A programme of semen cryopreservation for patients with malignant disease in a tertiary infertility centre: lessons from 8 years’ experience

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Introduction
In recent years, the survival rates of young men suffering from various types of cancer have improved dramatically due to advanced diagnostic techniques and better treatment modalities. In many cancer subjects, sperm quality is already reduced before receiving any treatment (Fossa et al., 1989; Meirov and Schenker, 1995). Further deterioration has been observed due to the damaging effect of chemotoxic agents on spermatogenesis, which may be temporary or permanent (Buchanan et al., 1975; Drasga et al., 1983; Johnson et al., 1984; Fossa et al., 1985; Hansen et al., 1990; Presti et al., 1993; Pont and Albrecht, 1997). Up to 90% of patients have azoospermia a few weeks after chemotherapy and only 20–50% resume spermatogenesis two to three years after completion of treatment (Kreuser et al., 1986; Nijman et al., 1987). Although the gonadotoxic effect of the agents depends on their type, dose and number of treatment cycles (Meirov and Schenker, 1995; Pont et al., 1997), it is impossible to predict who will have normal spermatogenesis and who will remain azoospermic. Many of these patients are young men who have neither started nor completed their families. This factor, combined with the developments in the treatment of male fertility, especially intracytoplasmic sperm injection (ICSI), motivated patients and clinicians to preserve the fertility potential of cancer patients before embarking on adjuvant therapy. For that purpose, we established in 1989 at Bourn Hall Clinic, a tertiary assisted conception centre, a programme of semen cryopreservation for patients with cancer. In this study, we present our experience from 8 years of this service.

Materials and methods

The programme
Between August 1989 and December 1997, 231 men diagnosed with malignant disease (mean age 28.0; range 15–56 years) were referred to our unit by oncology and/or haematology specialists in East Anglia, for semen cryopreservation before proceeding with chemotherapy. All patients were seen by a specially assigned nurse for the programme (mostly M.H.), and received an information leaflet about the programme. This was followed by consultation with one of the clinic’s medical team, when the logic of semen cryopreservation and the options for future use were discussed. Blood samples were taken for human immunodeficiency virus (HIV) and hepatitis B and C screening, independent counselling was offered, and informal consent taken. The patients gave written instruction whether the spermatozoa would be the property of their partner or disposed of in the event that they died from their illness.

Seventy-nine men (34.2%) had testicular tumours (group I), the majority having undergone unilateral orchidectomy; 121 men (52.4%) suffered from haematological malignancy (mostly leukaemia or lymphoma). Between August 1989 and December 1997, 231 men diagnosed with malignant disease (mean age 28.0; range 15–56 years) were referred to our unit by oncology and/or haematology specialists in East Anglia, for semen cryopreservation before proceeding with chemotherapy. All patients were seen by a specially assigned nurse for the programme (mostly M.H.), and received an information leaflet about the programme. This was followed by consultation with one of the clinic’s medical team, when the logic of semen cryopreservation and the options for future use were discussed. Blood samples were taken for human immunodeficiency virus (HIV) and hepatitis B and C screening, independent counselling was offered, and informal consent taken. The patients gave written instruction whether the spermatozoa would be the property of their partner or disposed of in the event that they died from their illness.

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lymphoma; group II) and 31 (13.4%) had cancer of different causes (group III; Table I). The three groups were similar in age and number of pregnancies achieved in the past. The time from diagnosis to referral was 4.2–8.6 weeks for all patients. Thirty-five patients (15.1%) had undertaken at least one course of chemotherapy before referral for semen cryopreservation.

**Semen cryopreservation**

The ejaculate was obtained by masturbation into two dry pots and the pre-freeze semen sample was analysed according to WHO guidelines (WHO, 1992). Post-thaw analysis was not done routinely because in many cases the sperm concentration was so low that performing this test would have jeopardized the amount available for freezing. Cryopreservation was performed on any semen sample containing motile spermatozoa by our routine protocol (Richardson, 1979). Semen was diluted with cryoprotectant and 0.7 ml aliquots were transferred to 1.5 ml screw-top plastic vials to enable future use without the need to thaw the whole sample. Samples were cooled by suspension in vapour phase nitrogen at a rate of approximately $-10^\circ C/min$, for 30 min, and then transferred to liquid nitrogen.

