

## CASE REPORT

# Recovery of spermatogenesis and successful conception after bone marrow transplant for acute leukaemia

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**A case is presented of a young adult male diagnosed with acute myeloid leukaemia who was treated with busulphan and cyclophosphamide, but not total body irradiation, with subsequent bone marrow transplantation. After surviving for 5 years, he and his wife experienced a period of infertility. Interestingly, the problem was found to be with the female partner and the man's sperm evaluation seemed normal. A successful pregnancy ensued following the surgical removal of a large endometrioma and treating a luteal phase deficiency. Though this may be the fifth case of proven parentage in cases of bone marrow conditioning and bone marrow transplantation in a male for control of leukaemia, we believe it is the first documented case showing a normal semen analysis despite treatment. Further studies are needed to see if chemical conditioning with busulphan and cyclophosphamide preserve the chances of sperm production better than total body irradiation.**

*Key words:* bone marrow conditioning/pregnancy/spermatozoa

### Introduction

It has been reported that all men undergoing total body irradiation for bone marrow conditioning had gonadal failure for up to 5 years (Sanders *et al.*, 1983). There are two recent reports, however, of paternity after bone marrow transplantation conditioning with total body irradiation in patients with chronic myeloid leukaemia (Facon *et al.*, 1993; Pakkala *et al.*, 1994). Despite pregnancy achieved only 3 years after bone marrow transplantation, no semen analysis was mentioned in one case (Facon *et al.*, 1993). Interestingly, despite establishing paternity, the male partner in the other case had hypogonadism and a zero sperm count, suggesting at best only transient sperm production with numbers mostly zero or close to it in the second case (Pakkala *et al.*, 1994).

Bone marrow conditioning with busulphan and cyclophosphamide theoretically is less likely to have a permanent adverse effect on sperm production. Two case reports have been

published with paternity established for two men with acute myeloid leukaemia who received conditioning for bone marrow transplantation with the above chemotherapy agents (Shepherd *et al.*, 1996; Lipton *et al.*, 1999). In one case, despite the paternity documentation, the sperm concentration was only  $\sim 1 \times 10^6/\text{ml}$  with a few motile spermatozoa (Shepherd *et al.*, 1996). No semen analysis report was mentioned in the second case (Lipton *et al.*, 1999).

The case presented here is apparently the fifth case report of paternity following bone marrow conditioning for bone marrow transplantation and the third case of paternity following the use of busulphan and cyclophosphamide conditioning for bone marrow transplantation in males with acute myeloid leukaemia (AML). However, it is the first case demonstrating preservation of a fertile sperm specimen despite the use of chemotherapy to treat the AML and the chemotherapy used for bone marrow conditioning.

### Case report

A 25 year old male was diagnosed with AML in 1987. Prior to chemotherapy, one sperm sample was cryopreserved in the office of a reproductive endocrinologist; the sample was not evaluated before freezing. The patient was treated with daunorubicin 45 mg/m<sup>2</sup>/day by rapid i.v. infusion and Ara-C 200 (mg/m<sup>2</sup>/day) for 5 days by continuous i.v. infusion. Subsequent bone marrow aspiration and biopsy after 14 days of therapy showed complete remission. After induction of remission, Ara-C 3 (gm/m<sup>2</sup>) was given over 3 days every 12 h every other day for six doses. Thereafter, Ara-C was given (100 mg/m<sup>2</sup> s.c.) every 12 h for 5 days every 28 days for 4 months. Daunorubicin (45 mg/m<sup>2</sup>) was also given by rapid i.v. infusion for one dose; the treatment ended in 1988. The patient's wife conceived in 1988 at the age of 24 years; unfortunately she miscarried in the first trimester.

While in remission, a bone marrow harvest was performed and the stem cells were treated with monoclonal antibodies raised in mice against human leukaemia cells, and then frozen. The patient relapsed in 1989 and he was then treated with bone marrow conditioning as follows: days 1-4 busulphan 1 mg/kg every 6 h, days 5 and 6 cyclophosphamide 60 mg/kg once daily; on day 9, autologous bone marrow transplantation was performed. Treatment was concluded by 1990.

The couple sought infertility treatment with another infertility centre in 1994. They were not successful and came to our centre in 1995. Their initial visit demonstrated a normal post-coital test with last intercourse having been 48 h before. A

semen analysis using a manual method with a microcell counting chamber performed in 1995 revealed the sperm concentration to be  $30.8 \times 10^6/\text{ml}$  with 54% motility, volume 1.8 ml, with no spermatozoon with rapid linear progression but 45.9% with slow or non-linear progression. Sperm viability was 94% and the hypo-osmotic swelling test was 82%. Sperm morphology using strict criteria found 5% normal and 1% slightly abnormal forms. Antisperm antibodies, using the immunobead test, were negative.

The female partner was diagnosed with premature luteinization, luteal phase deficiency, and a large endometrioma (54 mm by ultrasound). The endometrioma was removed by laparoscopy, and she was treated with leuprolide acetate 1 mg days 1–3 to prevent premature luteinization. Follicular maturation studies showed that she attained a mature follicle (19.3 mm average diameter with a serum oestradiol concentration of 203 pg/ml) and the oocyte was released without human chorionic gonadotrophin (HCG) injection. Progesterone support using progesterone vaginal suppositories 50 mg twice a day was given and she conceived that cycle. She delivered a healthy normal male by Caesarean section at 42 weeks.

