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Semen quality and cryopreservation in adolescent cancer patients

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BACKGROUND: Adult cancer patients are routinely offered pre-treatment sperm cryopreservation. However, only recently has the welfare of adolescent cancer sufferers gained momentum, including their infertility, and unsurprisingly relatively little is known about their semen quality and feasibility of cryopreservation. METHODS AND RESULTS: A total of 238 adolescent cancer patients referred to our centre between 1991 and 2000, from post-pubertal age up to 19 years 11.9 months, were included. Their semen was processed after appropriate counselling. Semen cryopreservation was possible in 205 of the initial 238 patients referred (86.1%). The pathology of the cancer cases included Hodgkin's lymphoma, non-Hodgkin's lymphoma, osteosarcoma, Ewing's sarcoma, acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), testicular, leukaemia, and others. The mean sperm counts were broadly uniform across the disease and age groups, except for the AML group. There was no cancer group analysed in which sperm could not be stored. Semen volume was broadly uniform across the disease groups, except the ALL and Ewing's sarcoma groups, which showed relatively lower and higher mean semen volumes respectively. Older adolescent patients appeared to have a higher mean semen volume. CONCLUSIONS: Semen cryopreservation was possible in most adolescent cancer cases regardless of age or diagnosis. In all cases the quality of the semen was potentially useful for assisted conception procedures. An offer to freeze sperm in all patients aged >12 years should be made. Adequate support and counselling of both the boys and their parents is essential.

Key words: adolescent/cancer/cryopreservation/semen/sperm

Introduction

There is a cumulative risk of 1 in 564 of developing cancer in the first 15 years of life (Stiller and Draper, 1998); in the UK there are 1200–1300 new cancer patients each year with current survival rates >60%, largely resulting from intensive treatment (Cancer Research Campaign, 1995). Whereas improved survival rates, quality of life concerns (Richards *et al*., 2000) and advances in reproductive technologies have led adult cancer patients to be routinely offered sperm cryopreservation (Aubier *et al*., 1989; Meirow and Schenker, 1995; Bahadur, 2000), this has only recently started to be offered to adolescent and younger cancer patients (Schover *et al*., 1998; Bahadur *et al*., 2000; Relander *et al*., 2000). More information is needed in relation to their semen quality, its feasibility for storage and whether some groups could clearly be excluded from sperm banking.

This information being presented on adolescent cancer patient semen is unique for its size and important for the increasing focus on adolescent patient fertility preservation. Rare reports on adolescent and childhood cancer patients do exist. However, these are on small patient numbers providing commentary on sperm number and motility increase with age against a background of unclear controls (Muller *et al*., 2000). Otherwise, they appear to cover adult cancer patients aged 20–63 years (Kliesch *et al*., 1996) and 15–22 years (Hovav *et al*., 2001); or applying non-age-matched controls (Kliesch *et al*., 1996). On the other hand, reports on childhood cancers seem to cover significant numbers of early to mid-adolescent patients (<1–16 years, mean age 12 years) (Nygaard *et al*., 1991; Relander *et al*., 2000). The necessity for a definition of 'adolescence' in relation to reproductive issues would thus seem to have been largely overlooked (Bahadur and Hindmarsh, 2000). It would seem appropriate to adopt biological and reproductive criteria and equate the onset of adolescence with puberty. However, although the end of puberty is generally taken to coincide with the end of growth, in the modern context this may include physical, reproductive, psychological and psychosocial development. It is therefore convenient to adopt an objective age point and we have defined this as age

