Intracytoplasmic sperm injection outcome of ejaculated spermatozoa from a man with mosaic Klinefelter’s Syndrome: case report and literature review

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Abstract

Objective: To report a case of abortion after intracytoplasmic sperm injection (ICSI) with ejaculated spermatozoa obtained from a man with mosaic Klinefelter’s syndrome.

Methods: Sperm nuclei from the patient were analyzed by fluorescence in situ hybridization, and the disomy frequencies for chromosome 18 and the sex chromosomes were determined. A literature review of the ICSI outcome of ejaculated sperm in patients with Klinefelter’s syndrome was also performed.

Results: A total of 108 spermatozoa nuclei were analyzed. Of these, 102 sperm cells were normal with an X18 (55.56%) or Y18 (38.89%) chromosome pattern. Three cells with XX18 (2.78%) and three cells with YY18 (2.78%) signals were detected. The fetus stopped developing in the eighth week. The karyotype determined by an analysis of the abortive tissue was 46, XY. The literature review identified a total of 12 patients who were analyzed in 11 reports. The fertilization rate was 80.9%, and the live birth rate per transfer was 71.4%.

Conclusions: ICSI with ejaculated spermatozoa from men with Klinefelter’s syndrome can lead to pregnancy, for which the risk of transmission of chromosomal aneuploidy is low.
Keywords
Klinefelter’s syndrome, intracytoplasmic sperm injection outcome, spermatozoa, infertility, fluorescent in situ hybridization, karyotype

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Introduction
With an incidence of approximately 1 in 1000 live births, Klinefelter’s syndrome is the most common sex chromosome abnormality in human males.\(^1\) This syndrome is usually associated with azoospermia or oligozoospermia, accounting for 4.6% of infertility cases among males.\(^2\) In approximately 15% to 20% of males with Klinefelter’s syndrome, a mosaic pattern is exhibited, usually involving two cell lines with karyotypes of 46, XY/47, XXY, and on rare occasions involving further cell lines such as those with karyotypes of 46, XY/47, XXY/48, XXXY.\(^3\) The other cases Klinefelter’s syndrome are not considered to involve mosaicism, as revealed by the cytogenetic examination of somatic cell lines.

Typical phenotypic abnormalities associated with Klinefelter’s syndrome include increased gonadotropin levels, decreased/normal testosterone levels, and small testicular volume. Men with non-mosaic Klinefelter’s syndrome are reported to be more severely affected than those with the mosaic form. It is also widely believed that men with mosaic Klinefelter’s syndrome are more androgenized than their non-mosaic counterparts.\(^4\)

Conventional testicular sperm extraction (TESE), in combination with intracytoplasmic sperm injection (ICSI), is used for men with azoospermia caused by Klinefelter’s syndrome.\(^5\) Microdissection testicular sperm extraction can improve sperm retrieval in men with nonobstructive azoospermia.\(^6\) However, only a few births following ICSI with ejaculated spermatozoa have been reported.\(^7,8\) Moreover, although ICSI has been successfully used for the treatment of azoospermia and oligozoospermia, the transmission of cytogenetic defects to offspring is a major concern. It is also unclear whether there is any difference in the risk of chromosome aneuploidy regarding the choice between using ejaculated sperm and testicular sperm.

We herein report a case of abortion after ICSI with ejaculated spermatozoa from a man with mosaic Klinefelter’s syndrome. Ejaculated spermatozoa were identified as being haploid by three-color fluorescence in situ hybridization (FISH) before ICSI.

Materials and methods

Patient
A 35-year-old man with a normal appearance consulted our hospital because of a history of primary infertility over a 9-year period. Physical examination revealed normal hair distribution and no gynecomastia. The volume of both testes was 5 mL. Analyses of two semen samples showed severe oligozoospermia, with sperm concentrations of \(0.2 \times 10^6/\text{mL}\) and \(0.1 \times 10^6/\text{mL}\). Motile spermatozoa were rare, but their precise morphology could not be assessed. Hormonal analysis showed a follicle-stimulating hormone (FSH) concentration of 23.2 mIU/mL (normal 1.5–12.4 mIU/mL), luteinizing hormone (LH) concentration of 13.1 mIU/mL.
(normal 1.7–8.6 mIU/mL), and testosterone concentration of 10.9 nmol/L (normal 9.9–27.8 nmol/L). The patient’s wife was a 33-year-old healthy woman with normal ovulatory cycles. Peripheral blood chromosome analysis of 50 patient cells revealed that 22 had a 47, XXY karyotype and 28 were 46, XY. Written informed consent was obtained from the patient for participation in the study, and the Ethics Committee of the First Hospital of Jilin University approved the study.

