Journal of Andrology, Vol. 25, No. 4, July/August 2004

Cryopreservation of Sperm From Adolescents and Adults With Malignancies

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ABSTRACT: Although cryopreservation of sperm is performed routinely in adults, only a small amount of information is available on its feasibility in adolescent patients with malignancies. Of 936 patients who were candidates for sperm cryopreservation, 851 (111 adolescents and 740 adults) were eligible for this retrospective analysis after excluding patients with relapses of the original or secondary cancers, known bitesticular lesions, or an unknown diagnosis. In general, patients were seen before initiation of treatment for malignancies. However, unilateral ablation of the testis was performed in 61% of patients with testicular cancer before cryopreservation of samples. Patients were grouped according to primary diagnosis and age. Measurements included testicular volume, semen analysis, and serum hormones (luteinizing hormone [LH], follicle-stimulating hormone [FSH], and testosterone). The youngest patient with an ejaculate containing sperm was 13.5 years old. No significant differences in any investigated parameter could be detected for any diagnosis among the 111 adolescents (age, <20 years). In contrast, adult patients with testicular cancer showed higher FSH values and lower sperm concentrations than adult patients with lymphomas, leukemias, and bone cancers. Patients younger than 16 years had lower ejaculate volumes than men older than 25 years, and testosterone levels were higher in patients aged 20–29 years than in the youngest patient group. Cryopreservation of sperm can be performed in adolescent patients with overall success rates (defined as the observation of at least a single motile sperm after the thawing procedure) similar to those observed in adults and should be recommended even to oncological patients younger than 15 years, provided that these patients can produce a semen sample.

Key words: Cancer, reproduction, fertility, childhood, male.
J Androl 2004;25:586–592

Today, some of the most common malignancies in adolescents and young adults, such as testicular tumors, acute leukemias, lymphomas, and osteosarcomas, can be cured by a combination of surgery, chemotherapy, and radiotherapy, and a considerable fraction of these patients survive long term. It is estimated that 1 in 1000 young adults survive a childhood malignancy (Relander et al, 2000; Howell and Shalet, 2001). However, with increasing long-term survival rates, the long-term toxicity that follows therapeutic intervention and the resulting quality-of-life issues become increasingly important (Kliesch et al, 2000).

Depending on the substances and dosages used, most chemotherapy or radiotherapy treatments affect gonadal function independently of the patient's pubertal status (Howell and Shalet, 2001). Theoretically, hormonal gonadal protection (Meistrich et al, 2000) and retransplantation of germ cell stem cells (Schlatt et al, 1999; Hovatta, 2001) preserved prior to chemotherapy offer the option of preserving spermatogenesis and fertility. However, to date, hormonal gonadal protection has not produced the desired effects in nonhuman primates (Meistrich et al, 2000; Kamischke et al, 2003) or in oncological patients treated with gonadotropin-releasing hormone analogs (Brennemann et al, 1994). Retransplantation of testicular stem cells is still in the early stages of development (Schlatt, 1999). At present, cryopreservation of sperm prior to oncological therapy represents the only option for maintaining reproductive capacity (Kliesch et al, 2000).

Although cryopreservation of sperm in adult patients is a routine procedure, only a small amount of information is available on the spermatogenetic capacity and feasibility of cryopreservation in adolescent oncological patients (Kliesch et al, 1996; Müller et al, 2000; Bahadur et al, 2002). In this study, we present an update of our previous study that consists of 72 additional adolescent patients (previously, 39 patients) (Kliesch et al, 1996).

Subjects and Methods

Subjects and Study Design

Between April 1989 and March 2003, 936 patients with malignant diseases who were younger than 40 years were referred to the Institute of Reproductive Medicine of the University (Münster, Germany) for cryopreservation of sperm. Patient selection

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Received for publication November 10, 2003; accepted for publication January 23, 2004.

depended on the counseling oncologist and the willingness of the patient (and his parents) to consider the preservation of fertility before the patient reported to the Institute of Reproductive Medicine. After excluding the patients with relapses of the original cancer, secondary cancers, known bitesticular testicular cancer, unilateral testicular cancer in combination with contralateral intraepithelial neoplasia, or an unknown primary diagnosis, 886 patients were eligible for the retrospective analysis. Of these patients, a further 35 were excluded because of incomplete data.