Using similar treatment, the couple attempted pregnancy again at the end of 1998. She conceived after 2 months of therapy but had an early first trimester spontaneous abortion. The semen analysis was repeated in 1999. The volume was 2.7 ml with a sperm concentration of  $12.5 \times 10^6/\text{ml}$  with 62.0% motility with 25% having rapid linear motion and 55% having slow or non-linear motion. The normal morphology was 12% using strict criteria and the hypo-osmotic swelling score was 79%. Antisperm antibodies were once again not present. The patient, now satisfied with the persistence of normal spermatogenesis, requested that the specimen frozen > 10 years should be discarded. Though no analysis had been performed before freezing, a concentration of  $4.3 \times 10^6/\text{ml}$  with 2% motility of the non-progressive type was found in the post-thaw specimen.

## Discussion

The demonstration of subsequent parenthood from men receiving total body irradiation in preparation for bone marrow transplantation did not provide evidence that spermatogenesis may resume despite this therapy but, merely showed that occasionally men may achieve pregnancies when hardly any spermatozoa are present (Facon *et al.*, 1993; Pakkala *et al.*, 1994). Nevertheless, complete azoospermia is usually the case (Sanders *et al.*, 1983).

The use of chemotherapy for leukaemia with busulphan and cyclophosphamide was hoped to provide bone marrow conditioning for transplantation without having such a devastating effect on future spermatogenesis in case of the patient's survival. Although two cases of parenthood after bone marrow conditioning with these agents have been reported, neither one demonstrated preservation of spermatogenesis, and failed to demonstrate any superiority of the treatment over total body irradiation (Shepherd *et al.*, 1996; Lipton *et al.*, 1999).

The case presented here was the first to demonstrate the preservation of spermatogenesis despite bone marrow condi-

tioning with busulphan and cyclophosphamide. The recovery of spermatogenesis was evident by 5 years of therapy but it is not clear whether the treatment did produce azoospermia for a time and how long it took for recovery, since there were no semen analyses performed during the 5 years after bone marrow transplantation.

The fact that this patient is still in remission from AML 10 years after diagnosis, and >9 years from busulphan and cyclophosphamide conditioning followed by bone marrow transplantation, demonstrates that this chemotherapeutic regimen can be effective for controlling this dreaded disease. Only time will provide the answer as to whether total body irradiation and this chemotherapy combination are equal in accomplishing bone marrow conditioning and maintaining long remission times. This case suggests that if equal efficacy for bone marrow conditioning is confirmed, the use of busulphan and cyclophosphamide may be a better choice than total body irradiation for a young male who may later wish to father a child.

However, one should not imply from this case report that drugs, e.g. cyclophosphamide, do not adversely affect spermatogenesis. Alkylating agents, e.g. cyclophosphamide, may be the class of chemotherapeutic drugs that have the greatest detrimental effects on spermatogenesis (Sweet *et al.*, 1996). Azoospermia following cyclophosphamide treatment has been reported in two patients receiving cyclophosphamide, but preservation of normal spermatogenesis was found in a patient with non-Hodgkin's lymphoma treated with this drug (Naysmith *et al.*, 1998). The significance of the case presented here is that the dosage of alkylating agents needed for bone marrow conditioning is usually higher than the dosage used in treatment protocols.

As pointed out by Naysmith *et al.* (1998), in cases when patients of reproductive age are confronted with the diagnosis of cancer, the patient and treating physician put all their efforts in treating the disease and do not always think to cryopreserve some semen specimens before therapy to preserve their future fertility potential. The patient described here did cryopreserve a sample but it was never used. Though this man was fortunate in retaining normal spermatogenesis, others, given exactly the same therapy, may develop permanent sterility. Thus, it is imperative for the treating physician to encourage the cryopreservation of a specimen prior to chemotherapy. Male patients of reproductive age, who are to be treated for cancer should be advised that, although spermatogenesis may be preserved, permanent azoospermia may ensue (Stuart *et al.*, 1990). Sometimes spermatogenesis may take up to 5 years to resume, but with cryopreserved spermatozoa, the couple may begin a family without further delay (Lampe *et al.*, 1997; Lass *et al.*, 1998).

Another reason for cryopreservation prior to therapy is the possibility of genotoxicity. In this case, the patient's first pregnancy that ended in miscarriage might have been related to a chromosome abnormality possibly related to the effect of the chemotherapy on the DNA. Though she was fortunate subsequently to have a normal full-term child, in some instances it may be safer to use the cryopreserved specimen first. In this case, this would not have been practical since the frozen-

thawed specimen demonstrated only a few poorly motile spermatozoa after thawing. The couple reported here chose not to thaw the specimen first, in case more therapy was needed for the husband and either his spermatozoa could not be used fresh for a period of time or the semen parameters might have decreased to subfertile values. We had also advised her that frequently this type of specimen would be poor, and in-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) would be recommended. She also chose to try for pregnancy with the fresh ejaculate to circumvent the expense and risk of IVF.

More studies are needed to compare the efficacy and risks of total body irradiation compared with chemotherapeutic bone marrow conditioning with agents, e.g. busulphan and cyclophosphamide. However, if the two techniques are proven to be equally effective, one reason for choosing the chemotherapeutic method is a possible better chance of sperm recovery in the future.

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