**Preparation of sperm nuclei**

Triple-color FISH with centromeric DNA probes (Beijing GP Medical Technologies, Beijing, China) for chromosomes 18, X, and Y was used to determine the sex chromosome constitution of spermatozoa. Prior to FISH, spermatozoa were prepared for FISH as described previously.9 Spermatozoa were washed three times by centrifugation for 10 minutes at 447 g in phosphate-buffered saline. The pellets were resuspended in fresh fixative (methanol: acetic acid, 3:1), then smeared on glass slides and air-dried. To render the sperm chromatin accessible to DNA probes, slides were incubated in 1 N NaOH at room temperature for 10 minutes. The slides were washed with distilled water, then with 2× sodium chloride/sodium citrate for 2 minutes, followed by dehydration through an ethanol series (70% → 85% → 100%) and air-drying.

**Fluorescence in situ hybridization analysis**

Using triple-color FISH for chromosomes 18, X, and Y, a total of 108 spermatozoa from the patient were scored. The probe mixture for triple FISH consisted of a repetitive DNA sequence of centromeric probes for chromosome X labeled green, for chromosome Y labeled red, and for chromosome 18 labeled azure. The sperm and probe mixture (10 µL) was applied to each slide under a 16 × 60 mm coverslip and the slides were then sealed with glass coverslip sealant.9 Hybridization was carried out for 4 hours at 42°C in a humidified chamber. The slides were counterstained with 10 µL of 4’,6-diamidino-2-phenylindole diluted in antifade mounting medium.9 Following FISH, the nuclei and fluorescent signals were viewed using a fluorescence microscope (DM4000B; Leica, Solms, Germany). Only intact, non-overlapping sperm heads with clear fluorescent signals were scored. Images were captured using a CCD camera and were saved on a computer using a video card. These images were stored and used after FISH processing to facilitate the identification and localization of each cell.

**ICSI protocol and embryo culture**

The long gonadotropin-releasing hormone agonist protocol was used in the luteal phase. Ovarian stimulation started when serum FSH levels were <5 mIU/mL, LH was <5 mIU/mL, estradiol was <50 pg/mL, follicular diameter was <5 mm, and endometrium thickness was <5 mm. Ovulation was triggered by the administration of human chorionic gonadotropin (hCG) when at least two follicles were 18 mm in diameter. Oocytes were retrieved 36 to 38 hours after the administration of hCG. Metaphase II oocytes were injected with normal morphology motile sperm, whenever possible, for ICSI. All ICSI procedures were performed as described previously.10 Fertilization and embryo culture were performed in Quinn’s-1026 medium (SAGE ORIGIO Inc., Cooper Surgical, Trumbull, CT, USA) with 10% serum protein substitute (SPS) (SAGE) and Quinn’s-1020 medium (SAGE) enriched with 5% human serum albumin (SAGE) in a 37°C incubator with 5% CO₂. At 17 to 19 hours after insemination, normal
fertilization was confirmed by the presence of two pronuclei. Blastocysts were cultured in Quinn’s-1029 medium (SAGE) containing 10% concentrated SPS at 37°C with 6% CO₂ and 89% N₂. After 72 hours fertilization, the modified PETER cleavage stage embryo scoring system was used to assess the day 3 embryo quality. Grade I embryos were uniform or slightly uneven with a fragmentation of <10%; Grade II embryos had a uniform or non-uniform blastomere size, and fragmentation amount of 10% to 20%; Grade III embryos had an accounted embryo amount of 21% to 50%; and Grade IV embryos contained >50% debris. According to the criteria, day 3 cell number 6–10 Grade I and II embryos were rated as high-quality (D2 to D3 embryonic development of a blastomere), and the remainder were regarded as poor-quality embryos.

**Results**

A total of 108 spermatozoon cells were analyzed by triple FISH, as shown in Table 1. Of these, 102 (94.44%) sperm cells were normal with an X18 (55.56%) or Y18 (38.89%) karyotype. Sex chromosome disomy was detected in 5.56% of nuclei, which included three cells with XX18 (2.78%) and three cells with YY18 (2.78%) signals. Our FISH analysis of spermatozoa in samples with a karyotype of 46, XY/47, XXY indicated normal spermatozoon frequencies ranging from 91.98% to 98.58% (Table 2).

