Successful Spouse Pregnancy of Male Patients with Severe Aplastic Anemia and Chronic Myelogenous Leukemia Using Spermatozoa Banked Prior to Bone Marrow Transplantation and Using the ICSI Procedure: *Case Reports*

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Purpose: To report two cases of successful spouse pregnancies which were conceived with long-term cryopreserved spermatozoa that had been collected prior to the male patients receiving a bone marrow transplant.

Methods: The first case is the pregnant wife of a 25-year-old man with chronic myelogenous leukemia, whose semen was collected before bone marrow transplant and then cryopreserved, thawed, and then injected into the wife's eggs via ICSI. The second case is a 28-year-old man with severe aplastic anemia who became a father after his wife's eggs were fertilized via ICSI with thawed spermatozoa.

Results: These two cases were achieved pregnancies.

Conclusions: These cases support research that men with malignancy have the chance of fathering their own genetic children. Therefore, it is important to increase the awareness of clinicians, oncologists, and patients to the new developments in preserving fertility for cancer patients.

KEY WORDS: ART; CML; ICSI; SAA; semen cryopreservation.

INTRODUCTION

The survival rates of young men who have suffered from various types of cancer have recently improved dramatically because of advanced diagnos-

tic techniques and better treatment modalities. A majority of the patients having leukemia have been able to survive by using anticancer drugs and bone marrow transplantation. However, these young male patients diagnosed with malignant diseases, treated with chemotherapy and radiotherapy, are concerned of the possibilities of permanent infertility caused by the diseases and treatment (1–3). Forthcoming assisted reproduction has given a chance for these married male partners and the unmarried young male patients diagnosed with malignant cancers to become genetic fathers (4,5). Since 1998, our hospital has cryopreserved the semen of married and unmarried young males diagnosed with malignancy. Now, we report two cases of successful pregnancy of which long-term cryopreserved spermatozoa were used to fertilize the spouses' eggs via ICSI.

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CASE REPORT

Case 1

A 25-year-old man presented the CHA Infertility Medical Center in March 1998 with chronic myelogenous leukemia (CML) and was referred to this unit for semen banking. After treatment of hydroxyurea and interferone-alpha, he banked three semen samples, with sperm concentrations ranging $2\text{--}6 \times 10^6/\text{mL}$, motility 16–44%. The three semen samples were frozen in 8 vials. This patient received radiotherapy on his whole body and a bone marrow transplant. After treatment, the condition of patient was completely mitigated. After transplantation, repeated semen analysis via high-speed centrifugation in 1999 and 2001 showed azoospermia.

First Cycle. This patient and his wife wanted to have children. In view of the low sperm count and motility, we decided to perform ICSI. Ovarian stimulation was carried out using a GnRH agonist in a long protocol, Buserelin acetate, 0.3 mg, injected every day on mid-luteal phase. And on day 3 pure FSH (Metrodin-HP[®], Serono) and HMG (Humegon[®], Organon) was applied for superovulation. Transvaginal ultrasound showed four follicles with a diameter of ≥ 18 mm. On day 12 a serum oestradiol concentration of 1337 pg/mL, 10,000 IU of HCG (Profasi[®], Serono), was administered and 10 oocytes were retrieved 36 h later.

Seven oocytes were normally fertilized and 3 days after oocytes retrieval, seven embryos were transferred, but the serum B-hCG level obtained a negative result.

Second Cycle. Ovarian stimulation was carried out without using a GnRH agonist. Superovulation was induced using recombinant human FSH (Gonal F[®], Serono) and HMG (IVF-M[®], LG) directly. Transvaginal ultrasound showed two follicles with a diameter of ≥ 18 mm. On day 10 a serum of oestradiol concentration of 1152 pg/mL, 10,000 IU of HCG (Profasi[®], Serono), was administered and 7 oocytes were retrieved 36 h later. Three oocytes were normally fertilized by ICSI and 3 days after oocytes retrieval, three embryos were transferred.

Two weeks later, the pregnancy test was positive using a serum of β -hCG 500 mU/mL. A transvaginal ultrasound scan at 6 weeks showed two live birth gestations with two 4-mm-sized fetuses with heart beats.

Case 2

A 28-year-old man presented the CHA Infertility Medical Center in July 1998 with severe aplastic ane-

mia (SAA) and was referred to this unit for semen banking. He banked two semen samples, with sperm concentrations of $35 \times 10^6/\text{mL}$, motility 27%, and $100 \times 10^6/\text{mL}$, motility 47%. The two semen samples were frozen in two vials. This patient received a bone marrow transplant, and afterwards the patient was determined to be completely healthy.

This patient and his wife intended to be parents and actively took steps for his wife to become pregnant in December 2001.

Her hysterosalpingography showed bilateral salpingeal obstruction, so we decided to perform ICSI and embryo transfer. Ovarian stimulation was carried out using a GnRH agonist in a long protocol. Buserelin acetate, 0.3 mg, injected on mid-luteal phase, and then reduced to 0.1 mg. On day 3 pure FSH (Metrodin-HP[®], Serono) and HMG (Pergonal[®], Organon) was injected for superovulation. Transvaginal ultrasound showed six follicles with a diameter of ≥ 18 mm. On day 9 a serum of oestradiol concentration of 4038 pg/mL, 10,000 IU of HCG (Profasi[®], Serono), was administered and 11 oocytes were retrieved 36 h later. Five oocytes were normally fertilized and 3 days after oocytes retrieval, five embryos were transferred.

