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Fertility preservation in adolescent males: experience over 22 years at Rouen University Hospital

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BACKGROUND: Sperm banking is a suitable procedure to prevent infertility after cancer therapy in male adolescents. We evaluated the feasibility of semen preservation in 156 adolescents aged between 13 and 20 years and then we assessed fertility outcome after treatment.

METHODS: Age, urogenital history, indications for cryopreservation, histological diagnosis and semen parameters were recorded. Fertility status after treatment was assessed by a questionnaire addressed to those patients who had utilized sperm storage. Post-treatment semen analysis was performed for 22 patients.

RESULTS: Cryopreservation was possible in 88.5% of cases. Azoospermia was detected in 2.6% of the patients at the time of diagnosis. Malignant disease accounted for 84% of our male adolescents. In this type of disease, semen parameters were significantly altered only among patients with metastatic malignant bone tumour. After treatment, nine patients presented azoospermia, five patients achieved pregnancy spontaneously, two achieved it after assisted reproductive technique using fresh ejaculated spermatozoa and one following sperm donation. Three failed with cryopreserved sperm.

CONCLUSIONS: Semen cryopreservation is possible for most adolescents and, regardless of disease type, may be a means of preserving fertility prior to gonadotoxic treatment that might impair the spermatogenesis process.

Key words: adolescents / cryopreservation / fertility / outcome / semen parameters

Introduction

Cancer is the third most frequent cause of mortality in young people (Gatta *et al.*, 2003). In France, the annual incidence of adolescent cancer is ~200 per million, similar to the incidence reported in other European countries, and ~700 new cases are recorded annually (Gatta *et al.*, 2003; Desandes *et al.*, 2004).

The types of cancer that occur in adolescents differ markedly from those described in younger children and older adults: embryonic tumours are frequent in children but rare in adolescents (1%). Epithelial tumours are more frequent in adults than in adolescents, where they account for only one-third of all cancers. Thus, the most common tumours in adolescents are lymphoma and acute leukaemia, gonadal germ cell tumours and sarcoma (Gatta *et al.*, 2003; Desandes *et al.*, 2004).

The 5 year overall survival rate of adolescents treated for cancer has considerably improved from 50% in the 1970s to nearly 75% in the 1990s. This improvement was related to the progress made in diagnosis and treatment (Gatta *et al*., 2003; Desandes *et al*., 2004). However, the long-term effects of treatments have a recognized toxicity on gonads and lead to infertility (Brougham *et al*., 2003). Fertility is a major concern of childhood and adolescent cancer survivors and ~15–30% of cured cancer patients become infertile after treatment (Schrader *et al*., 2001). Moreover, this concern also applies to other gonadotoxic treatments used in the situation of adolescent benign disease such as varicocele, testicular torsion and epididymitis. The strategy used to improve fertility preservation depends on sexual maturity. In young males and pubertal boys, cryopreservation of ejaculated semen should be considered as an established and successful technique (Bahadur *et al*., 2002b), despite the reluctance of oncologist clinicians and parents to refer adolescent patients for sperm storage. However, only few studies have focused on sperm quality at the time of diagnosis or after the end of treatment (Muller *et al*., 2000; Bahadur *et al*., 2002b; Jedrzejczak *et al*., 2004). Moreover, it is also important to assess the future fertility of this population.

We performed a retrospective study in a population of young men aged up to 20 years who consulted for sperm cryopreservation between 1984 and 2006 at Rouen University Hospital Centre d'Etude et de Conservation des Oeufs et du Sperme humain (CECOS). The main objectives of this study were to evaluate: (i) the feasibility of sperm banking in adolescents, (ii) pre-freeze and post-thaw sperm parameters according to disease type and stage and (iii) sperm quality after gonadotoxic treatment and the outcome of fertility. We expected to confirm the feasibility of sperm banking in adolescents and to establish recommendations concerning fertility preservation.

