

Brief Report

BIRTHS AFTER INTRACYTOPLASMIC INJECTION OF SPERM OBTAINED BY TESTICULAR EXTRACTION FROM MEN WITH NONMOSAIC KLINEFELTER'S SYNDROME

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KLINFELTER'S syndrome is a form of hypergonadotropic hypogonadism and infertility resulting from a supernumerary X chromosome (47,XXY), with an incidence of approximately 1 case in 500 phenotypic males.^{1,2} Some men with Klinefelter's syndrome who have chromosomal mosaicism (46,XY/47,XXY) are fertile. Men with nonmosaic, or complete, Klinefelter's syndrome usually have azoospermia, and only a few have any spermatogenesis.^{3,4}

Intracytoplasmic sperm injection, in which a spermatozoon is injected into an ovum *in vitro*, is an effective treatment for male-factor infertility. However, the complete absence of spermatozoa presents a particular clinical challenge. Postorchitis atrophy and genetic anomalies are the main causes of nonobstructive azoospermia, which is characterized by germ-cell aplasia, maturation arrest, or hypospermatogenesis. In such cases, testicular extraction of sperm has proved useful in obtaining sufficient sperm for fertilization with intracytoplasmic sperm injection.^{5,6} We report on two couples, in each of which the man had nonmosaic Klinefelter's syndrome, who underwent testicular sperm extraction and intracytoplasmic sperm injection, which resulted in the delivery of healthy infants.

CASE REPORTS

Couple 1

A healthy 32-year-old man and a healthy 32-year-old woman were evaluated after two years of primary infertility. A testicular-biopsy specimen obtained from the man one year earlier contained only Sertoli cells. He had a gynecoid habitus, scant facial hair, slight gynecomastia, bilaterally atrophic testes, and small bi-

lateral varicoceles. His serum gonadotropin concentrations were high, and his serum testosterone concentration was low. Analysis of three semen specimens showed normal volumes and fructose concentrations; a single, abnormal, nonmotile sperm was seen in one specimen. On the basis of an analysis of 50 peripheral-blood leukocytes, his karyotype was 47,XXY. The woman was normal.

We performed a single *in vitro* fertilization cycle with intracytoplasmic injection of sperm obtained by testicular extraction. During this cycle, the woman was treated first with a gonadotropin-releasing hormone agonist (leuprolide), administered subcutaneously, to inhibit gonadotropin secretion and then with a combination of human menopausal gonadotropin and follicle-stimulating hormone, administered intramuscularly, to stimulate the development of ovarian follicles. Fifteen oocytes were retrieved approximately 34 to 36 hours after chorionic gonadotropin had been given intramuscularly. Ten oocytes were fertilized, and three were transferred into the uterus, but no pregnancy resulted. No embryos were considered suitable for cryopreservation.

For the second *in vitro* fertilization cycle, which was performed six months later, the woman received leuprolide and follicle-stimulating hormone. After chorionic gonadotropin had been administered, 13 oocytes were retrieved by ultrasonographically guided transvaginal needle aspiration; 12 were at the second stage of metaphase. Bilateral testicular biopsy to obtain sperm was performed at the same time.⁶ Before undergoing the biopsy, the man had received testolactone (100 mg orally twice daily) for three months. The biopsy specimens yielded 600 mg of seminiferous tubules. On microscopical examination, the tubules contained mostly Sertoli cells and only a few spermatogenic elements; extensive Leydig-cell hyperplasia was also seen (Fig. 1). A search of fresh tissue under higher magnification identified approximately 10 sperm with twitching movements only. Enough sperm were obtained by centrifugation of the tissue to fertilize each of the 12 ova by injection of a spermatozoon immobilized by crimping the tail.⁵ Eight oocytes were normally fertilized, as indicated by the presence of two pronuclei and two extruded polar bodies. The couple declined preimplantation genetic testing. Because a proportion of the blastomeres were fragmented (>20 percent) in the three embryos selected for transfer, assisted hatching (creation of an artificial breach in the zona pellucida) was performed according to previously reported methods.⁷ The embryos were transferred into the uterus three days after fertilization. No embryos were cryopreserved. The woman was given intramuscular progesterone in oil (50 mg daily) until fetal cardiac activity was confirmed by ultrasonography.⁷

Serum concentrations of the beta subunit of chorionic gonadotropin, measured twice weekly, increased to 94 mIU per milliliter on day 11 after the embryo transfer. Ultrasonography performed 32 days later revealed two asymmetric intrauterine sacs, of which one had a fetal heartbeat. Amniocentesis at 20 weeks' gestation showed a fetal karyotype of 46,XY. The pregnancy was normal, and the woman delivered a healthy 2778-g boy at 38.5 weeks' gestation.

