CASE REPORT

Pregnancy achieved by intracytoplasmic sperm injection using cryopreserved semen from a man with testicular cancer

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A successful pregnancy was achieved by intracytoplasmic sperm injection (ICSI) using cryopreserved semen from a man with testicular cancer. He was a victim of right testicular seminoma, and was azoospermic after right orchidectomy and radiotherapy. The wife had had three successive failures of intrauterine insemination (IUI) using semen that was cryopreserved before radiotherapy. The couple then underwent in-vitro fertilization (IVF) treatment. ICSI was performed because the sperm motility was extremely poor after thawing. Eight of 12 injected oocytes had normal fertilization and embryo cleavage. After replacement of four embryos, a singleton pregnancy developed. She delivered a healthy male baby at 39 weeks gestation. In addition to IUI and IVF, ICSI further provides male patients with cancer an improved chance of fathering a child. Any men diagnosed with cancer who have not yet finished their families should have their spermatozoa frozen before treatment, regardless of its quality.

Key words: cryopreserved semen/intracytoplasmic sperm injection/testicular cancer

Introduction

Advances in treating patients with testicular cancer have remarkably improved long-term survival. Unfortunately, standard treatment modalities, such as surgery, chemotherapy and radiotherapy, may adversely affect fertility. Cryopreservation of semen combined with intrauterine insemination (IUI) or in-vitro fertilization (IVF) provides such patients with the opportunity to have a child. However, patients with testicular cancer often have poor semen quality, and cryopreservation results in further impairment of sperm motility, which leads to limited success with assisted reproduction (Bracken and Smith, 1980; Sanger et al., 1992). Criteria have been defined for selection and rejection of semen for preservation in some andrology laboratories (Zagars, 1991). In these circumstances, doctors are often not likely to recommend semen cryopreservation for men with testicular cancer. Fortunately, the recently introduced technique of intracytoplasmic sperm injection (ICSI) has proved to be highly effective in cases of severe oligoasthenoteratozoospermia (Van Steirteghem et al., 1993). It may now be possible to help cancer patients with poor semen samples. Here we present the first case report of successful pregnancy by ICSI using cryopreserved semen from a man with testicular cancer. This documentation may encourage doctors to recommend and accept semen cryopreservation for all male cancer patients who desire a child after cancer treatment.

Case report

This couple had suffered from infertility for 4 years since their marriage. The husband, aged 34 years, was diagnosed as having a right testicular tumour during an infertility work-up in April 1994. When histological examination revealed seminoma, he received right orchidectomy. There was no evidence of nodal metastasis on computed tomography examination of pelvis and abdomen. This couple had already failed to achieve any pregnancy for 2.5 years at that time. After discussion with the urologist, he decided to have adjuvant radiotherapy rather than watch-and-wait for a natural conception. Prior to radiotherapy, six samples of semen were cryopreserved for future assisted reproductive treatment. The quality of prefreeze semen was poor. The sperm counts ranged from 10 to 20 × 10^6/ml with motility of 10-30%. The semen was diluted (1:1) with freezing medium (test yolk buffer, Irvine Scientific, Santa Ana, CA, USA), cooled, and stored in liquid nitrogen. The radiotherapy was performed using a linear accelerator for para-aortic and ipsilateral iliac lymph nodes with a total dose of 30 Gy. Testicular shielding was employed to reduce scattered irradiation to the left testis. The post-radiation course was smooth, but an azoospermic state developed thereafter.

The wife, aged 32 years, had regular menstrual cycles, and underwent an infertility work-up, including basal body temperature, hormone profile and hysterosalpingography that showed normal findings. Beginning July 1994, IUI using the cryopreserved semen was attempted three times without success, and poor motility of spermatozoa was noted after thawing. The couple finally decided to enter our IVF programme for further treatment in October 1995. At that time, 18 months after radiotherapy, the husband was still azoospermic. She received ovarian stimulation with a combination of gonadotrophin-releasing hormone agonist (GnRHα; Supremon; buserelin acetate, Hoechst, Frankfurt, Germany),
follicle stimulating hormone (FSH; Metrodin; Serono, Rome, Italy) and human menopausal gonadotrophin (HMG; Pergonal; Serono). When the leading follicles reached a mean diameter of 18 mm with a serum oestradiol level of 1216 pg/ml, 10 000 IU of human chorionic gonadotrophin (HCG; Profasi; Serono) was given. Oocyte retrieval was carried out 34 h later through the vagina under sonographic guidance and 14 oocytes were recovered.

The prefreeze semen analysis showed a count of $19 \times 10^6$ spermatozoa/ml with 25% motility, whereas a post-thaw analysis revealed a count of $8 \times 10^6$/ml with 1% motility. The morphological study using Kruger's strict criteria revealed 31% with normal forms, 39% with slightly abnormal forms, and 30% with severely abnormal forms. The thawed semen was prepared by washing and swim-up procedure, adding it to 3 ml human tubal fluid (HTF) with 15% heat-inactivated maternal serum, and centrifuging at 300 g for 10 min. The pellet was re-suspended with 1 ml of culture medium, and then 1 ml of culture medium was carefully layered over. After incubation in a humidified atmosphere of 5% CO$_2$ at 37°C for 60 min, the supernatant was recovered. The sample contained only $1 \times 10^4$ motile spermatozoa. Because the post-thaw motility and recovery of spermatozoa were poor, ICSI was considered to be the best choice to achieve fertilization. After thorough explanation and discussion, informed consent was obtained from the couple.