Materials and Methods

Patients

We performed a review of our cryopreservation database, including all boys and young men who cryobanked sperm between January 1984 and December 2006 in the CECOS of Rouen University Hospital. Patients included in the study were aged up to 20 years. All patients signed an informed consent to both cryopreservation and follow-up. The CECOS of Rouen University hospital was created on January 1984 and, as recommended by the French federation of CECOS, an informed consent for sperm banking was included in the management of all patients consulting for sperm banking. For young adolescent males younger than 18 years, the informed consent was signed both by the patient and by his parents. In our centre, since 2000 we tried to develop a successful sperm banking programme with the collaboration of oncologist and urologist teams. We have recommended that all young males 12 years of age and older should be invited for sperm banking before any disease or treatment likely to adversely affect the spermatogenesis process (chemotherapy, radiotherapy and surgery for testis disease). A rapid and flexible access to sperm storage was proposed to accommodate acutely ill young patients. Clinical and biological data were recorded for each patient according to the data collected at the time of the first semen collection. Information concerning follow-up is routinely sent to our centre by the urologists or oncologists who have counselled patients for sperm banking.

Age and urogenital history were assessed for each patient: cryptorchism, varicocele, genital and urinary infectious disease, scrotal injury and testis torsion. Indicators for the preservation of fertility were recorded.

An accurate histological diagnosis was also determined in cases of malignant disease. A disease diagnosis was obtained for all the patients included in the study according to urological and oncological information. A testicular cancer simplified histological diagnosis was used to distinguish between pure seminoma, embryonic carcinoma (EC) and mixed tumour (MT). Malignant lymphoma was defined as Hodgkin's lymphoma (HL) or non-HL (NHL). Acute leukaemias were classified in two phenotypes: acute lymphocytic leukaemia with precursors B or T and acute myelocytic leukaemia. Factors suggesting a poor prognosis were not evaluated in this study. Histological diagnosis was also assessed for other cancers. Disease stage was defined as metastatic or non-metastatic.

The type of treatment was recorded in the database for each disease, in particular for cancer: surgery (e.g. orchidectomy in testicular tumour), chemotherapy, radiotherapy and bone marrow transplantation.

Fertility status was assessed by a questionnaire sent to those patients who annually maintained sperm storage. All reproductive events that occurred after treatment were recorded [pregnancies achieved spontaneously or after assisted reproductive techniques (ART), number of children].

Semen samples

Semen samples were collected by masturbation at our laboratory. Semen analysis was performed according to World Health Organisation recommendations (World Health Organisation, 1999) after liquefaction for 20 min at 37°C. Sperm freezing was carried out after dilution into a cryoprotectant medium [locally prepared Ackerman medium until 1995 and Spermfreeze® (JCD, Lyon, France) from 1996 to 2006] taking into account spermatozoa number and motility. Before 1989, a manual cryopreservation procedure was performed using nitrogen vapour. From 1989 to 2006, a rapid controlled protocol without seeding was used to freeze samples with an automatic apparatus [Minicool LC40 or 40PC (Air Liquide Santé, Paris, France)]. One straw was thawed at 37°C for 5 min and sperm inspection (numeration and motility) was performed directly without removing the cryoprotectant and without washing the sample. This procedure followed the general practice of the French federation of CECOS.

The following sperm parameters were taken into consideration: volume (ml), sperm concentration (SC, 10^6/ml), total sperm count (TSC, 10^6/sample), forward motility ($a+b$: %), morphology (% of normal forms, classification of David) (David *et al*., 1975), number of straws per sample, TSC per straw (10^6/straw), post-thaw forward motility ($a+b$: %) and total number of forward motile spermatozoa per straw (10^6/straw). Any time of abstinence was discarded from the analysis, since it was not specified for most of the patients. The absence of this parameter may limit the interpretation of variations in semen parameters.

Other variables were explored: the number of semen samples obtained, the delay between the end of treatment and the provision of semen samples, the number of failed samples for each patient, the number of patients who underwent testicular sperm extraction (TESE) or provided a post-masturbation urine sample. Aspirated sperm and those obtained by urine production were evaluated only qualitatively for the presence or absence of spermatozoa, and were not included in the calculation of mean sperm count and motility mentioned above.