Measurements of Testes Volumes

Sonographic evaluation of scrotal content was performed with a sector scanner (7.5 MHz; Siemens, Erlangen, Germany), as is routinely practiced at our institute (Behre et al, 2000). Estimations of testicular volumes were performed either with the help of a Prader orchidometer or, since 1990, by ultrasonography, because a previous study at our institute showed a reasonable correlation of orchidometer and ultrasound measurements without systematic over- or underestimations in infertile patients (Behre et al, 1989).

Hormone Assays

Venous blood was sampled between 0800 and 1300 hours during every visit. Blood samples for endocrine determinations were separated by centrifugation at 800 × *g* and stored at −20°C until evaluation. Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were determined by highly specific routine immunoassays using methods published previously (Schürmeyer et al, 1984; Bals-Pratsch et al, 1986, 1988; Jockenhövel et al, 1990; Behre et al, 1992, 1994; Lemcke et al, 1996; Kamischke et al, 1998; von Eckardstein et al, 2000). The reference range of values for adult men in our laboratory was as follows: LH, 2–10 IU/L; FSH, 1–7 U/L; and testosterone, greater than 12 nmol/L.

Semen Analysis

Semen samples were analyzed according to guidelines established by the World Health Organization (1987, 1992, 1999) and were subjected to rigid internal (Cooper et al, 1992) and external (Cooper et al, 1999) quality control. In cases of extremely low sperm counts or azoospermia, the ejaculates were centrifuged, and analysis (including Papanicolaou staining) to assess sperm morphology was performed on the sediment. Azoospermia was defined as no sperm found after centrifugation and analysis of the pellet. Patients were requested to abstain from sexual activity for 48 hours to 7 days before investigation.

Cryopreservation of Sperm

After semen analysis, samples were immediately processed for cryopreservation. Semen was mixed rapidly with an equal volume of sterile, commercial, glycerol-based cryoprotectant (Steritec; Steripharm, Berlin, Germany) and dispensed into straws. The straws were placed into cassettes and frozen (according to a protocol from Cooper et al [1992]) in a liquid nitrogen freezer (IceCube 1810; SY-LAB, Punkersdorf, Austria). After freezing, an aliquot was thawed, and sperm motility was determined as described elsewhere (Cooper et al, 1992). Cryopreservation was defined as successful if, after freezing and thawing, at least a single motile sperm could be observed in the aliquot.

Statistics

Variations between the age and diagnostic groups were evaluated by the Kruskal-Wallis test. In case of an overall $P < .05$ in the analysis of variance, differences between the age and diagnostic groups were tested by the Dunn multiple comparisons post hoc procedure. Comparisons between the age groups within a single diagnosis were performed by the Mann-Whitney *U* test. All analyses were performed using statistical software GraphPadPrism for Windows (Version 2.01; GraphPad Software Inc, San Diego, Calif). Two-sided *P*-values $<.05$ were considered significant. In general, results are given as mean ± standard error of the mean.

Results

Subjects

The 851 patients were grouped according to the Cancer Survival Epidemiologists as adults (20 years or older) and adolescents (younger than 20 years) (Bahadur and Hindmarsh, 2000). Patient characteristics and diagnoses are presented in Tables 1 and 2. To date, 1 former adolescent patient desiring paternity has used his cryopreserved semen for assisted reproduction (ART). In 2 in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles, a pregnancy with an early abortion was achieved. Eleven adult patients used their cryopreserved semen samples for ART. Nine clinical pregnancies occurred, which resulted in 11 live births (including 3 sets of twins) and 1 abortion.