Table I. Age, sperm count and semen volume for disease and healthy groups

Disease/group	*n*	Age (years)		Sperm count ($\times 10^6$/ml)		Semen volume (ml)	
		Mean	SEM	Mean	SEM	Mean	SEM
Hodgkin's	36	16.44	0.34	55.56	7.3	1.41	0.16
Non-Hodgkin's	6	16.83	0.7	91.67	22.72	1.75	0.17
Osteosarcoma	51	16.38	0.24	59.14	6.75	1.68	0.15
Ewings sarcoma	24	16.67	0.4	49.42	9.01	2.06	0.29
ALL	7	17.43	0.48	57.43	23.15	1.02	0.42
AML	3	14.33	0.67	18	11.14	1.67	0.67
Testicular cancer	17	17.82	0.31	31.94	4.81	1.99	0.19
Leukaemia	23	16.78	0.35	38.96	8.31	1.50	0.19
Lymphoma	11	16.17	0.52	66.67	15.55	1.31	0.33
Other	27	16.96	0.29	36.41	8.88	1.26	0.19
Total cancer patients	205	16.67	0.12	50.63	3.2	1.59	0.07
Healthy donors	71	22.89	0.34	84.51	3.39	2.96	0.16

ALL = acute lymphoblastic leukaemia; AML = acute myeloid leukaemia.

19 years 11.9 months in accordance with the cancer survival epidemiologists.

In part, the discrepancy between the treatment of adults and adolescents arises due to controversial questions of maturity, sensitivity and legal precepts in relation to gaining consent in this group. In this regard we have recently introduced our approach to the handling of adolescent cancer patients (Bahadur *et al*., 2001). Apart from these discreet handling procedures for adolescent males, semen samples may have to be kept for much longer periods than their adult counterparts, as post-treatment adolescents can expect many decades of life ahead of them (Richards *et al*., 2000). However, there is also a reluctance by some parents and clinicians to refer the patient to bank semen due to doubts as to whether he would be able to masturbate or whether the quality of the semen would be viable. This paper aims to address these doubts.

Materials and methods

All male adolescent cancer patients who were referred to our unit in the period 1991–2000 were seen within 3 days. Patients were counted as adolescent if adjudged by the referring clinician to have undergone puberty on the basis of testicular examination and pubic hair distribution and to be no older than 19 years 11.9 months. Patients with sperm obtained by intrusive methods were excluded from this study and only the first attempt to produce the ejaculate has been included. All patients were counselled and gave consent before being asked to produce the samples (Bahadur *et al*., 2001).

The semen analyses were performed in accordance with WHO guidelines (World Health Organization, 1987, 1992) using a Neubaur counting chamber or Makler counting chamber. Sperm freezing was performed using an egg yolk-based medium containing 10% glycerol (Peek *et al*., 1982; Pilikian *et al*., 1982; Mahadevan and Trounson, 1983), mixed with an equi-volume of cryoprotectant, and usually 1 ml aliquoted vials were made. The patient groups were categorized according to disease types that appear in Table I.

A healthy cohort of potential sperm donors was chosen for illustration. Only the first semen sample presented before consideration for becoming a sperm donor in our donor insemination programme was included.

Mean, SD and SEM for age, sperm count ($\times 10^6$/ml), motility (%) and volume (ml) were calculated using the Statistical Package for Social Science (SPSS, version 6.1).

Table II. Sperm count and semen volume for age-specific adolescent cancer patients

Age (years)	*n*	Sperm count ($\times 10^6$/ml)		Semen volume (ml)	
		Mean	SEM	Mean	SEM
12	3	7.33	3.71	0.60	0.31
13	2	17.5	7.5	0.85	0.15
14	18	41.44	10.91	1.06	0.21
15	36	42.17	6.17	1.26	0.14
16	32	54	7.77	1.34	0.18
17	32	45.72	6.64	1.60	0.20
18	45	57.64	7.31	1.88	0.13
19	37	62.81	9.25	2.18	0.20

Results

A total of 238 adolescent cancer patients was referred to our unit. Of these, 205 were able to produce a semen sample (86.1%). The mean age of those able to produce a sample was 16.67 years (SD 1.78) and the mean age of those unable to produce a sample was 15.50 years (SD 2.28). The healthy cohort consisted of 71 donors, mean age 22.89 years (SD 2.84).

The disease bands are shown in Table I and included Hodgkin's lymphoma, non-Hodgkin's lymphoma, osteosarcoma, Ewing's sarcoma, acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), testicular, leukaemia, and others. The sperm counts were broadly similar across the disease bands although those in the AML group appeared to be lower. Semen volume was broadly uniform across the disease groups except the ALL and Ewing's sarcoma groups, which showed relatively reduced and increased mean semen volumes respectively (Table I). Older adolescent patients appeared to have a higher mean semen volume (Table II).

