CASE REPORT

Births Using Frozen-Thawed Testicular Spermatozoa and Frozen-Thawed Embryos in Two Azoospermic Patients with Nonmosaic Klinefelter's Syndrome

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Abstract

This study presents healthy births with two azoospermic nonmosaic Klinefelter's syndrome patients using frozen-thawed testicular spermatozoa and frozen-thawed embryos. In the first couple, male partner underwent a diagnostic testicular sperm extraction (TESE). Spermatozoa were recovered and cryopreserved. Subsequent intracytoplasmic sperm injection (ICSI) cycle using the frozen-thawed testicular spermatozoa resulted in delivery of healthy female twins with normal karyotypes. The second couple underwent simultaneous TESE-ICSI cycle and after recovery of spermatozoa, twenty-one metaphase II oocytes were injected with testicular spermatozoa. Nineteen oocytes showed fertilization with two pronuclei. Ten embryos at pronuclear stage were frozen and the remaining nine were cultured for further cleavage. No pregnancy was obtained by the transfer of four fresh embryos on day three. Later, after endometrial preparation, ten embryos frozen at pronuclear stage were thawed and transfer of two grade 1 and two grade 3 embryos, resulted in delivery of a healthy girl with a normal karyotype. As a conclusion, it is possible to achieve pregnancies using frozen-thawed spermatozoa and embryos in cases with Klinefelter's syndrome if spermatozoa are obtained.

Keywords: Klinefelter's syndrome, testicular spermatozoa, frozen-thawed spermatozoa, frozen-thawed embryo

Özet

Nonmozaik Klinefelter Sendromlu İki Azoospermik Hastada Dondurulmuş-Çözülmüş Testiküler Spermatozoa ve Dondurulmuş-Çözülmüş Embriyo Kullanılarak Sonuçlanan Doğumlar

Bu çalışmada, nonmozaik Klinefelter sendromlu iki azoospermik hastada dondurulmuş-çözülmüş testiküler sperm ve dondurulmuş-çözülmüş embriyo transferi ile gerçekleşen iki sağlıklı doğum ele alındı. İlk çiftte azoospermik olan erkeğe diyagnostik olarak testiküler sperm ekstraksiyonu (TESE) uygulandı ve sperm bulunarak dondurudu. Daha sonra yapılan intrasitoplazmik sperm enjeksiyonu (ICSI) siklusunda dondurulmuş-çözülmüş sperm kullanıldı ve bu siklus sağlıklı ve normal karyotipli ikiz kız bebeğin doğumu ile sonlandı. İkinci çift eşzamanlı olarak ICSI-TESE siklusuna hazırlandı ve TESE işleminde bulunan spermler kullanılarak elde edilen yirmi bir metafaz II oositin 19'u döllendi. Pronükleer aşamada 10 oosit dondurudu ve kalan dokuz embriyodan 4'ü 3. günde transfer edildi, ancak gebelik gerçekleşmedi. Daha sonra dondurulmuş 10 embriyo çözüldü ve ikisi iyi, ikisi kötü kalitede olmak üzere 4 embriyonun transferi normal karyotipli bir kız çocuğunun doğumu ile sonlandı. Sonuç olarak, Klinefelter sendromlu hastalarda sperm bulunması durumunda fazla sperm ya da embriyoların dondurulması ve çözülmesi ile gebelik mümkündür.

Anahtar sözcükler: Klinefelter sendromu, testiküler spermatozoa, dondurulmuş-çözülmüş spermatozoa, dondurulmuş-çözülmüş embriyo