Testes Volumes

Testes volumes of adolescents with testicular tumors or other cancers were not different from those of adults, except for those of adolescents with bone carcinoma, who had testes volumes below adult sizes (Table 2). In the adolescent patients, the testes volumes among different diagnoses showed no significant differences, whereas in the adult patients, in the analysis of variance, significant variations could be detected. These variations, however, remained nonsignificant in the post hoc analysis (Table 2). All adolescents showed normal or above-normal testicular volumes (Figure 1) according to the ranges established by Zachmann et al (1974). In the adolescents, testicular volume (Figure 1) was significantly correlated with age ($r = 0.24$, $P = .0096$), ejaculate volume ($r = 0.26$, $P = .0058$; data not shown), testosterone ($P = .0048$; Figure 2a), and sperm count ($P = .0064$; Figure 2b), while no significant correlation could be found with FSH (data not shown).

Table 1. *(Mean ± SEM) unilateral testicular volume; ejaculate volume; sperm concentration; total motile sperm; sperm count; LH, FSH, and testosterone values; and numbers of patients with successful cryopreservation (as evidenced by motile sperm after freezing and thawing) in the different age groups**

Age Group, y	Mean Unilateral Testicular Volume, mL	Ejaculate Volume, mL	Sperm Concentration, million/mL	Sperm Count, million/ejaculate	Total Sperm Motility, %	FSH, U/L	LH, U/L	Testosterone, nmol/L	Successful Cryopreservation, n (%)
<15 (n = 11)	16.3 ± 1.6	1.5 ± 0.3	38.9 ± 13.9	38.0 ± 10.2	38 ± 7	4.4 ± 0.8	3.3 ± 0.6	10.5 ± 2.2	10 (91)
15–<16 (n = 15)	18.5 ± 1.5	1.7 ± 0.3	56.0 ± 24.4	73.0 ± 23.6	48 ± 5	4.1 ± 1.3	2.9 ± 0.6	15.5 ± 3.6	14 (93)
16–<17 (n = 19)	19.4 ± 1.3	3.4 ± 0.5	46.6 ± 15.0	174.4 ± 58.7	48 ± 4	4.5 ± 0.5	4.4 ± 0.5	14.7 ± 1.9	19 (100)
17–<18 (n = 21)	18.9 ± 0.8	2.4 ± 0.2	49.9 ± 20.1	113.2 ± 35.6	57 ± 4	4.7 ± 0.7	4.1 ± 0.5	18.9 ± 1.6	21 (100)
18–<19 (n = 19)	19.9 ± 1.5	3.0 ± 0.3	36.6 ± 11.4	119.7 ± 38.4	56 ± 5	6.7 ± 1.9	5.5 ± 1.0	17.9 ± 1.7	17 (89)
19–<20 (n = 26)	21.0 ± 1.4	3.0 ± 0.4	34.7 ± 6.5	106.9 ± 20.5	48 ± 5	4.0 ± 0.5	4.2 ± 0.5	17.2 ± 1.5	22 (85)
20–<25 (n = 189)	20.8 ± 0.5	3.6 ± 0.1†	34.8 ± 3.7	135.0 ± 18.9	49 ± 2	5.8 ± 0.4	5.1 ± 0.3†	19.0 ± 0.6‡†	165 (87)
25–<30 (n = 271)	23.2 ± 0.2	3.9 ± 0.1‡†	32.9 ± 3.1	127.0 ± 11.6	50 ± 1	6.4 ± 0.4	4.8 ± 0.2	19.8 ± 2.4‡	248 (92)
30–<35 (n = 191)	22.9 ± 0.6	4.2 ± 0.2‡†c	37.2 ± 4.0	135.2 ± 13.6	47 ± 2	7.2 ± 0.4¶	5.0 ± 0.3	16.3 ± 0.5	171 (90)
35–<40 (n = 89)	21.9 ± 0.9	3.9 ± 0.2‡†§	27.8 ± 5.3	104.6 ± 22.4	48 ± 2	8.4 ± 0.6†‖¶	4.9 ± 0.3	15.8 ± 0.7	77 (87)

* LH indicates luteinizing hormone; FSH, follicle-stimulating hormone.
† $P < .05$ compared to 15–<16.
‡ $P < .05$ compared to <15.
§ $P < .05$ compared to 17–<18.
‖ $P < .05$ compared to 19–<20.
¶ $P < .05$ compared to 20–<25.