A decision was taken from the beginning of the programme to freeze any sample with motile sperm, even if it was below the required minimum for standard in-vitro fertilization (IVF) (in our unit a total of $2 \times 10^6$ motile spermatozoa/ml). Of 231 men referred to our unit for sperm cryopreservation, successful freezing was done in 191 cases. In 40 cases (17.3%), cryopreservation was not performed because of complete azoospermia in the ejaculate in spite of repeated attempts ($n = 32$), or the presence of immotile spermatozoa only ($n = 2$). Six patients were not able to produce a sample and they were excluded from this analysis. Patients were asked to give a sample every 2–3 days until they had a total of 12 ampoules frozen. However, many of them had to start chemotherapy immediately and therefore gave only one or two samples (mean = $7.7 \pm 4.4$ ampoules/patient). For each patient, means of the following sperm variables were calculated: volume, concentration (spermatozoa/ml), motility and total motile spermatozoa/ejaculate.

**Data analysis**

Statistical analysis of the data was performed by the Stat View statistical package (Abacus Concepts Inc, Berkeley, CA, USA).

### Table I. Origin of cancer in testicular (group I), haematological (group II) and solid tumour (group III) cancer patients

<table>
<thead>
<tr>
<th>Testicular cancer (group I)</th>
<th>No. of patients</th>
<th>Haematological malignancy (group II)</th>
<th>No. of patients</th>
<th>Solid tumours (group III)</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminoma</td>
<td>17</td>
<td>Aplastic anaemia</td>
<td>1</td>
<td>Bladder carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Non-seminoma</td>
<td>47</td>
<td>Leukaemia</td>
<td>22</td>
<td>Ampula of Vater carcinoma</td>
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</tr>
<tr>
<td>Unspecified</td>
<td>15</td>
<td>Lymphoma</td>
<td>98</td>
<td>Gastric carcinoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hodgkin’s</td>
<td>64</td>
<td>Colon carcinoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Hodgkin’s</td>
<td>34</td>
<td>Glyoma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver carcinoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Melanoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Malignant thymoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medulloblastoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nasopharyngeal carcinoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neuroblastoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sarcoma</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Haemangiosarcoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Osteosarcoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rhabdomyosarcoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leiomyosarcoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other sarcoma</td>
<td>6</td>
</tr>
</tbody>
</table>

**Totals** 79                                      121                                      27

Mean ± SE were calculated for all variables with the exception of semen parameters and time, for which the median and range were calculated because of non-normal distribution. A Mann–Whitney U-test, chi-square test and Spearman correlation test where used when appropriate. $P < 0.05$ was considered statistically significant.

### Results

Among the 231 patients diagnosed with cancer, only 225 gave at least one semen sample for cryopreservation. Of these, 112 patients (49.8%) had reduced sperm quality, i.e. $<10^6$ motile spermatozoa per ejaculate. Patient age did not correlate with sperm count or motility ($r = 0.063$ and $-0.035$, respectively).

Fifty-five men (23.8%) had fathered a child before their illness, while the remaining 176 had not achieved any pregnancies; most of them were single. There was no difference in semen variables between patients who had children before their illness and those who did not, although the former were significantly older (33.1 ± 7.3 versus 25.9 ± 6.5 years; $P < 0.001$).

Of the 35 patients who had had chemotherapy treatment before attending the clinic for semen cryopreservation, 12 (34%) were azoospermic compared with 20 azoospermic men out of 190 men without previous chemotherapy (10.5%; $P = 0.006$). Moreover, sperm concentration and motility were significantly reduced in patients after chemotherapy (Table II). Because of the strong effect of previous chemotherapy on sperm quality, these patients were excluded from further analysis.

The median time from diagnosis of cancer to the first semen assessment at Bourn Hall was 3 weeks (range 1 day to 16 weeks) and was not correlated to semen parameters, although men in group II were referred for semen cryopreservation earlier than men in group I (2 versus 3.5 weeks; $P = 0.004$).

Men with testicular cancer (group I) had significantly lower sperm concentration and motility than patients with other forms of malignancy (Table III). In group I, 16 men had
In our clinic we do not actively follow up patients after completion of their chemotherapy; thus, we do not have data on their survival or whether they have been able to conceive spontaneously. Patients are advised to give semen samples about 6 months into remission to assess restoration of fertility capacity, but very few chose to do so.

Following chemotherapy, only six couples have attended the clinic so far for assisted conception treatment using the frozen semen. Three male partners had Hodgkin’s lymphoma, one had non-Hodgkin’s lymphoma, one had testicular cancer and the sixth had acute hairy cell leukaemia. In all patients, the method of treatment was tailored depending on the quality of the frozen spermatozoa and the standard criteria of our unit. Therefore, two couples were treated by intrauterine insemination (IUI) which resulted in delivery of a healthy girl in each case. Two other couples underwent IVF; one conceived at the first attempt and had healthy twin girls, and the other achieved only biochemical pregnancy after numerous IVF and frozen embryo replacement cycles. The remaining two patients required ICSI treatment because of the poor sperm quality and both are currently pregnant beyond the first trimester (Table V).