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Introduction

Klinefelter's syndrome was first described as an endocrine disorder characterized by small firm testes, gynaecomastia, hypogonadism and elevated follicle stimulating hormone (FSH) levels. Currently, it is the most common form of chromosome aneuoploidy in humans and its reported incidence is 0.1-0.2% in the general population, but a higher frequency of 1.8-3.5% has been reported in infertile men attending andrology units (1). Genetic surveys have reported up to 0.7% of oligozoospermic and 11% of azoospermic men have an abnormal 47, XXY karyotype (2). Although, men with nonmosaic Klinefelter's syndrome show azoospermia and considered to be sterile, spermatozoa are rarely observed in the ejaculate of these patients and exceptional cases of proven spontaneous paternity have been reported (1). After the first successful recovery of spermatozoa from azoospermic men with Klinefelter's syndrome by means of testicular sperm extraction (TESE) in 1996 (3), the progress in assisted reproduction technologies with intracytoplasmic sperm injection (ICSI) and TESE have provided the opportunity to become biological fathers for these men and successful treatment outcomes in large study groups have been recently reported (4-6). Furthermore, a few successful pregnancies and healthy births using frozen-thawed testicular spermatozoa have been published (5-8). With Klinefelter's syndrome, however, according to our knowledge, only one birth and one ongoing pregnancy have been reported so far by the transferral of frozen thawed embryos obtained from the patients (9,10).

In this report, births of healthy female twins using frozen thawed testicular spermatozoa and a healthy girl after the transfer of frozen-thawed embryos obtained by ICSI using testicular spermatozoa from azoospermic patients with nonmosaic Klinefelter's syndrome were presented.

Case 1

A 33-year-old man and his 31-year-old wife applied to our IVF center in June 2005 after 5 years of primary infertility. The male partner had azoospermia in repeated semen analysis and he had undergone bilateral testicular biopsy 2 years previously demonstrating Sertoli cell-only syndrome. Physical examination was normal and no gynaecomastia was observed. Both testes were small and firm. Laboratory tests revealed a FSH level of 33.6 IU/L and a total testosterone level of 4.03 ng/ml. Chromosome analysis in 30 metaphases showed a 47, XXY karyotype. The medical history of the female partner did not reveal any abnormality and the results of gynecologic investigation with transvaginal ultrasonography and day-three hormone analysis were normal. After informing the couple on the outcome of TESE procedure, a diagnostic TESE was planned. The couple received genetic counseling and were extensively informed about preimplantation and prenatal genetic diagnosis.

TESE was performed by microdissection technique as previously described (6). Briefly, the testis was widely opened in an equatorial plane and using an operation microscope of x15-20 power magnification, microdissection was carried out. Enlarged seminiferous tubules were selected, removed and tissue pieces were placed in 0.5 ml bicarbonate buffered sperm wash media (Sperm Preparation Medium, Medicult, Denmark) at 37°C. Tissue dissected under stereomicroscope using sterile pincers and one drop of the suspension was evaluated under the phase contrast microscope at x200 magnification. Suspensions of the motile sperm found on the right testis were centrifuged at a force of 800xg. The pellet was resuspended to desired volume with sperm wash media. For sperm freezing, the cell suspension was slowly mixed 1:1 with Sperm Freezing Medium (Medicult, Denmark); aspirated into sterile high-security straws (CryoBio System, France); cooled over liquid nitrogen vapor and plunged directly into liquid nitrogen.