Couple 2

A healthy 34-year-old man and a healthy 33-year-old woman were evaluated after five years of primary infertility. The man had a gynecoid habitus, bilaterally atrophic testes, gynecomastia, and a moderate-size left varicocele. Analysis of three semen samples showed low volume (1.2 ml) and normal fructose concentrations but no sperm. His serum gonadotropin concentrations were high, and his serum testosterone concentration was low. Bilateral testicular-biopsy specimens obtained approximately one year earlier contained only Sertoli cells. On the basis of an analysis of 40 peripheral-blood leukocytes, his karyotype was 47,XXY. The woman was normal.

The woman was given leuprolide, human menopausal gonadotropin, and follicle-stimulating hormone. Forty mature oocytes were retrieved by transvaginal ultrasonography after the administration of chorionic gonadotropin. Simultaneous testicular biopsy,

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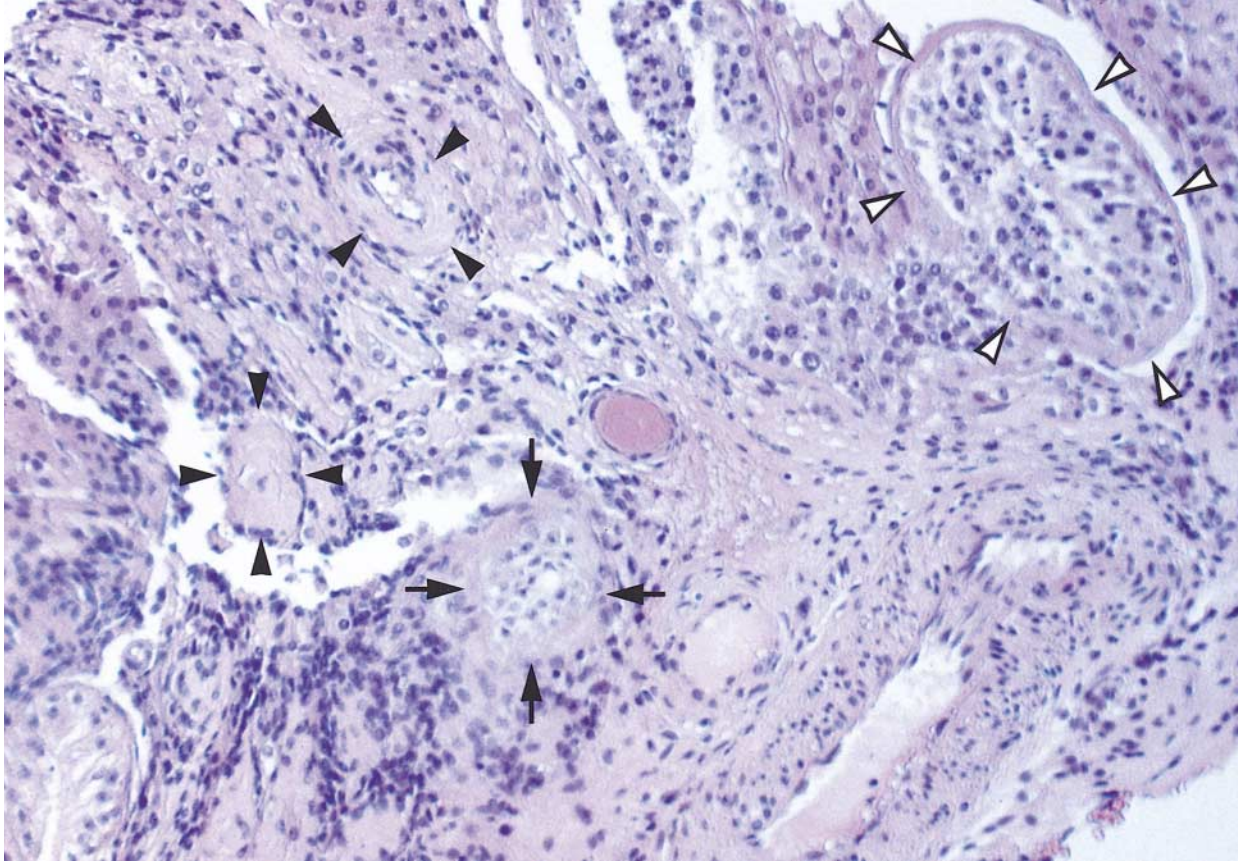


Figure 1. Testicular-Biopsy Specimen Obtained from the Male Partner of Couple 1 during Testicular Extraction of Sperm (Hematoxylin and Eosin, $\times 100$).