ICSI was attempted for the couple. On the day of ICSI, 14 oocytes were obtained, and the patient provided a sufficient amount of motile ejaculated spermatozoa. The spermatozoa were aspirated into an injection needle and injected into the 11 metaphase II oocytes. Eight oocytes were fertilized and cleaved. Two of five high-quality embryos (8I, 8II) were transferred into the uterine cavity 3 days later. Four weeks later, we observed the gestational sac, but miscarriage occurred six weeks after embryonic transfer (ET). The karyotype as determined by analysis of the abortive tissue was 46, XY. In the following two frozen ET cycles, two thawed embryos (8II, 7II) and one thawed embryo (7II) were transferred into the uterine cavity. Both hCG tests 14 days after ET were negative.

Through a literature review, we identified 12 patients with Klinefelter’s syndrome who had undergone ICSI with ejaculated spermatozoa. Their ICSI outcomes and clinical characteristics are shown in Table 3. Fourteen ET cycles were carried out for these 12 patients. The mean patient age was 30.4 years (range, 24–36 years), the median testicular volume was 4.1 ± 1.9 mL, and the fertilization rate was 80.9% (72/89). A total of 14 healthy babies from eight couples (75%) were delivered, and the live birth rate per transfer was 71.4% (10/14). The karyotypes of all newborn babies were normal. One case of pregnancy ended in an early abortion, and analysis of the aborted material revealed a normal karyotype of 46, XX.

**Discussion**

In subjects with mosaic Klinefelter’s syndrome, 46, XY/47, XXY, spermatogenesis is associated with the presence of 46, XY cells among germ cells and other surrounding cells. However, Vidal et al. and

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**Table 1.** Fluorescent in situ hybridization of patient’s spermatozoa with probes specific for chromosomes X, Y, and 18

| FISH results | Presumed karyotype | No. of spermatozoa | % of spermatozoa |
|--------------|--------------------|--------------------|------------------|
| X18          | 23,X               | 60                 | 55.56            |
| Y18          | 23,Y               | 42                 | 38.89            |
| XX18         | 24,XX              | 3                  | 2.78             |
| YY18         | 24,YY              | 3                  | 2.78             |
Chevret et al.\textsuperscript{24} proposed that a few 47, XXY germ cells would be able to complete meiosis and produce mature spermatozoa. Patients with non-mosaic Klinefelter’s syndrome also produce spermatozoa, which can result in fertilization and the development of a healthy baby.\textsuperscript{13,25} The risk of transmitting chromosomal aneuploidy when using spermatozoa from patients with Klinefelter’s syndrome varies, and many reports on this issue have been published. Chromosomal disomy has previously been found with an incidence of 1.47\% to 3.91\% in mosaic Klinefelter’s syndrome, along with chromosomal diploidy with an incidence of 0\% to 1.7\%.\textsuperscript{9,14,26–30} Foresta et al.\textsuperscript{31} analyzed 10,000 spermatozoa from two patients with non-mosaic Klinefelter’s syndrome, for which the incidence of disomy varied from 11.34\% to 18.13\%.

As well as the risk of chromosome aneuploidy, the origin of spermatozoa should be taken into account prior to ICSI. Ben-Ami et al.\textsuperscript{32} showed that the outcome of ICSI was worse after using ejaculated spermatozoa than those which had undergone TESE cycles. It is likely that spermatozoa suffer oxidative stress and nuclear DNA damage during transit through the male genital tract.\textsuperscript{33} However, it is unclear whether ejaculated spermatozoa are more vulnerable to damage in patients with Klinefelter’s syndrome.

Although a higher aneuploidy rate has been identified in embryos for whom the father has Klinefelter’s syndrome, almost no abnormal babies born with ICSI without preimplantation genetic diagnosis have been reported worldwide. One possible reason for this is that abnormal embryos are more likely to fail to survive until birth. Moreover, noninvasive prenatal screening appears to be useful to further reduce the risk of abnormal babies.\textsuperscript{34} A limitation of our study is the lack of data on karyotypes and Y chromosome microdeletion screening associated with pregnancy.