Four months later, the couple underwent ICSI cycle and controlled ovarian hyperstimulation was achieved using down regulation with leuprolide acetate 1 mg daily (Lucrin, Abbott, Switzerland) and recombinant follicle stimulating hormone (recFSH) 225 IU for 10 days (Puregon, Organon, Netherlands). On day 11 of the cycle, serum concentration of estradiol was 3289 pg/ml and there were about 10 follicles with a diameter between 16-20 mm. Final maturation of oocytes was induced with a 10 000 IU human chorionic gonadotropin (hCG) (Pregnyl, Organon, Netherlands); and, 15 oocytes were collected under transvaginal ultrasound guidance 35 hours after the hCG administration. The cryopreserved spermatozoa were thawed on the oocyte retrieval day. Straws were poured out into sterile test tubes, mixed well and one drop was evaluated under phase contrast microscope. Post-thaw sperm suspensions were layered on a 80% gradient solution (SupraSperm, Medicult, Denmark), centrifuged for 25 minutes at a force of 600xg and the pellet was washed twice with sperm wash media (Sperm Preparation Medium, Medicult, Denmark). The washed sperm suspensions were placed in sterile Petri dishes (Falcon 1006, Becton and Dickinson, USA), covered with oil (Liquid Paraffin, Medicult, Denmark), evaluated on the inverted microscope and motile sperm cells were collected using the micromanipulator system (Narishige, Japan). Fourteen metaphase-II oocytes were injected with motile spermatozoa and 12 of them showed fertilization with two pronuclei. Fertilized oocytes were cultured in BlastAssist medium (BlastAssist, Medicult, Denmark) and four good quality eight-cell embryos were transferred on day 3 using Wallace transfer catheter (1 embryo retained in the transfer catheter was retransferred immediately). Luteal phase was supported by vaginal administration of progesterone. A pregnancy test, carried out 12 days after transfer, showed a serum β-hCG level of 376 mIU/ml which increased to 814 mIU/mL two days later. A twin pregnancy with visible heart beats was detected at sixth gestational week. Amniocentesis for karyotyping was rejected by the couple. A cesarean section was performed and two healthy girls weighing 1900 g and 2200 g were born at 37 weeks of gestation in June 2006.

Cord blood chromosomal analysis of infants revealed normal female karyotypes of 46, XX.

Case 2

A 21-year-old man and his 20-year-old wife applied to our IVF center in September 2005 after 2 years of primary infertility due to azoospermia in repeated semen analysis. Physical examination of male partner was normal and no gynaecomastia was seen. Both testes were small and firm. Laboratory tests revealed a FSH level of 54 IU/L and total testosteron level of 4.99 ng/ml. Chromosome analysis in 30 metaphases showed a 47, XXY karyotype. The medical history, physical examination and all laboratory tests of female partner did not reveal any abnormality. The couple were informed on the outcome of the TESE procedure, and were given genetic counselling. After down regulation with leuprolide acetate 1 mg daily (Lucrin, Abbott, Switzerland), ovarian hyperstimulation was achieved by recFSH starting on day 3, 225 IU for the first 6 days and 150 IU for the last 3 days (Puregon, Organon, Netherlands). On day 12 of the cycle, when there were about 15 follicles with a diameter between 16-20 mm, ovulation was triggered with a 10 000 IU hCG (Pregnyl, Organon, Netherlands). On the day of oocyte recovery, the male partner first underwent the TESE procedure as stated above. After the TESE procedure, oocyte pick-up was performed under transvaginal ultrasound guidance 35 hours after the hCG administration. Twentyone metaphase-II oocytes were injected with motile spermatozoa and 19 of them showed fertilization with two pronuclei. Ten pronuclear stage embryos were frozen. For this purpose, embryos were aspirated into high security straws and frozen in Dulbecco's Phosphate Buffered Saline (PBS), 1,2-propanediol and sucrose solutions (Embryo Freezing Pack, Medicult, Denmark). A programmable freezer was used for controlled-rate cooling (Planer Kryo 360-1.7, UK). Manual seeding was applied after cooling to –7°C. After completion of the freezing program, straws were transferred into liquid nitrogen and stored at –196°C. The remaining nine embryos were cultured in BlastAssist medium (BlastAssist, Medicult, Denmark) for further cleavage. No pregnancy was achieved by the transfer of four fresh embryos, one grade 1 and three grade 3, on day three.

Three months later, after endometrial preparation by estradiol valerate, ten embryos freezed at the pronuclear stage were thawed by removing straws from liquid nitrogen and placing them in water at 30°C for 1 minute. Embryos were transferred through sucrose, 1,2-propanediol, and PBS solutions (Embryo Thawing Pack, Medicult, Denmark), washed and finally placed in culture media. Two grade 1 and two grade 3 embryos were transferred. A pregnancy test carried out 12 days after transfer showed a serum β-hCG level of 107 mIU/ml, which increased to 258 mIU/ml two days later. A singleton pregnancy with visible heart beat was detected at seventh gestational week. Amniocentesis for karyotyping was rejected by the couple. At 37 weeks of gestation in June 2006, the patient was delivered by cesarean section and a healthy girl weighing 3120 g was born. Cord blood chromosomal analysis of infant revealed a normal female karyotype of 46, XX.