All statistical analyses were performed using Statview® for Windows (Abacus Concepts, Inc., Berkeley, CA, USA). Quantitative variables were expressed as mean (± SEM). All parameters were evaluated according to disease diagnosis, tumour histotype and presence of urogenital history. When justified, non-parametric tests were performed to

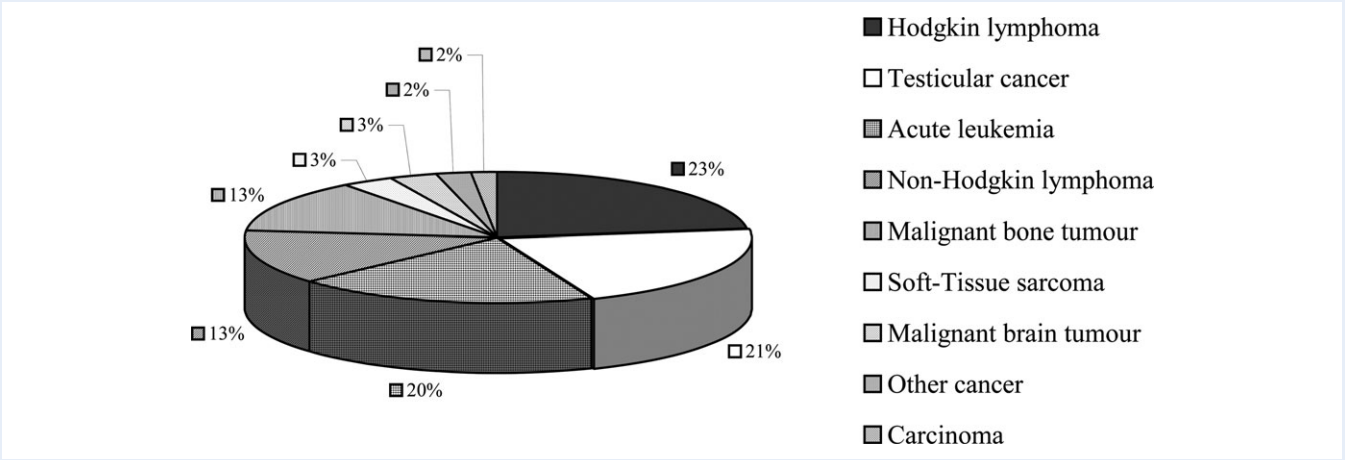

Figure 1 Distribution (%) of malignant disease.

compare results between the different subgroups. A *P*-value below 0.05 was considered as statistically significant.

Results

Population studied

Data were collected from 156 patients aged between 13 and 20 years (average 17.81 ± 0.14). From 1984 to 1999, we received 70 adolescent patients (45%). From 2000 to 2006, after the introduction of the adolescent sperm banking programme, 86 young patients (55%) were referred to our centre.

Malignant disease accounted for 84% of patients ($n = 131$) (Fig. 1). The mean age of patients for each type of cancer was not significantly different. Only 5.3% of these patients presented with aurogenital history: cryptorchism (2.3%), scrotal injury (0.8%), testis torsion (0.8%), inguinal hernia (0.8%) and hydrocele (0.8%). All the patients with HL were treated with polychemotherapy combined with radiotherapy in 79% of cases. A bone marrow transplantation was performed in 21% of patients. Patients with TC presented with a MT (52%), an EC (41%) or a seminoma (S) (4%). It is worth noting that only one patient with TC had urogenital history (hydrocele). An adjuvant treatment was performed in 89% of TC patients, chemotherapy being most frequently used (81%). Most of the patients with AL (83%) had acute lymphocytic leukaemia. All the patients were treated with polychemotherapy combined with radiotherapy in 72% of cases. A bone marrow transplantation was carried out in 52% of patients. A total of 29.4% of patients with NHL had Burkitt lymphoma. All the patients were treated with polychemotherapy combined with radiotherapy in 29.4% of cases. A bone marrow transplantation was performed in 29.4% of patients.

The majority of patients with non-malignant disease wished to cryobank sperm because of urological disease (Fig. 2). Twenty percentage of these patients had presented with previous urogenital history: cryptorchism (12%) and genital infectious (8%).