From age 12 years upwards, in our cohort a reasonable enough sperm count and volume was obtained to consider cryopreservation for future use (Table II). Tables III and IV

Table III. Age, sperm count, motility and semen volume for healthy donors and adolescents with Hodgkin's disease and with osteosarcoma

	Healthy donors (n = 71)		Hodgkin's (n = 36)		Osteosarcoma (n = 51)	
	Mean	SEM	Mean	SEM	Mean	SEM
Age (years)	22.89	0.34	16.44	0.34	16.38	0.24
Sperm count ($\times 10^6$/ml)	84.51	3.39	55.56	7.30	59.14	6.75
Sperm motility (%)	68.45	0.84	51.76	2.82	48.84	2.43
Volume (ml)	2.96	0.16	1.41	0.16	1.68	0.15

Table IV. Age, sperm count, motility and volume of healthy donors and all adolescent cancer patients

Parameter	Healthy cohort (n = 71)		All cancer (n = 205)	
	Mean	SEM	Mean	SEM
Age (years)	22.89	0.34	16.67	0.12
Sperm count ($\times 10^6$/ml)	84.51	3.39	50.63	3.2
Motility (%)	68.45	0.84	45.05	1.51
Volume (ml)	2.96	0.16	1.59	0.07

provide the age, sperm concentration, motility and volume analyses of the three largest groups—Hodgkins disease, osteosarcoma and healthy donors cohort—as well as the whole adolescent cohort. Tables III and IV illustrate the sperm counts, motility and semen volume of the disease groups to be broadly lower than the healthy cohort.

Discussion

Our study demonstrates that the majority of adolescent cancer sufferers are able to produce a semen sample. Those boys that produce a sample, including boys of 12 years of age, have sperm that is suitable for assisted reproductive technologies in all cases, and sperm banking should be routinely offered to all adolescent patients.

Where semen was produced, the sperm count appeared broadly unaffected within the single year age bands. Within the adolescent group, there appears little difference in the sperm count and volume in relation to most of the disease groups, thereby indicating some uniformity in testicular maturation having been achieved once post-pubertal. It also reinforces the view that childhood testes are by no means quiescent as shown in the marmoset model and therefore even greater care needs to be exercised in protecting children's fertility before treatment (Kelner *et al*., 2002). It is undeniable that any strict policy of referral to bank sperm, based on age, would have denied significant numbers of adolescent cancer patients the chance to store their sperm. For instance, if the threshold age of $<$16 years were applied, this would have amounted to 28.8% (59/205) of the successful group being denied the chance to store sperm. Provided the patient has understood the issues (Bahadur *et al*., 2001), it seems unreasonable to deny a 12 year old a chance to bank semen. It may, however, be prudent to consider patients from 10 years upwards if they have fully understood the issues. This is to overcome possible inconsistent interpretation by individual specialists applying a subjective pubertal classification (Tanner, 1989). There was no disease group in which sperm could not be stored. In all cases the quality of the semen was potentially useful for assisted conception procedures.

Having a broad policy of storing 1 ml vials, containing semen and cryoprotectant mixed in equi-volume, it is therefore reasonable to obtain 3–4 vials per ejaculate for an adolescent cancer patient, and we aim for 2–3 ejaculates. However, the number of vials made could be increased, depending on individual circumstances, such as the inability to provide a second or third ejaculate for sperm banking, or on the semen quality. The 33 patients who were unable to produce semen, representing 13.9% of the cohort, deserved an in-depth report (Bahadur *et al*., 2002) considering the complex clinical management issues that may follow, as well as sensitive patient and parent involvement.