The specimen contains a seminiferous tubule with hypospermatogenesis (white arrowheads), a tubule with only Sertoli cells (arrows), and sclerotic tubules (black arrowheads). Extensive intertubular Leydig-cell hyperplasia is also present.

after the man had been treated with testolactone for three months, resulted in the recovery of 19 spermatozoa, which were used to fertilize 19 oocytes by intracytoplasmic sperm injection. Normal fertilization was confirmed in 12 oocytes. This couple also declined preimplantation genetic testing. Three embryos were transferred to the woman's uterus three days after fertilization.⁷ She received daily intramuscular injections of progesterone in oil (50 mg) until fetal cardiac activity was confirmed by ultrasonography.

On day 11 after the embryo transfer, the serum concentration of the beta subunit of chorionic gonadotropin was 239 mIU per milliliter. Ultrasonography performed 32 days later revealed two intrauterine sacs, both with fetal heartbeats. Amniocentesis at 20 weeks' gestation showed that the fetal karyotypes were 46,XY and 46,XX. The infants, a boy and a girl, were delivered by cesarean section at 35.5 weeks' gestation; their birth weights were 2551 g and 2410 g, respectively. Both were normal.

METHODS

The testicular sperm extraction involved an open operation performed simultaneously with oocyte retrieval from the woman after programmed ovarian stimulation. The procedure was performed through a median scrotal incision with the use of local or general anesthesia. Up to 500 mg of tissue was obtained from each testis. To obtain individual tubules, the tissue was dispersed between glass slides, minced in artificial human tubal fluid, and then passed through a 24-gauge catheter. If no spermatozoa were

identified in the final suspension, further biopsies were performed immediately. To obtain spermatozoa for intracytoplasmic injection of sperm into oocytes, the suspension was centrifuged at $1800\times g$ for five minutes. The resulting pellet was examined for motile (i.e., twitching) spermatozoa. If none were identified, the suspension was reincubated at 37°C in a humidified 5 percent carbon dioxide atmosphere for up to two hours in order to enhance observable kinetic characteristics.⁶

DISCUSSION

Klinefelter's syndrome is thought to occur because of an error in meiosis.⁸ The most important testicular lesions are spermatogenic arrest and the predominance of Sertoli cells.⁹ However, not all affected men have complete suppression of sperm production; in particular, men with chromosomal mosaicism may have small foci of spermatogenesis. The paucity of these foci in men with nonmosaic Klinefelter's syndrome has frustrated attempts to overcome this common form of male-factor infertility.

Intracytoplasmic sperm injection is an effective remedy for many types of male-factor infertility,¹⁰ but its usefulness is predicated on the availability of at least

one living spermatozoon to achieve fertilization. In otherwise normal men with nonobstructive azoospermia, small numbers of spermatozoa can sometimes be recovered by testicular biopsy and extraction for subsequent intracytoplasmic injection. Previous application of these techniques in men with nonmosaic Klinefelter's syndrome has resulted in fertilization and pregnancy, but not in birth.¹¹⁻¹⁴ Our results in two men indicate that this approach can be successful in men with nonmosaic Klinefelter's syndrome.

Among men with nonobstructive azoospermia, including those with Klinefelter's syndrome, who agree to undergo testicular sperm extraction, some will be found to have no sperm. At present, there is no way to predict which men will have no sperm.⁶ Couples should be prepared for the possibility that no sperm will be recovered and consider the use of donated sperm, if necessary. Testolactone (an aromatase inhibitor) is routinely given to men with nonobstructive azoospermia, including those with Klinefelter's syndrome (as in this study), to optimize semen production.

During a three-year period, we have attempted testicular sperm extraction in 70 men with nonobstructive azoospermia, 6 of whom had either mosaic or nonmosaic Klinefelter's syndrome. This report describes the first two couples in which the man had nonmosaic Klinefelter's syndrome. Three of the four men in the other couples also had nonmosaic Klinefelter's syndrome, and spermatozoa were successfully retrieved in one of the four. With intracytoplasmic sperm injection, this couple conceived and now has an ongoing pregnancy.

Although Klinefelter's syndrome is a nonheritable genetic condition that almost always results in sterility, meiosis is possible. However, spermatozoa from men with mosaic Klinefelter's syndrome contain an extra sex chromosome more often than do spermatozoa from normal men.^{15,16} Therefore, there is a possibility that chromosomal errors will be transmitted to the offspring of men with Klinefelter's syndrome. The normal karyotypes of the three infants described here support previously reported data on the safety of intracytoplasmic sperm injection in the treatment of oligospermia and azoospermia.¹⁷ As in all cases of severe male-factor infertility requiring intracytoplasmic

sperm injection, genetic screening and prenatal testing should be strongly recommended.

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