Semen parameters before treatment

During the period of the study, personnel changed only in 1995. From 1984 to 1995, 31 adolescent males cryopreserved sperm in our laboratory, representing only 20% of the entire cohort. Both semen analysis and cryopreservation procedures were unchanged during the study period, except that Ackerman home made freezing

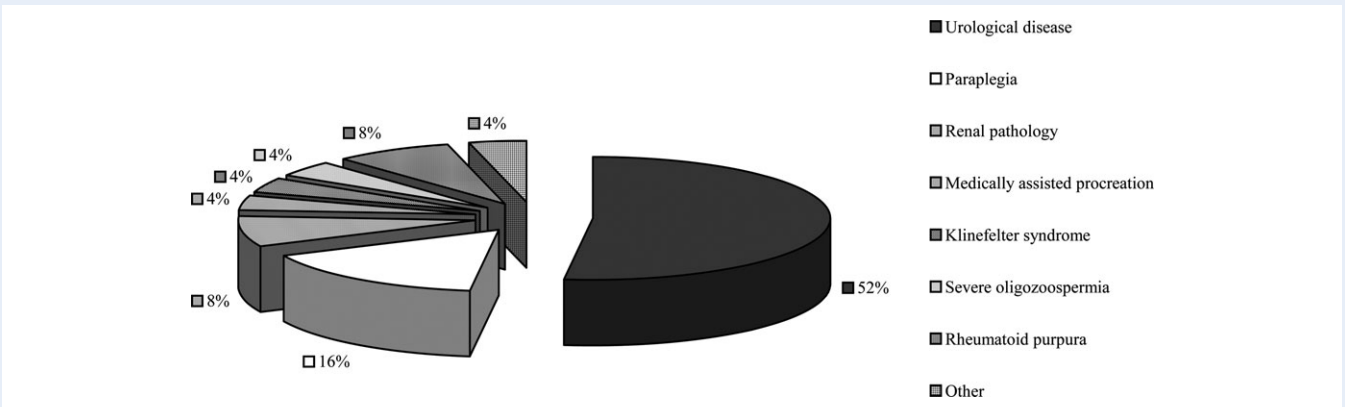

Figure 2 Distribution (%) of non-malignant disease.

medium was replaced by Spermfreeze® (JCD) in 1995. Furthermore, during the period from 1996 to 2006, semen analysis and freezing procedures were evaluated annually during the internal seminar of the French federation of CECOS. The above points ensure that variations in semen analysis and freezing procedure have been minimized.

Number of semen samples

The mean number of semen samples obtained was 2.40 ± 0.90 per patient. Patients presenting with AL, however, provided fewer samples than patients presenting with other types of cancer (1.50 ± 0.2, Table I).

Twenty-one patients (13.5%) failed to collect semen samples on at least one occasion and 18 patients (11.5%) could not collect any semen sample. These patients were not significantly younger ($P = 0.08$) compared with the mean age of the population studied (17.05 versus 17.81). The majority of patients who failed to collect any sample presented with cancer and 40% of them are no longer alive. The remainder (16.7%) presented with paraplegia or malformative urological disease and they all provided post-masturbation urine samples. Spermatozoa were found only in urine samples of paraplegic patients.

Semen volume

Semen volume was normal (≥2 ml) for 67% of patients and increased significantly with age (0.28 ml ± 0.09 at 14 years old versus 3.25 ml ± 0.25 at 20 years old). No significant differences in volume could be found between different types of tumour (Table I).

Sperm concentration and total sperm count

SC and TSC were within normal ranges (≥20.10^6/ml and ≥40.10^6/sample) for 61% of patients. Azoospermia was present in 2.6% of patients at the time of diagnosis. SC and TSC did not vary significantly between the different types of tumours (Table I) but were significantly lower in metastatic than in non-metastatic MBT (9.51 ± 5.18 versus 63.34 ± 13.96, $P = 0.014$ and 17.10 ± 10.32 versus 151.89 ± 42.69, $P = 0.014$).

Pre-freeze forward motility

Pre-freeze forward motility was normal (≥50%) in 23.72% of patients (Table I). Pre-freeze forward motility was significantly lower in AL compared with TC ($P = 0.001$), NHL ($P = 0.0007$) and MBT ($P = 0.03$). No significant difference was observed relating to disease histotype in patients with malignant disease (Table I).

Number of straws

Eighteen patients (11.5%) did not produce a single sperm straw. Nine failed to collect any sample, including one patient with malformative urological disease who provided post-masturbation urine samples from which no spermatozoa were retrieved. Four patients presented with azoospermia, three before treatment of AL relapse and one patient with Klinefelter syndrome. Two adolescents did not provide a sample at the time of diagnosis and presented with azoospermia after treatment. Finally, the spermatozoa from three patients with very poor semen quality were not frozen, this occurring 17–18 years ago. The number of straws did not significantly differ between tumour types in those patients with cancer (Table I).