The question of ethical controls will become increasingly prominent in this field involving adolescent cancer patients and their fertility, as studies on healthy age-matched subjects are likely to encounter widespread recruitment difficulty or disapproval. Even in adult patients, ethical controls appear to have been applied to alleviate the use in research of sperm cryopreserved before cancer treatment (De Mas *et al*., 2001). Despite this limitation in recruiting healthy adolescent age-matched controls and with unequal numbers, significant conclusions on semen quality seem to have been reported (Kliesch *et al*., 1996). Given the exclusive nature of our cohort and the increasing clinical importance of the topic, we have undertaken analyses against a healthy cohort which are not age-matched, and remarks have to be kept in perspective and for illustration purposes only. Without age-matched controls the analysis of variance followed by Duncan's test was not performed for our study. The sperm counts were broadly uniform across the disease groups, with the exception of the AML group, but lower than the healthy cohort (Table I). In Hodgkin's disease and osteosarcoma, lower sperm numbers, motility and volume were seen in relation to the healthy cohort (Table III). The sperm quality for the whole adolescent cancer patient cohort was poorer in relation to the healthy sperm donors in terms of count, motility and volume (Table IV). This mirrors the findings in adult disease with significantly impaired sperm

quality in adult Hodgkin's disease and non-Hodgkin's disease being reported compared with healthy donors (Botchan *et al*., 1997). Pre-treatment sperm quality is usually impaired among adult cancer patients (Chapman *et al*., 1981; Vigersky *et al*., 1982). The semen volumes were broadly uniform across the disease groups, except the ALL and Ewing's sarcoma groups, which had relatively lower and higher mean semen volumes respectively (Table I). It is interesting to note that our older adolescent patients may have a higher semen volume (Table II).

In boys, testicular failure may be a result of Leydig cell dysfunction or germinal epithelium dysfunction, or both. Direct irradiation of the testes in total body irradiation results in permanent Leydig cell failure and ablation of the germinal epithelium. This results in infertility and a need for lifelong testosterone therapy (Shalet *et al*., 1985; Castillo *et al*., 1990) following its initiation at ~12–13 years. Cytotoxic chemotherapy can cause germinal epithelium damage which could possibly be reversed. Although sperm may recover after a prolonged period of time, it is clear that there is a fertility deficit after cancer treatment amongst long term male survivors (Byrne *et al*., 1987; Siimes and Rautonen, 1990). Even the pre-pubertal state would not seem to protect the gonads from the effects of treatment (Aubier 1989; Shafford, 1993), although testicular function appears to be worse if patients are treated post puberty (Hieken *et al*., 1996).

Study of the outcome of pregnancy and offspring born after childhood cancer do not provide evidence of germ cell mutagenesis, manifested in increased congenital malformations, neonatal mortality or cancers in offspring. However, much larger patient numbers would be needed to rule out any of these associations with confidence (Li *et al*., 1979; Otake *et al*., 1990; Yoshimoto *et al*., 1990; Hawkins, 1991, 1994; Dodds *et al*., 1993; Byrne *et al*., 1998). With incomplete registrations of spontaneous abortions, miscarriages and elective abortions coupled with methodological problems, caution is needed in interpreting these data.

It has been noted that young men offered sperm banking before treatment for Hodgkin's disease often appear to use denial to avoid acknowledging the possibility of future infertility (Cella and Najavits, 1986). However, men with testicular cancer who remained childless after treatment were clearly distressed about their infertility (Rieker *et al*., 1990). It is interesting to note that in a quality-of-life analysis, a great majority of younger cancer survivors saw their cancer experience as potentially making them better parents (Schover *et al*., 1999). It would appear therefore that the undoubted sensitivity of adolescent cancer patients concerning fertility considerations should lead clinicians to offer positive encouragement to try to have sperm cryopreserved rather than ignoring the issue.

In conclusion, sperm banking in adolescent cancer patients was a practical and feasible option in the majority (86.1%, 205/238) of our cohort and all producers were able to provide samples by masturbation, which would be suitable for assisted reproductive technologies. In our cohort, the minimum age at which sperm could be produced and cryopreserved was 12 years.

Adequate counselling and support to adolescent cancer patients and their parents is essential, both in terms of coming to terms with their sexual maturity and in the event of their failing to produce semen.

The high level of successful sperm banking should be reassuring to physicians, parents and the adolescent cancer patients in relation to any uncertainty to the patients' future fertility potential. Additionally, the positive news should provide a much needed psychological boost before embarking on chemo- or radiotherapy.

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