Pregnancy was confirmed with a serum of B-hCG of 87.6 mU/mL 2 weeks later. A transvaginal ultrasound scan at 6 weeks showed a living embryo. Gestation culminated with the birth of a healthy male baby that weighed 3.75 kg of weight.

DISCUSSION

The only effective way for young cancer patient to ensure future fertility is via semen cryopreservation (6). In case of patients with CML the average diagnosed age is 45–50. And, the age of patients diagnosed with SAA is 25 (7). Bone marrow transplantation is the most operative therapy for CML and SAA, but immunosuppressive drug or radiotherapy disrupt spermatogenesis and could result in infertility.

On the other side, malignant cancer itself may produce a quite contrary result to reproducibility, without any anticancer drug treatment or radiotherapy (4,8). For example, the patient with testicular cancer showed reduced sperm count and motility before medical treatment when compared with other patient with malignant cancer (9–11). The sperm characteristics of Hodgkin lymphoma patients were extremely impaired in comparison with those of non-Hodgkin lymphoma patient.

And then, most cases with impaired sperm quality before treatment showed a poor outcome of ART

after thawing (11–13). There are some cancer malignancies and other malignancies that occur during a patient's reproductive age. Therefore when a patient is diagnosed with CML or SAA, sperm cryopreservation should be done as soon as possible.

In case 1, the patient attended 6 months later after diagnosis of CML and showed reduced sperm count and motility, but cryopreservation of semen was possible. His semen was collected three times with an interval of 2–3 days.

Lass (5) recommended that a properly designed program for cryopreservation of semen for cancer patients had to be developed, and advocated that any sperm sample containing motile spermatozoa should be frozen, without regard to its quality. According to count and motility of postthaw sperm, an adequate ART technique, that is IVF and embryo transfer, and ICSI, should have been decided (14). Males diagnosed to be malignant have showed reduced sperm count and motility. However, most of them have been able to successfully use sperm cryopreservation. In recent days, malignant cancer patients and their female partners have been able to achieve pregnancy due to the newly developing ART techniques, ICSI.

Therefore, it is very important for cryopreservation of semen immediately in male patients during their reproductive ages that are diagnosed malignant. All—clinicians, patients, and spouses—have to recognize the necessity of sperm cryopreservation for the future parenthood of the cancer inflicted. In conclusion we report two cases of successful pregnancies following long-term cryopreserved spermatozoa prior to BMT for CML and SAA.

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REFERENCES

1. Horne G, Atkinson A, Brison DR, Radford J, Yin JAL, Edi-Osagie ECO, Pease EHE, Lieberman BA: Achieving pregnancy against the odds: Successful implantation of frozen-thawed embryos generated by ICSI using spermatozoa banked prior to chemo/radiotherapy for Hodgkin's disease and acute leukaemia: Case report. *Hum Reprod* 2001;16:107–109
2. Tournaye H, Camus M, Bollen N, Wisanto A, Van Steirteghem AC, Devroey P: In vitro fertilization techniques with frozen-thawed sperm: A method for preserving the progenitive potential of Hodgkin patients. *Fertil Steril* 1991;55:443–445
3. Bokemeyer C, Schmoll HJ, van Rhee J, Kuczyk M, Schuppert F, Poliwoda H: Long-term gonadal toxicity after therapy for Hodgkin's and non-Hodgkin's lymphoma. *Ann Hematol* 1994;68:105–110.
4. Meirow D, Schenker JG: Cancer and male infertility. *Hum Reprod* 1995;10:2017–2022
5. Lass A, Akagbosu F, Abusheikha N, Hassouneh M, Blayney M, Avery S, Brinsden P: A programme of semen cryopreservation for patients with malignant disease in a tertiary infertility centre: Lessons from 8 years' experience. *Hum Reprod* 1998;13(11):3256–3261
6. Padron OF, Sharma RK, Thomas AJ Jr, Agarwal A: Effects of cancer on spermatozoa quality after cryopreservation: A 12-year experience. *Fertil Steril* 1997;67(2):326–331
7. Keating MJ: Cecil textbook of medicine: Chronic leukemia, 19th rev edn. Philadelphia: Saunders Press, 1992
8. Fossa SD, Aass N, Molne K: Is routine pre-treatment cryopreservation of semen worthwhile in the management of patients with testicular cancer? *Br J Urol* 1989;64:524–529
9. Berthelsen JG: Sperm counts and serum follicle-stimulating hormone levels before and after radiotherapy and chemotherapy in men with testicular germ cell cancer. *Fertil Steril* 1984;41:281–286
10. Botchan A, Hauser R, Yorgev L, Gamzu R, Paz G, Lessing JB, Yavetz H: Testicular cancer and spermatogenesis. *Hum Reprod* 1997;12:755–758
11. Khalifa E, Oehninger S, Acosta AA, Morshedi M, Veeck L, Bryzyski RG, Muasher SJ: Successful fertilization and pregnancy outcome in in-vitro fertilization using cryopreserved/thawed spermatozoa from patients with malignant diseases. *Hum Reprod* 1992;7:105–108
12. Vigersky RA, Chapman RM, Berenberg J, Glass AR: Testicular dysfunction in untreated Hodgkin's disease. *Am J Med* 1982;73:482–486
13. Sanger WG, Olson JH, Sherman JK: Semen cryobanking for men with cancer—Criteria change. *Fertil Steril* 1992;58:1024–1027
14. Chen SU, Ho HN, Chen HF, Huang SC, Lee TY, Yang YS: Pregnancy achieved by intracytoplasmic sperm injection using cryopreserved semen from a man with testicular cancer. *Hum Reprod* 1996;11:2645–2647