### Discussion

This study presents the experience of Bourn Hall Clinic which has established a programme that combines the facilities of sperm cryopreservation with the whole range of sophisticated assisted reproductive technologies for the treatment of the female partners of male cancer patients.

Few authors have demonstrated impaired sperm quality in testicular cancer patients (Berthelsen, 1984; Fossa et al., 1989; Meirow and Schenker, 1995; Botchan et al., 1997a). The aetiologies for reduced sperm quality are fibrosis of the seminiferous tubules (Berthelsen and Skakkebaek, 1983), the presence of sperm antibodies (Guazzieri et al., 1985), and endocrine activity of raised levels of β-human chorionic gonadotrophin (β-HCG) and α-fetoprotein (Berthelsen and Skakkebaek, 1983; Carroll et al., 1987; Meirow and Schenker, 1995). Moreover, the majority of patients have produced semen samples after orchidectomy and the 50% reduction in germinal epithelium may contribute to the lower sperm concentration (Botchan et al., 1997a). Impaired spermatogenesis is related neither to the clinical stage of the disease nor to the duration and severity of the symptoms (Hendry et al., 1983; Hansen et al., 1990; Fossa et al., 1994).

The effect of the type of testicular cancer on spermatogenesis is not clear. While Botchan et al. (1997a) and Padron et al. (1997) have shown a higher sperm count in seminoma patients compared with non-seminoma patients, others failed to demonstrate such a difference (Thachil et al., 1981; Fossa et al., 1984). In our study the sperm parameters were similar in seminoma and non-seminoma testicular cancer groups.

Few studies have found decreased spermatogenesis in lymphoma patients compared with healthy volunteers (Chapman et al., 1981; Hendry et al., 1983; Marmor et al., 1986; Botchan et al., 1997b). Data of semen parameters in different types of cancer disease in the same centre are very
limited, and only recently Padron et al. (1997) demonstrated similar semen quality in men with Hodgkin’s disease, leukaemia and testicular cancer. In this study we have found, in a relatively large series, that the sperm quality of testicular cancer patients was significantly lower compared with spermatozoa of men with any other type of malignant disease. It seems therefore that the local effect of testicular cancer is stronger than the systemic influence of cancer on spermatogenesis.

Our results confirm the findings of Botchan et al. (1997b) that patients with Hodgkin’s disease have a lower sperm quality than those with non-Hodgkin lymphoma. The reason for this difference is not clear and probably is unrelated to the clinical stage or duration of symptoms (Viviani et al., 1991; Shekarriz et al., 1995). The effect of prolonged hyperthermia, frequently observed in these cases, could be a possible cause of the decrease in sperm quality. Malignant diseases other than testicular cancer, lymphoma or leukaemia are fortunately quite rare in the younger population; therefore, information about spermatogenesis in these situations is scarce. A few case reports which included endocrine, brain, thyroid and adrenal tumours were summarized by Meirow and Schenker (1995). In this study we have shown that men with soft tissue or solid tumours (group III) have spermatogenesis decreased to a level similar to that in patients with haematological malignancy, but significantly better than in testicular cancer patients. In the solid tumour patients (group III), there was a delay of a few weeks to referral for semen cryopreservation compared with other types of cancer. The causes of the delay were a lower level of awareness for semen banking by the medical team due to lack of data about the effectiveness of this option, and a longer investigation period to achieve accurate diagnosis and staging of the primary disease in solid tumours. Although there was no correlation between the time lag and sperm parameters, it is vital to send these patients for semen cryopreservation immediately after diagnosis, to enable them to give enough semen samples before starting chemotherapy that most will require. Reassuringly, Agarwal et al. (1995) have demonstrated that in cancer patients, semen after abstinence of 24–48 h has a post-thaw quality similar to that of semen obtained after a longer abstinence period; therefore, the period between diagnosis and starting chemotherapy can be shortened, without jeopardizing the frozen sperm quality.

Our results also confirm the deleterious effect of chemotheraphy on sperm quality. The effects of cytotoxic agents on short- and long-term spermatogenesis have been investigated mainly in testicular cancer patients (Drasga et al., 1983; Johnson et al., 1984; Fossa et al., 1985; Hansen et al., 1990; Aass et al., 1991) and were summarized in detail by Pont and Albrecht (1997). Alkylating agents have the most deleterious effect on sperm quality, and the total dose of the agent and number of treatment cycles are also important. After more than four cycles of cis-platinum-based chemotherapy, spermatogenesis was altered compared with non-chemotherapy treatment patients (Presti et al., 1993; Meirw and Schenker, 1995; Pont and Albrecht, 1997). Most of the patients will be azoospermic 7–8 weeks after beginning treatment because of the action of chemotoxic drugs on rapidly proliferating spermatogonia. If these cells survive, spermatogenesis restarts 12 weeks after treatment and the degree of recovery depends on the number of surviving germ cells. Eventually, spermatogenesis will occur in 20–50% of patients (Padron et al., 1997). Assa et al. (1991) demonstrated that impairment of gonadal function was correlated to sperm quality before treatment rather than to the treatment itself.