| Table 2. Incidence of chromosome anomalies in spermatozoa from patients with mosaic Klinefelter’s syndrome |
|-----------------|-----------------|-----------------|
| Reference       | Karyotype of patients | No. of spermatozoa | X-bearing Y-bearing |
| Chevret, 1996\textsuperscript{26} | 46,XY (90\%)/47,XXXXY (10\%) | 27,097 | 52.78 |
| Martini, 1996\textsuperscript{27} | 46,XY (97.5\%)/47,XXXXY (2.5\%) | 38,000 | 46.7 |
| Lim, 1999\textsuperscript{28} | 46,XY (70\%)/47,XXXXY (30\%) | 17,001 | 46.74 |
| Rives, 2000\textsuperscript{9} | 46,XY (95\%)/47,XXXXY (5\%) | 20,814 | 49.68 |
| Morel, 2000\textsuperscript{29} | 46,XY (70\%)/47,XXXXY (30\%) | 3,581 | 47.1 |
| Morel, 2000\textsuperscript{29} | 46,XY (70\%)/47,XXXXY (30\%) | 3,581 | 50.5 |
| Bielskas, 2000\textsuperscript{14} | 46,XY (98.3\%)/47,XXXXY (1.7\%) | 358 | 44.13 |
| Blanco, 2001\textsuperscript{30} | 46,XY (35\%)/47,XXXXY (65\%) | 553 | 42.86 |
| Yu et al. \textsuperscript{43,27} | | | |
Table 3. ICSI outcome of ejaculated spermatozoa in patients with Klinefelter’s syndrome

| Reference                  | Female age (years) | Male age (years) | Mean volume of testis (mL) | Count of spermatozoa (n) | Mature oocytes (n) | Fertilization rate (%) | Embryo transferred cycle (n) | Transferred embryos (n) | Outcome | Karyotype                        |
|---------------------------|--------------------|------------------|---------------------------|--------------------------|--------------------|------------------------|-----------------------------|-------------------------|---------|----------------------------------|
| Harari, 1995              | 28                 | 35               | 2.5                       | <100 (motile)            | 8                  | 100 (8/8)              | 2                           | 3                       | Fail    | –                                |
| Hinney, 1997              | 33                 | 24               | 6                         | <0.1 x 10⁶/mL            | 8                  | 50 (4/8)               | 1                           | 3                       | Early abortion | Normal                           |
| Bourne, 1997              | 29                 | 26               | 3                         | –                        | 13                 | 77 (10/13)            | 2                           | 4                       | Healthy twin | Normal                           |
| Kitamura, 2000            | 32                 | 36               | 6                         | 1 x 10⁶                  | 1                  | 100 (1/1)             | 1                           | 1                       | Fail    | –                                |
| Kitamura, 2000            | 26                 | 30               | 2                         | 1 x 10⁶                  | 1                  | 0                      | 0                           | 0                       | –       | –                                |
| Bielanska, 2000           | 36                 | 35               | –                         | 0.1 x 10⁶/mL             | 19                 | 79 (15/19)            | 1                           | 3                       | Twin    | Normal                           |
| Crüger, 2001              | 28                 | 28               | 1                         | 9 (motile)               | 4                  | –                      | 1                           | 2                       | Healthy girl | Normal                           |
| Tachdjian, 2003           | 28                 | 27               | 6.4                       | –                        | 9                  | 56 (5/9)              | 1                           | 2                       | Healthy twin | Normal                           |
| Komori, 2004              | 23                 | 31               | 4                         | 0.35 x 10⁶/mL            | 3                  | 100 (3/3)             | 2                           | 4                       | Healthy girl | and boy, respectively             |
| Akashi, 2005              | 33                 | 35               | 6                         | 0.4 x 10⁶                | 6                  | 83 (5/6)              | 1                           | 3                       | Healthy girl | –                                |
| Zhang, 2012               | 22                 | 24               | 5                         | 0.7 x 10⁶/mL             | 11                 | 100 (11/11)           | 1                           | 2                       | Healthy twin | 46, XY and 46, XY, inv(9) (p11q21) |
| Gurbuz, 2015              | 30                 | 34               | 3.4                       | 2 x 10⁶                  | 11                 | 91 (10/11)            | 1                           | 2                       | Healthy twin | Normal                           |
failure. An analysis of abortive tissue would provide useful information for further study.

**Conclusion**

This study indicates that ejaculated spermatozoa in patients with mosaic Klinefelter’s syndrome differ in the associated incidence of chromosomal abnormalities, but the risk of transmission of chromosomal aneuploidy for ICSI using ejaculated spermatozoa is low.

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**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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