The cumulus cells of oocytes were removed by needle dissection in HTF medium containing 80 IU/ml hyaluronidase (type IV-S; Sigma, St Louis, MO, USA), pipetting, and washing. A total of 12 metaphase II (MII) oocytes was obtained for micromanipulation. The sperm sample was centrifuged again, 300 g for 5 min, and was adjusted to 50 μl final volume. A droplet (5 μl) of 10% polyvinylpyrrolidone (PVP; mol. wt 360 000, Sigma) in HTF medium added with the sperm wash (1 μl) and four droplets (5 μl) of 0.01 M HEPES HTF medium with 0.5% human serum albumin containing MII oocytes (one oocyte in each droplet) were placed in a Petri dish and covered with pre-equilibrated mineral oil to prevent evaporation. Micromanipulation was performed with the aid of two micromanipulators and microsyringes (Narishige, Tokyo, Japan) mounted on an inverted microscope (Olympus, Tokyo, Japan) in a modified incubator. A single spermatozoon was immobilized and picked up using the injecting micropipette. The oocyte was held at the 9-o'clock position by gentle suction with a holding micropipette, while the polar body was at the 6 or 12-o'clock position. The zona pellucida was penetrated at the 3-o'clock position. The cytoplasm was aspirated into the injecting pipette, and the oolemma was considered to be broken when a rush of cytoplasmic flow occurred. A spermatozoon was finally released into the cytoplasm for each oocyte. A total of 12 sperm injections was performed, and no oocyte damage was observed.

Eight two-pronuclear zygotes with a second polar body were observed 16 h later. Among them, four 4-cell embryos with grade 1–2 morphology and four 2-cell embryos with grade 1–2 morphology subsequently developed. The four 4-cell embryos were laparoscopically transferred into the right Fallopian tube. Progesterone in oil 25 mg daily, as well as HCG (Pregnyl; Organon, Oss, Holland) 1500 IU on days 2, 5, and 8, were administered IM after transfer. The serum β-HCG concentration was 93.5 mIU/ml 14 days later. A singleton pregnancy developed and a healthy male baby was delivered at 39 weeks gestation. The husband remains well without evidence of recurrence of tumour. The regular follow-up semen analysis showed a recovery of sperm output 24 months after the radiotherapy.

Discussion

This report documents the successful application of ICSI in establishing pregnancy using a poor frozen-thawed semen sample, preserved before radiotherapy, from a man with testicular cancer. This is the first such report in English literature. The reported cumulative pregnancy rates after IUI with cryopreserved semen from men with cancer range from 20 to 45% (Sanger et al., 1992). IVF has been successful, with a lower number of motile spermatozoa than has IUI, and may achieve a higher pregnancy rate. However, the fertilization rates in IVF using these cryopreserved semen samples are still low and some fertilization failures may occur (Tournaye et al., 1991). Generally, these cancer patients have only a restricted number of semen samples preserved. Currentpractice is to try IUI for several cycles. If a pregnancy does not occur, then the option of IVF is pursued. ICSI has a high fertilization rate, requires a minimal number of spermatozoa, and may secure a successful treatment. The poor semen from our patient with testicular cancer seemed more vulnerable to cryopreservation. The prefreeze sample with $19 \times 10^6$ spermatozoa/ml with 25% motility was reasonable, whereas the post-thaw condition revealed a poor count of $8 \times 10^6$/ml with 1% motility. After sperm washing, only $1 \times 10^4$ motile spermatozoa were available. Therefore, we chose ICSI instead of conventional IVF to treat this particular couple.

Although the remaining testis in patients treated this way is protected by shielding, the small amount of scattered irradiation usually induces acute depression of spermatogenesis which poses a threat to fertility potential. Fortunately, the majority of patients with post-radiotherapy azoospermia start to recover sperm output at variable times from 12 months onwards. The recovery may be related to the gonadal dose and the severity of impairment to spermatogenesis before radiotherapy (Hansen et al., 1990). In many patients this impairment appears to be reversible, as shown by much higher recovery counts from the single remaining testis compared with the relatively poor output from two testes at the time of diagnosis (Zagars, 1991). Post-radiotherapy paternity has been well documented, and no increased chromosomal abnormalities have been found in these babies (Fossa et al., 1986; Zagars, 1991). For those patients without recovery of spermatogenesis, the semen cryopreserved before radiotherapy can be used to achieve a pregnancy with the aid of assisted reproduction treatment. Furthermore, the incidence of miscarriages or birth defects in the reported pregnancies from cryopreserved semen in male cancer patients is not increased (Sanger et al., 1992).

Post-treatment fertility represents an important concern in patients who have undergone unilateral orchidectomy for
testicular cancer. For some patients with non-seminomatous testicular cancer suffering from anejaculation due to retroperitoneal lymph node dissection, Hultling et al. (1995) have successfully used transrectal electroejaculation and IVF to help those couples achieve pregnancies. The improvements in treatment modality and assisted reproduction treatment provide these patients with a realistic opportunity to father children. The exclusion criteria for semen cryopreservation should be abandoned, considering successful pregnancies with even extremely poor semen are now possible with ICSI. Therefore, any men with a diagnosed cancer who have not yet finished their families should have their spermatozoa frozen, regardless of its quality. The strategy regarding how many semen samples should be cryopreserved, sufficient to establish a pregnancy, without delaying cancer therapy merits further elucidation. The optimal choices (IUI, IVF, or ICSI) for each individual and the cumulative pregnancy rates using these methods deserve further study.

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