Discussion

Men with nonmosaic Klinefelter's syndrome predominantly show azoospermia. Occasionally, very few men have low rates of spermatozoa in ejaculated semen. Only exceptional cases of proven spontaneous paternity have been reported in the literature (1), but ICSI is now the treatment modality in patients with Klinefelter's syndrome. A few reports described successful pregnancies and deliveries after ICSI with ejaculated spermatozoa. After the first report of successful sperm recovery from azoospermic men with Klinefelter's syndrome by means of TESE in 1996 (3), surgical sperm retrieval has currently revealed spermatozoa in up to 72% of patients with nonmosaic Klinefelter's syndrome selectively referred to centers specializing in assisted reproduction techniques (6). A clinical pregnancy rate between 40% and 56.4% were also reported using the TESE-ICSI procedure (5,6). Moreover, testicular tissue can be successfully cryopreserved in patients with nonmosaic Klinefelter's syndrome. Friedler et al. (5) demonstrated that outcome of ICSI using cryopreserved-thawed testicular spermatozoa seems comparable with that following use of fresh testicular spermatozoa. They reported two pregnancies, which were obtained after ICSI using cryopreserved-thawed testicular spermatozoa resulting in one delivery of twins and one early spontaneous abortion. Thereafter, birth of healthy male twins (7) and one abortion, delivery of two singleton girls and one singleton boy in four pregnancies achieved by ICSI of frozen thawed testicular spermatozoa have also been reported (8). Adult onset of declining spermatogenesis in a man with nonmosaic Klinefelter's syndrome has been reported and age has been shown to be a limiting factor for successful sperm retrieval in these patients (11). Therefore, early sperm retrieval and cryopreservation for future management of infertility can be mandatory in patients with Klinefelter's syndrome.

As fertilization and cleavage rates and quality of embryos were not different than other patients undergoing ICSI, excess good quality embryos can be frozen for subsequent use if pregnancy is not achieved or if the couple desire further pregnancy (4-6). But, only one birth and one ongoing pregnancy have been reported after the transfer of frozen-thawed embryos in these patients (9-10). The first case reported, which resulted in the birth of a healthy male, involved transfer of frozen thawed blastocyst after ICSI of frozen thawed testicular spermatozoa (9). The second case was an ongoing pregnancy after frozen thawed embryo transfer. As far as we know, this report is the third in the literature on the usage of frozen thawed embryos with successful pregnancies. It is therefore recommended that every effort should be done to freeze excess embryos in patients with nonmozaic Klinefelter's syndrome.

Most infants born after ICSI with sperm from men with Klinefelter's syndrome have a normal karyotype, as would be expected from the presence of high proportion of chromosomally normal spermatozoa in these men (1). However, an increased risk of sex chromosome and autosome abnormalities has also been confirmed in these patients (12,13). Therefore, the probability of transmitting abnormal sex chromosome material to offsprings was discussed with the couples and preimplantation genetic diagnosis was offered before embryo transfer. Both of the couples whose cases have been reported here, opted to continue treatment without any preimplantation and prenatal diagnostic intervention.

In conclusion, we report here successful pregnancy outcomes using frozen-thawed testicular spermatozoa from two cases of non-mosaic Klinefelter's syndrome and frozen-thawed embryos resulting in births of healthy babies with normal karyotypes. As the outcome of ICSI is succesful using frozen thawed spermatozoa and the transfer of frozen thawed embryos provides successful pregnancies, every effort should be done to freeze spermatazoa and excess embryos in patients with non-mozaic Klinefelter's Syndrome.

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