Hormone Assays

Variations among the age groups but not among the diagnostic groups could be detected for LH, FSH, and testosterone (Tables 1 and 2). For FSH, significant differences were observed between patients with testicular cancer and patients with lymphomas, leukemias, and bone cancers (Table 2). FSH values were higher in adults than in adolescents with testicular cancers (Table 2). All other hormone values between adolescent and adult patients with the same diagnoses showed no significant differences (Table 2).

Semen Analysis

Apart from lower ejaculate volumes, no significant differences between the age groups could be detected for sperm concentration, sperm count, total motile sperm, progressive motile sperm, and sperm with normal morphology (Table 1). In the adolescents, among the different diagnoses, no significant differences could be detected for semen parameters (Table 2). In the adults, differences were observed between patients with testicular cancer and patients with lymphomas, leukemias, and bone cancers (Table 2). Mean ± standard error of the mean abstinence times were not significantly different in patients with testicular cancer (adolescents, 9 ± 2 days; adults, 7 ± 1 day), leukemias (adolescents, 14 ± 4 days; adults, 10 ± 2 days), bone cancer (adolescents, 12 ± 6 days; adults, 11 ± 3 days), and other types of cancer (adolescents, 12 ± 4 days; adults, 5 ± 1 day). However, significant differences could be detected between adolescent patients (13 ± 3 days) and adult patients (8 ± 2 days) with lymphomas.

Cryopreservation of Sperm

No differences among the age groups (Table 1) or within the diagnostic groups (Table 2) could be detected for the percentage of total motile sperm after dilution with the cryoprotectant, the percentage of total motile sperm after freezing and thawing, and the overall success of the cryopreservation procedure, which is defined as the observation of at least a single motile sperm after freezing and thawing. On the basis of this minimal requirement, ''successful'' cryopreservation of sperm was not possible in 8 of 111 (7%) adolescents or in 79 of 740 (11%) adults.

Discussion

Cryopreservation of sperm in adults has been performed for decades, and it is well known that spermatozoa survive long-term cryobanking (Sanger et al, 1992). However, for various reasons, the possibility of cryopreservation of sperm from adolescent cancer patients has until now received little attention (Kliesch et al, 1996; Schover

Table 2. *(Mean ± SEM) unilateral testicular volume; ejaculate volume; sperm concentration; sperm count; LH, FSH, and testosterone values; total postthaw motility; and numbers of patients with successful cryopreservation (as evidenced by motile sperm after freezing and thawing) in adolescents (age, <20 years) and adults (≥20–40 years) with respect to the oncological diagnosis**

