non-mosaic, or complete Klinefelter's syndrome have azoospermia and only a few have spermatogenesis. In 11 % of azoospermic patients, testicular failure was caused by Klinefelter's syndrome [1] due to a numeric sex chromosome aberration (47, XXY) explained by meiotic non-disjunction[2]. About 15 % are mosaic cases, usually with two cell lines: 47, XXY/46, XY. The others are considered non-mosaic, upon cytogenetic examination of somatic cell lines. In these individuals, the testicular tubules become fibrotic and hyalinized, the tubule lumen is gradually obliterated and germ cells disappear with time. In the adult XXY

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testes, virtually all germ cells disappear [3]. Occasionally, single foci of spermatogenesis do exist in the testes of Klinefelter's syndrome [4, 5], explaining the cases of sperm production and presence in the ejaculate [5, 6]. Indeed, in non-mosaic Klinefelter's syndrome, pregnancies have been reported using intracytoplasmic sperm injection (ICSI) with ejaculated spermatozoa [7, 8]. In other cases, ICSI using testicular spermatozoa retrieved surgically is the sole mode of treatment to be offered, besides sperm donation, as no mature, viable spermatozoa may be found in the ejaculate.

To date, the births of 19 neonates have been reported following ICSI with the use of spermatozoa from patients with non-mosaic Klinefelter's syndrome [6, 7, 9–12]. Many neonates were normal, but XXY neonates were also seen.

We report a male patient with a low-incidence mosaic Klinefelter's syndrome, whose ejaculated spermatozoa were identified as being haploid by FISH before ICSI, leading to the successful pregnancy of his wife and the birth of a healthy baby girl.

2 Case report

2.1 Patient

A 35-year-old man with a normal appearance consulted our hospital because of sterility over a 5-year period. A physical examination revealed normal hair distribution and no gynecomastia. The volume of the right testis was 5 mL and that of the left, 7 mL. Two semen analyses with an average volume of 0.15 mL \pm 0.05 mL showed severe oligozoospermia. Retrograde ejaculation did not exist. Average sperm concentration was 4.0×10^5 mL. Total motility of the sperm was 33 % (forward progressive motility was about 10 %). Morphology was not precisely assessed. Hormonal analysis was performed showing a follicle-stimulating hormone (FSH) concentration of 21.2 mIU/mL (normal 2.9–8.2), luteinizing hormone (LH) concentration of 9.2 mIU/mL (normal 1.8–5.2), testosterone at 2.0 ng/mL (normal 3.2–10.3) and prolactin 14.0 ng/mL (normal 1.5–9.7). The patient's wife was a 33-year-old healthy woman with normal ovulatory cycles and a normal hysterosalpingography.

2.2 Fluorescence in situ hybridization (FISH) analysis

A triple colour FISH with centromeric DNA probes (Vysis Inc., Downers Grove, IL, USA) for chromosome

18 (Spectrum aqua), X (spectrum orange) and Y (spectrum green) was used to determine the sex chromosome constitution of interphase lymphocytes and spermatozoa. Prior to FISH, sperm nuclei were decondensed by slide incubation in a solution of 5 mmol/L dithiothreitol (DTT) and 1 % Triton X-100. In order to determine the sex chromosome composition, FISH using a combination of probes was used (Vysis Inc.).

Analyses were done using an Olympus BX60 epifluorescence microscope equipped with specific filter sets for FITC, Texas Red, Aqua and a multiband pass filter for DAPI/Texas Red/FITC.

2.3 ICSI attempt

ICSI attempts were made for the couple. Ovarian stimulation was achieved through a combination of gonadotrophin-releasing hormone (GnRH agonist, Suprecur nasal; Hoechst, Tokyo, Japan), purified FSH (Fertinorm P; Serono, Tokyo, Japan) and human menopausal gonadotrophin (hMG, Humegon; Sankyo, Tokyo, Japan). When the leading follicle reached a mean diameter of 16 mm, 10000 units of human chorionic gonadotrophin (hCG, Profasi; Serono, Switzerland) were administered. Vaginal ultrasound-guided follicle puncture took place 35 h after hCG injection. Seven oocytes were retrieved and cultured in Quinn's Advantage Fertilization Medium (Sage BioPharma, Inc., Pasadena, CA, USA). The cumulus corona cells were initially removed by exposure to 60 IU/mL of hyaluronidase for up to 1 min.