Of the 35 patients in our study who gave semen samples after receiving chemotherapy, about one-third were azoospermic. The time gap from treatment to semen cryopreservation ranged from a few weeks to 5 years; therefore, some were in the recovery phase. The remaining patients produced semen samples of sufficient quality for freezing (1–13

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Time from freezing (years)</th>
<th>Treatment</th>
<th>Sperm count ($\times 10^6$/ml)*</th>
<th>Motility (%)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairy cell leukaemia</td>
<td>1</td>
<td>IUI</td>
<td>74</td>
<td>50</td>
<td>Healthy girl</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>1.5</td>
<td>IUI</td>
<td>58</td>
<td>14</td>
<td>Healthy girl</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>3</td>
<td>IVF</td>
<td>28</td>
<td>29</td>
<td>NP</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma ICSI</td>
<td>5</td>
<td>ICSI</td>
<td>13.2</td>
<td>16</td>
<td>Ongoing pregnancy</td>
</tr>
<tr>
<td>Testicular cancer ICSI</td>
<td>2</td>
<td>IVF</td>
<td>1.6</td>
<td>50</td>
<td>Failed to fertilize</td>
</tr>
</tbody>
</table>

* Sperm count and motility are of the thawed sperm used for the treatment.
* Husband died before the delivery.
* FER = frozen embryo replacement; ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination; IVF = in-vitro fertilization; NP = not pregnant.
ampoules, median = 8.5). Our results indicate that every effort should be made to refer cancer patients for semen cryopreservation before proceeding with chemotherapy. However, for some patients when there is no alternative but to start chemotherapy immediately as a life-saving procedure, it is still worth attempting semen cryopreservation during or after that treatment. The same policy should be applied to patients whose primary disease recurs.

In the present study, only six couples among 191 (3.1%) have returned for infertility treatment to date. This low rate was similar to that reported by other centres (Fossa et al., 1989; Sanger et al., 1992) and may have been due to several reasons, including death, awaiting possible recovery of gonadal function, a short interval since the original illness, and uncertainty about whether recovery may be complete and thus deciding not to have children. However, the largest proportion comprised young men who had not yet established family life.

At present, fertility specialists can provide a wide range of relatively successful assisted conception treatments, depending upon the quality of the frozen spermatozoa, including intracervical insemination (Le Lannou et al., 1995), IUI (Hendry et al., 1983; Rhodes et al., 1985; Scammell et al., 1985; Rothmann et al., 1986), IVF (Naz et al., 1985; Rowland et al., 1985; Rothmann et al., 1986; Davis et al., 1990; Tournaye et al., 1991; Khalifa et al., 1992; Sanger et al., 1992) and ICSI (Chen et al., 1996). In addition, cryopreserved spermatozoa extracted from an orchidectomized seminoma-bearing testis was recently used successfully (Yavetz et al., 1997).

In our unit, all three methods were used as appropriate, and all six female partners have conceived: two in IUI cycles, two from IVF cycles (one of them with frozen embryo replacement cycle), and the remaining two with ICSI. Four girls were born and two other singleton pregnancies are ongoing.

In summary, men with malignant disease—and especially with testicular cancer—have a reduced sperm quality at the time of diagnosis of their illness. However, most of them have sufficient suitable spermatozoa for freezing. Any sperm sample containing motile spermatozoa should be frozen, regardless of its quality. The recent progress in assisted reproductive techniques, and especially in the field of micromanipulation, can secure the fertility potential of these men—especially those younger individuals who have not yet completed their family—by providing them with the opportunity to have semen samples frozen for future use. Our results indicate that the probability of such men fathering their own genetic children is quite high. It is essential therefore, to increase the awareness of general practitioners, oncology teams and patients themselves to the new developments in preserving fertility for cancer patients. We recommend that a properly designed programme of semen cryopreservation for cancer patients should be developed in leading tertiary assisted conception centres, which have the facilities and experience for cryopreservation and can offer a whole range of appropriate sophisticated assisted reproductive treatments.

References


Semen cryopreservation for cancer patients


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