Post-thaw forward motility

Sperm forward motility significantly decreased after thawing (Table II). In cancer patients, post-thaw sperm forward motility was lower in metastatic than in non-metastatic MBT (5.41 ± 3.56 versus 19.48 ± 2.51, $P = 0.019$). For the other tumours, no differences were detected relating to disease histotype (Table I).

The total number of forward motile spermatozoa per straw did not significantly differ in patients with malignant disease relating to tumour type, excepted for metastatic MBT in comparison with non-metastatic MBT (0.11 ± 0.07 versus 2.80 ± 0.80, $P = 0.016$) (Table I).

Testicular sperm extraction before treatment

During the study period, TESE was proposed before treatment to five patients. Four patients accepted: a 16-year-old patient with azoospermia and acute myelocytic leukaemia, a 16-year-old patient with cryptospermia and a testicular EC, a 13-year-old patient with a malignant brain tumour who failed to collect any semen sample and a 20-year-old patient with paraplegia with poor semen quality in a post-masturbation urine sample. Spermatozoa were retrieved from the first three of these patients. A patient with azoospermia and Klinefelter syndrome refused the procedure.

Semen parameters after treatment

In our population, a total of 22 patients (including 19 cancer patients) provided a semen sample for analysis after treatment. These patients had the following conditions: HL ($n = 8$), MBT ($n = 4$), NHL ($n = 3$), TC ($n = 2$), AL ($n = 1$), other cancer ($n = 1$) and urological disease ($n = 3$). Both age and semen volume were significantly higher after treatment than before treatment (Table III). However, no differences were detected in SC, TSC, forward motility or morphology (Table III). A total of nine patients [(HL ($n = 4$), MBT ($n = 3$), NHL ($n = 1$), testis torsion ($n = 1$)] presented with azoospermia after treatment. Four patients [(HL ($n = 2$), MBT ($n = 1$), NHL ($n = 1$)] underwent bone marrow transplantation.

Outcome after treatment

The outcome after treatment of the 138 patients (88.5%) who cryobanked sperm is shown in Fig. 1. Patients who did not express each year their choice regarding their straws were considered as lost to follow-up. Seventy-one patients were asked for their fertility status, including 60 cancer patients considered in remission after treatment. Sixty-four (90%) responded to the questionnaire. Most of them ($n = 56$) were <30 years old. Ten patients attempted to achieve a pregnancy with their partner. Seven succeeded: five conceived spontaneously, two fathered children after ART using their own fresh ejaculated sperm. Two other patients used their own sperm straws for ART, but they did not achieve pregnancy. One patient transferred his sperm straws to another CECOS, but no further information was obtained concerning the use of the cryopreserved spermatozoa. Finally, one patient obtained two children following sperm donation.

Discussion

Our study demonstrates not only the feasibility of semen cryopreservation in French adolescents but also the low rate of utilization of cryopreserved sperm after treatment in this population.

Table I Summary of semen parameters at the time of semen cryopreservation and factors (histotype) evaluated in the comparison of patients with specific types of malignant disease and in the total population studied