On the day of transvaginal oocyte aspiration, the husband produced fresh ejaculated sperm. The semen was washed twice in human tubal fluid medium by centrifugation at $300 \times g$. As the sperm count was small, the centrifuged sperm pellet was made to float with 30 μ L of fertilization medium and placed on the dish. The sperm which moved out were used for ICSI. Motile sperm were inseminated into each culture dish containing 1 mL of culture medium and one oocyte. One moving sperm was immobilized by rubbing its tail with a sperm-injection needle under a phase-contrast microscope with Hoffmann modulation. The immobilized sperm was aspirated into the injection needle and injected into the six metaphase II oocyte. The inseminated six oocytes were washed twice with human tubal fluid medium, transferred into another culture dish and then cultured for 20 h. Twenty hours after ICSI, oocytes were observed under a dissecting microscope and the number of pronuclei

was determined. Oocytes that had two pronuclei were defined as fertilized and cultured in Quinn's Advantage Cleavage Medium (Sage BioPharma, Inc., Pasadena, CA, USA) for an additional 24 h. Three good quality four-cell embryos were transferred into the uterine cavity of the patients 2 days after oocyte retrieval according to the guidelines for ET of the Japan Society of Obstetrics and Gynecology. Pregnancy was defined as the presence of a fetus detected by vaginal ultrasonography 4 weeks after ET.

Two supernumerary blastocysts were not cryopreserved because of the couple's intention.

At first, we examined 114 mitosis and 111 (97.4 %) XXY cells and three (2.6 %) XY cells were observed in the metaphase. We could not determine whether this case was mosaic or not. So we more precisely examined 1000 lymphocytes to determine the sex chromosome constitution of interphase using FISH procedure. Nine hundred and sixty-one (96.1 %) XXY cells and 39 (3.9 %) XY cells were observed. So we thought that this case was a low-incidence, mosaic Klinefelter's syndrome. Using FISH, we examined 200 ejaculated spermatozoa and no hyperploidy was found. We estimated that the risk of this parent having a child with Klinefelter's syndrome after an ICSI procedure was minimal. One ICSI attempt led to clinical pregnancy and a healthy baby girl.

Since consent could not be obtained from the baby's parents, neither prenatal nor postnatal diagnosis could be performed.

3 Discussion

With the introduction of ICSI, a patient with Klinefelter's syndrome has an increased chance of fathering a child. The risk of transmission of gonosomal aneuploidy using spermatozoa from a non-mosaic Klinefelter's syndrome patient is various and many reports have been published. Up to now, the risk has been considered low. This is not surprising, as it has been demonstrated recently that 47,XXY spermatogonia are capable of undergoing meiosis and completing the spermatogenic process, culminating in the formation of cytogenetically normal spermatozoa [13, 14].

Among patients with non-mosaic Klinefelter's syndrome, the rate of haploid spermatozoa varied from 92.25 % from an analysis of 2206 spermatozoa from one case [15], to 84.63 % and 76.47 %, the scoring of

10 000 spermatozoa from two patients [16]. While sex chromosomal hyperploidy has been found with an incidence of 0.9-2.5 % in the mosaic form and its incidence in the non-mosaic form has varied from 2.52 % [17] to 3.48 % [15] to 21.76 % [16].

The other report showed the result of multicolor FISH and chromosome painting in premeiotic cells, pachytenes, post-reductional cells (secondary spermatocytes or spermatids) and spermatozoa from an XXY, a mosaic XY/XXY and XYY male. It showed that probably all Klinefelter males who produce spermatozoa in any numbers are XY/XXY mosaics, taking into account that XXY cells are meiotically incompetent. So the risk of ICSI in apparently non-mosaics may be lower than expected [18].

However, another report showed that a 47,XXY fetus was conceived by a couple, the husband of which had non-mosaic Klinefelter's syndrome and the conception was prenatally diagnosed as 47,XXY and terminated [19].

In this case, we evaluated the aneuploidy rate of ejaculated spermatozoa to improve the precision of genetic counseling. All of the 200 spermatozoa that were analyzed by FISH for sex-chromosome ploidy were euploid.

In clinical practice, Klinefelter's syndrome has been treated by ICSI or TESE-ICSI using ejaculated spermatozoa, and over 10 studies have reported cases of pregnancy and delivery. This case suggests that the risk of ICSI in mosaic Klinefelter's syndrome can be successfully treated with ICSI procedure when spermatozoa are present in the ejaculate and are euploid for sex chromosome after FISH analysis.

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