Disease	Age, y	Mean Unilateral Testicular Volume, mL	Ejaculate Volume, mL	Sperm Concentration, million/mL	Sperm Count, million/ejaculate	FSH, U/L	LH, U/L	Testosterone, nmol/L	Total Postthaw Motility, %	Successful Cryopres-ervation, n (%)
Testicular cancer										
Adolescents (n = 28)	17.6 ± 0.3	20.9 ± 1.3	3.2 ± 0.4	36.9 ± 12.9	95.0 ± 23.5	6.3 ± 1.4	4.4 ± 0.8	17.8 ± 2.2	33 ± 3	27 (96)
Adults (n = 457)	29.2 ± 0.2	23.1 ± 0.4	4.0 ± 0.1†	24.3 ± 1.7	93.1 ± 6.4	7.9 ± 0.3†	5.1 ± 0.2	19.0 ± 1.4	26 ± 1	410 (90)
Lymphomas										
Adolescents (n = 36)	17.5 ± 0.3	19.4 ± 0.9	2.6 ± 0.3	29.6 ± 5.5	92.6 ± 21.8	4.1 ± 0.4	4.3 ± 0.4	17.0 ± 1.4	27 ± 3	34 (94)
Adults (n = 151)	27.5 ± 0.4‡	27.5 ± 0.4	3.6 ± 0.2†‡	45.3 ± 5.2‡	172.3 ± 20.6†‡	4.8 ± 0.3‡	4.7 ± 0.2	17.4 ± 0.6	27 ± 2	134 (89)
Leukemias										
Adolescents (n = 13)	17.5 ± 0.5	19.8 ± 1.2	2.1 ± 0.3	80.2 ± 22.3	190.9 ± 80.1	5.7 ± 1.2	5.1 ± 0.7	13.0 ± 1.9	22 ± 5	11 (85)
Adults (n = 55)	27.1 ± 0.7‡	20.2 ± 0.8	3.5 ± 0.2†	67.8 ± 12.4‡	236.9 ± 56.0†	4.6 ± 0.5‡	5.3 ± 0.3	15.2 ± 0.9	22 ± 3	47 (86)
Bone cancer										
Adolescents (n = 20)	17.0 ± 0.4	16.9 ± 1.0	2.4 ± 0.3	34.1 ± 7.6	95.1 ± 23.2	3.7 ± 0.5	3.7 ± 0.4	15.9 ± 1.5	28 ± 4	19 (95)
Adults (n = 45)	27.0 ± 0.8‡	20.9 ± 1.1†	3.6 ± 0.3	49.3 ± 7.6‡	183.3 ± 33.8	4.3 ± 0.7‡	3.9 ± 0.3	17.1 ± 1.1	24 ± 3	40 (89)
Other cancers										
Adolescents (n = 14)	17.1 ± 0.4	19.5 ± 1.9	2.6 ± 0.5	69.4 ± 30.6	133.2 ± 49.3	3.9 ± 0.7	3.4 ± 0.6	15.3 ± 2.1	31 ± 6	12 (86)
Adults (n = 32)	30.1 ± 0.9	21.6 ± 1.4	4.4 ± 0.3†	38.7 ± 8.7	169.6 ± 42.9	4.7 ± 0.6	4.7 ± 0.5	17.7 ± 1.3	28 ± 4	30 (94)

* LH indicates luteinizing hormone; FSH, follicle-stimulating hormone.
† $P < .05$ compared to adolescents with the same diagnosis.
‡ $P < .05$ compared to adults with testicular cancer.

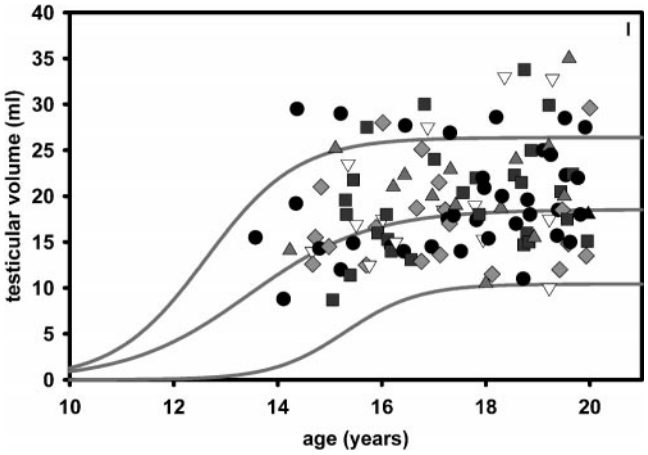

Figure 1. Age vs unilateral testicular volume in adolescent patients with testicular cancer (gray squares), malignant Hodgkin lymphoma (black circles), leukemia (gray triangle), bone cancer (gray diamond), and other types of cancer (white triangle). Gray lines represent the mean and 95% confidence intervals of normal testicular volumes during normal adolescence and are taken from Zachmann et al (1974).