Semen parameters (mean ± SEM)	HL	TC	AL	NHL	MBT	All patients ($n = 156$)	Comparison (P-value)	Specific significant factors
Number of samples obtained per patient	2.80 ± 0.17	2.60 ± 0.13	1.50 ± 0.2	2.80 ± 0.25	2.50 ± 0.24	2.40 ± 0.09	AL < HL, $P = 0.0001$ AL < TC, $P = 0.0004$ AL < NHL, $P = 0.0007$ AL < MBT, $P = 0.0049$	None
Volume (ml)	2.29 ± 0.23	2.99 ± 0.26	1.91 ± 0.23	2.18 ± 0.35	2.07 ± 0.29	2.38 ± 0.11	NS	None
Initial sperm count (10^6/ml)	29.94 ± 5.67	25.17 ± 4.99	46.73 ± 14.73	52.66 ± 8.59	45.40 ± 11.49	34.66 ± 3.17	NS	In MBT, metastasis < non metastasis $P = 0.014$
Total sperm count (10^6/ejaculate)	64.13 ± 12.26	77.04 ± 19.09	83.75 ± 30.06	116.18 ± 26.48	106.96 ± 32.86	81.58 ± 8.31	NS	In MBT, metastasis < non metastasis $P = 0.014$
Initial forward motility ($a + b$: %)	29.30 ± 2.50	35.70 ± 2.38	23.30 ± 1.66	33.45 ± 1.70	31.79 ± 4.55	29.88 ± 1.15	AL versus HL, NS AL < TC, $P = 0.0016$ AL < NHL, $P = 0.0007$ AL < MBT, $P = 0.03$	None
Number of straws	7.89 ± 0.88	9.27 ± 1.19	6.68 ± 1.25	9.31 ± 1.76	6.44 ± 1.01	8.19 ± 5.23	NS	None
Total sperm count per straw (10^6)	5.31 ± 0.81	4.14 ± 0.74	10.02 ± 3.08	9.67 ± 1.34	9.20 ± 2.28	6.53 ± 0.58	TC<NHL, $P = 0.0017$	In MBT, metastasis < non metastasis $P = 0.0237$
Post-thaw forward motility ($a + b$: %)	10.94 ± 1.73	13.31 ± 1.78	8.90 ± 2.00	16.33 ± 2.09	15.47 ± 2.66	12.41 ± 0.80	NS	In MBT, metastasis < non metastasis $P = 0.019$
Total number of forward motile spermatozoa per straw (10^6)	0.82 ± 0.18	0.68 ± 0.16	1.31 ± 0.51	1.65 ± 0.4	2.03 ± 0.66	1.09 ± 0.13	NS	In MBT, metastasis < non metastasis $P = 0.016$

HL, Hodgkin's lymphoma; TC, testicular cancer; AL, acute leukaemia; NHL, non-Hodgkin's lymphoma; MBT, malignant bone tumour; $a + b$, spermatozoa with forward motility; n, number of patients; NS, not significant.

Table II Pre-freeze and post-thaw forward motility (mean ± SEM)

	Pre-freeze forward motility (*A*: %)	Post-thaw forward motility (*B*: %)	Variation of forward motility (*C*: %)	Comparison (*P*-value)
HL	29.30 ± 2.50	10.94 ± 1.73	66.66	$P < 0.0001$
TC	35.70 ± 2.38	13.31 ± 1.78	62.72	$P < 0.0001$
AL	23.30 ± 1.66	8.80 ± 2.00	62.23	$P = 0.0015$
NHL	33.45 ± 1.70	16.33 ± 2.09	51.18	$P = 0.0006$
MBT	31.79 ± 4.55	15.47 ± 2.66	51.34	$P = 0.002$
All patients ($n = 156$)	29.88 ± 1.15	12.41 ± 0.79	58.46	$P < 0.0001$

HL, Hodgkin's lymphoma; TC, testicular cancer; AL, acute leukaemia; NHL, non-Hodgkin's lymphoma; MBT, malignant bone tumour; Non-cancer, 'non-cancer' group; *C*, $[(A - B)/A] \times 100$; *n*, number of patients.

In our study, the distribution of the different malignant tumours is representative of the population of male adolescents who present with a cancer (Houlgatte *et al*., 2002; Desandes *et al*., 2004,2006; for review, Horwich *et al*., 2006; Young *et al*., 2007). However, even if testicular abnormalities such as cryptorchidism are known to be risk factors for testicular germ-cell tumours (Horwich *et al*., 2006), only one TC patient from our study had a previous urogenital pathology. More than 50% of the patients with non-malignant disease cryobanked sperm because of urological pathology such as varicocele, testis torsion or necrosis. When treatment of varicocele is proposed, sperm cryopreservation should be performed because of the risks of surgery on the testis (Paduch *et al*., 2001). This is also the case for the management of testis torsion or trauma.