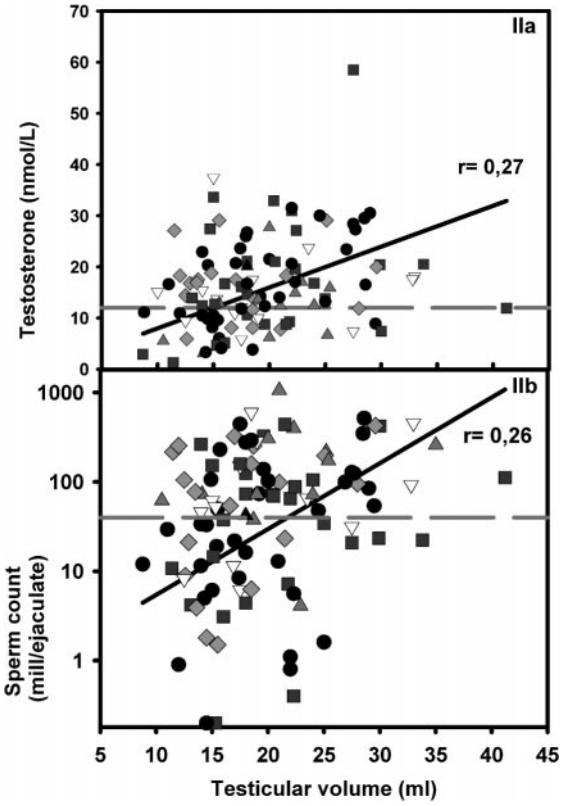

Figure 2. Unilateral testicular volume vs **(a)** serum testosterone and **(b)** sperm counts in adolescents with testicular cancer (gray squares), malignant Hodgkin lymphoma (black circles), leukemia (gray triangle), bone cancer (gray diamond), and other types of cancer (white triangle) prior to cryopreservation. Regression lines were calculated on the basis of all points shown in the graphs, regardless of the underlying disease, and are shown as black lines. Horizontal dashed gray lines represent the lower normal limit (testosterone and sperm count).

et al, 1998; Müller et al, 2000; Bahadur et al, 2002). Nevertheless, cryopreservation of sperm may be even more important in adolescents than in adults, since adolescents have not yet started their families. In our study, only 2 adolescent patients were married, and only 1 had fathered children; by contrast, of the 740 adults, 26% were married, and 14% had become fathers before oncological therapy. However, in a quality-of-life analysis of former oncological patients, about 80% viewed themselves as potential parents, and the vast majority of younger cancer survivors saw their cancer experience as preparing them to be better parents (Schover et al, 1999).

Cryopreservation of sperm is often not discussed with the adolescent patient because examiners harbor uncertainties about semen quality and the sexual maturity of the patient. In addition, there may be difficulties in discussing this sensitive topic with the adolescent and his parents (Bahadur, 2000; Müller et al, 2000). Few data are available on the physiology of semen parameters in pubertal males. In addition, oncological patients may have impaired semen quality even before therapy (Handelsman, 2000). Indeed, in our study, only 17% of the adults and 23% of the adolescents showed normozoospermia as defined by World Health Organization guidelines. Sperm survival may be further decreased because of the freezing and thawing procedure; moreover, in the patients of our study, the total sperm motility was uniformly and severely decreased in the samples after thawing (Table 3). However, despite the poor postthawing sperm survival rates obtained in most oncological patients, ICSI offers the possibility of a pregnancy even if only a single motile sperm is present after thawing (Chen et al, 1996). On the basis of these criteria, successful cryopreservation of sperm was achieved in 93% of the adolescent patients of our study, which is slightly better than the 89% successful cryopreservation obtained in the adult patients of our study. Furthermore, cryopreservation may be considered successful even if only immotile but viable sperm are present. As in other diseases, pregnancies in patients with malignancies have been achieved with immotile but viable sperm, as evidenced by the hypo-osmotic swelling test (Ved et al, 1997).

One possible explanation for these relatively good results, especially in the adolescents, is that only 25% of the patients suffered from a testicular tumor as opposed to 62% of the adults, and the testicular tumor per se may be associated with impaired semen parameters (Petersen et al, 1998).