Although semen cryopreservation is an established and successful method in young adult males, this procedure is not systematically proposed to these young adolescents. Oncologists offer semen cryopreservation to <25% of their adolescent patients (Schover *et al*., 2002). They consider this procedure to be less effective in adolescents, especially for the youngest males and they generally underestimate the toxicity of cancer therapy to the male gonad. They also do not feel they have enough time to discuss and propose sperm banking because therapy cannot be delayed (Ogle *et al*., 2008). Furthermore, Ogle *et al*. (2008) have suggested that sperm banking is too expensive. However, the cost of sperm preservation does not seem to be a limiting factor for the practice of this procedure. In France, sperm banking has been included in the cost of cancer disease treatment for several years and in our centre the introduction of a sperm banking programme for young patients is the only factor to have modified the number of patients undergoing this procedure annually. This programme involves collaboration with physicians, communication and counselling for young patients, as suggested by Chapple *et al*. (2007). The majority of our patients were able to produce a semen sample by masturbation, in agreement with results reported by Bahadur *et al*. (2002a). The same study also demonstrated a negative influence on the ability to produce a semen sample, if the patients were accompanied. In our centre, adolescents <18 years are always accompanied by their parents to sign the informed consent. They are then interviewed separately to evaluate the feasibility of sperm storage. The difficult question of masturbation is discussed to ensure that the adolescent is able to produce a semen sample, because the presence of signs of puberty is not sufficient to ensure this. However, we do not discuss the effect of abstinence so as to avoid embarrassing the young adolescents. All the patients are interviewed after each semen sample to transmit information about semen quality. In the situation of ejaculation failure, the search for spermatozoa in a urine sample could be proposed (Bahadur *et al*., 2002a; our study). A post-masturbation urine sample is easily produced and is also a less invasive procedure than other options such as TESE and penile vibratory stimulation or electro ejaculation. The majority of our patients who failed to obtain a sample had cancer and 40% are no longer alive. They may have been in a bad general condition or might have needed to start their treatment as soon as possible.

Table III Age and semen parameters at the time of diagnosis and after treatment in a group of 22 patients (mean ± SEM)

Parameters	Before treatment	After treatment	Comparison (*P*-value)
Age (years)	17.82 ± 0.7	27.36 ± 1.23	$P < 0.0001$
Delay after treatment (years)		4.50 ± 0.68	
Volume (ml)	1.96 ± 0.26	2.93 ± 0.29	$P = 0.009$
Sperm count (10^6/ml)	40.80 ± 8.77	20.31 ± 5.87	NS*
Total sperm count (10^6)	66.34 ± 14.83	68.08 ± 22.25	NS*
Forward motility ($a+b$: %)	32.04 ± 2.89	39.17 ± 3.42	NS*
Normal morphology (%)	52.44 ± 6.53	57.69 ± 3.51	NS*

$a + b$, spermatozoa with forward motility; *n*, number of patients; NS, not significant.
*$P > 0.05$.

Normal semen volumes and SCs were observed in a majority of our patients. Although spermatogenesis starts in the pre-pubertal period and mature spermatozoa can be found at Stage III of the Tanner classification (with testis volume above 5 ml), spermatozoa production is

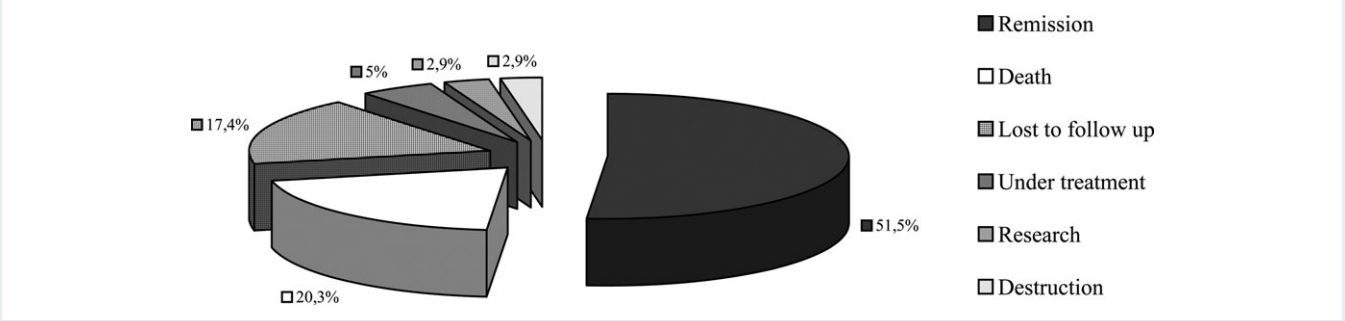

Figure 3 Outcome of the 138 patients who provided sperm straws.

generally qualitatively effective only at the age of 13–14 years (Guerin *et al*., 2005). The youngest patient included in our study was 13 years old and failed to obtain semen samples. A bilateral TESE was performed and spermatozoa were retrieved from the right testis. Semen volume significantly increased with age, in agreement with other data (Kamischke *et al*., 2004). Furthermore, semen volume and sperm count appeared to be unaffected by the type of disease. However, azoospermia, oligospermia and asthenozoospermia were also observed in our population and this may be due to the disease itself. A period of abstinence may impair sperm motility if very long. However, fever, pain and anorexia may also lead to spermatogenesis impairment. In TC, disorders of urogenital development, primary endocrine dysfunction and the presence of controlateral testis disease, environmental xenoestrogens or substances produced by the tumour itself could be implicated (Gandini *et al*., 2003). Finally, a reduction of post-thaw motility was also observed regardless of the subgroup of population, in agreement with previous data (Agarwal *et al*., 1995). Although the majority of our patients presented with moderate to severe sperm alterations, there was no disease group where sperm could not be stored and in all cases, straws could potentially be used for ART, in agreement with recent reports (Bahadur *et al*., 2002b; Ginsberg *et al*., 2008).

In our study, semen parameters were investigated in 22 patients after treatment. Nine of them (40%) presented azoospermia and four patients received a bone marrow transplantation. Bahadur *et al*. (2005) observed a similar frequency, with 37% of the patients presenting permanent post-treatment azoospermia. In our patients, SC, TSC and sperm motility did not differ significantly between pre- and post-treatment situations. However, a significant increase in post-treatment semen volume was observed. This is probably due to the increase in age after treatment (Table III) and may reflect increasing testicular maturity.

In our population, sperm cryobanking was terminated for many patients (26%). The most frequent reason for sperm disposal was patient death (Fig. 3). Patients, who asked for their spermatozoa to be destroyed or used in a research programme, did not explain their choice. In agreement with other studies (Hallak *et al*., 1998; Lass *et al*., 2001; Agarwal *et al*., 2004; Chung *et al*., 2004; Pacey *et al*., 2007), only a relatively small proportion of patients (2.2%) returned to use their cryopreserved samples for ART. The vast majority of our patients who maintained their sperm storage were <30 years old (76%). This may explain the particularly low rate of straw utilization, considering the current mean age for first conception in France (29.60 years for women, data from INSEE; no data retrieved for men). However, several additional reasons may be involved: recovery or waiting for possible recovery of gonadal function, short period of original illness, no wish to father children, anxiety regarding risks for the children, uncertainty about long-term health and suitability to be parents. In our population, five patients conceived spontaneously and two fathered children after ART using ejaculated spermatozoa, attesting to the recovery of gonadal function. Despite the low rate of straw utilization, most authors have concluded that sperm banking might be strongly encouraged for patients with malignant disease (Lass *et al*., 2001).

Thus, sperm cryopreservation should be offered routinely to adolescents exposed to gonadotoxic treatment. Age must not be a discriminative parameter. It is generally difficult to predict which patients will remain azoospermic after treatment, except in the case of treatment for bone marrow transplantation. The high level of successful sperm storage observed in our data should encourage physicians to refer their young patients as soon as possible for semen cryobanking. Semen samples might be obtained by masturbation from male adolescents. If young boys fail to produce a sample, they might alternatively provide post-masturbation urine samples. In addition, physicians should ask their patients about erectile function. Medication might also be offered to young men presenting with an erectile dysfunction. Other methods of semen collection are available but are proposed only exceptionally because of their potential negative psychological impact, such as penile vibratory stimulation and electro ejaculation (Schmiegelow *et al*., 1998; Muller *et al*., 2000; Hovav *et al*., 2001; for review, Brougham *et al*., 2003). Adolescents should be referred for semen cryopreservation immediately after the diagnosis and before the beginning of treatment, to store sufficient semen samples. It is reasonable to obtain 2–3 ejaculates per patient because treatment may dramatically reduced semen quality (Ginsberg *et al*., 2008). When patients present azoospermia, TESE could be a useful technique to obtain spermatozoa and must be discussed. We recommended semen cryopreservation for adolescent males, whatever disease type, before gonadotoxic treatment which may impair spermatogenesis.

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