The adolescent patients of our study had normally developed testicular volumes for persons that age (Zachmann et al, 1974) and, except for 1 patient, all of the them showed normal or above-normal testicular volumes, irrespective of the underlying diagnosis (Figure 1). Spermarche occurs between 11 and 17 years when testic-

Table 3. *Age; disease; unilateral testicular volume; ejaculate volume; sperm concentration; sperm count; LH, FSH, and testosterone values; and success of cryopreservation (as evidenced by motile sperm after freezing and thawing) in the 11 patients younger than 15 years**

Disease	Age, y	Unilateral Testicular Volume, mL	Ejaculate Volume, mL	Sperm Concentration, million/mL	Sperm count, million/ejaculate	FSH, U/L	LH, U/L	Testosterone, nmol/L	Postthaw Motile Sperm, %
Malignant Hodgkin lymphoma	13.6	15.5	3.9	10.1	39.4	8.1	3.6	6.0	2
Malignant Hodgkin lymphoma	14.1	8.8	3.0	5.4	16.2	7.9	3.2	11.1	0
Leukemia	14.2	14.1	0.5	146.0	73.0	2.9	1.5	3.1	23
Malignant Hodgkin lymphoma	14.4	19.2	1.0	73.5	73.5	3.9	2.9	14.2	36
Malignant Hodgkin lymphoma	14.4	29.5	1.0	54.0	54.0	2.2	1.9	8.9	51
Neuroblastoma	14.7	14.0	1.3	35.5	46.2	5.9	3.4	15.4	1
Osteoblastoma	14.7	12.6	0.6	14.8	8.9	1.1	1.5	5.9	25
Osteoblastoma	14.7	15.5	1.9	0.7	1.5	6.5	3.8	25.1	1
Non-Hodgkin lymphoma	14.8	14.3	1.6	3.1	5.0	1.9	4.2	3.3	18
Ewing sarcoma	14.8	21.0	1.2	82.0	98.4	2.2	1.5	7.8	45
Osteoblastoma	14.9	14.5	0.7	2.6	1.8	6.1	8.9	10.3	11

* LH indicates luteinizing hormone; FSH, follicle-stimulating hormone.

ular volumes are between 5 and 20 mL (Hirsch et al, 1979; Nielsen et al, 1986; Kulin et al, 1989; Schaefer et al, 1990). All of the adolescents of our study had testicular volumes greater than 5 mL, and only 1 patient with a mediastinal teratoma (age, 19 years) showed azoospermia. In the adolescents, testicular volumes were correlated with age (Figure 1), serum testosterone (Figure 2a), and sperm count (Figure 2b). However, development of testicular volume was a weak predictor of endocrine maturation, since 41 adolescents had serum testosterone values below the normal adult limit of 12 nmol/L, despite normal or above-normal age-adjusted testicular volumes.

The adolescent patients of our study generally abstained from sexual activity for longer periods than the adults of our study with the same disease. Although this is significant only in patients with lymphomas, abstinence time has not been correlated with sperm concentration, but it has been related positively to ejaculate volume (Cooper et al, 1993; Rolf et al, 1996). The ejaculate volume of the adolescents, which is often low, therefore provides biological evidence for not fully developed androgenicity, a finding that was also observed by Bahadur et al (2002).

To compensate for the low ejaculate volume, several semen samples from the adolescents should be requested, or, if sperm concentrations are high, the ejaculate should be diluted before it is mixed with the cryoprotectant, as very few sperm will be needed from 1 straw for ICSI. However, the reduced ejaculate volume that is often observed is not a major problem in cryopreservation of sperm from adolescents. Because no adverse prognostic factors for successful cryopreservation in adolescents have been identified, cryopreservation of sperm should be offered to all oncological patients, even to those younger than 15 years, who will be subjected to potentially irreversible oncological treatments and who are able to produce semen samples.

Acknowledgments

This work was supported in part by the German Federal Ministry of Health. The clinical collaboration of physicians from the University of Münster as well as technicians at the Institute of Reproductive Medicine are gratefully acknowledged. We are grateful to Anita Broschk for data documentation and to Susan Nieschlag, MA, for language editing.

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