Clinical and biochemical correlates of successful semen collection for cryopreservation from 12–18-year-old patients: a single-center study of 86 adolescents

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BACKGROUND: Cryopreservation of semen should be offered to adults before gonadotoxic treatment. However, the experience with semen collection in adolescents is still limited. The objective of this study was to evaluate potential correlates of successful semen sampling in adolescents.

METHODS: A total of 86 boys (aged 12.2–17.9 years), referred for cryopreservation of semen prior to gonadotoxic treatment were included. Age, testicular volume, diagnosis and reproductive hormones were evaluated as correlates of successful semen collection.

RESULTS: Median sperm concentration was 9.6 (range 0–284) million/ml. Of the 86 included boys, 76 (88.4%) had spermatozoa in their ejaculate. Of the 76 patients for whom a semen sample was obtained, 71 (93.4%) had motile spermatozoa eligible for cryopreservation. Of the 86 boys, 74 produced a semen sample by masturbation, whereas semen samples were obtained from 12 patients by penile vibration or electroejaculation. The youngest patient with an ejaculate containing motile spermatozoa was 12.2 years old, and the smallest testicular volumes in boys associated with motile spermatozoa in the ejaculate were 6–7 ml. Testicular volume correlated with sperm concentration (R = 0.283, P = 0.046), and the percentage of motile spermatozoa (R = 0.410, P = 0.003). Chronological age, but not reproductive hormones, also correlated with sperm concentration (R = 0.25, P = 0.049).

CONCLUSIONS: Semen was successfully collected and cryopreserved in 71 out of 86 boys and adolescents. Testicular volume, but not age or reproductive hormone levels, was indicative of successful semen collection. Regardless of their age, adolescent boys with testicular volumes of more than 5 ml should be offered semen banking prior to gonadotoxic treatment or other procedures that could potentially damage future fertility.

Key words: childhood cancer / infertility / sperm concentration / testis size / cryopreservation

Introduction

During the last decades, the treatment efficiency has improved significantly for child and adolescent cancer and the overall number of survivors has increased substantially. Five-year survival for all cancers is 80–90% in adolescents and young adults (Gatta et al., 2009). With an increasing treatment intensity and increased survival rate, side effects in cancer survivors have become a more frequent issue, primarily in the area of chemotherapy and radiation therapy. When the survival rate increases, the side-effects of treatment also increase. One of the frequent side effects of cancer treatment is permanent male sterility, and the proportion of patients who becomes oligo- or...
azoospermic is large (Tournaye et al., 2004). The gonadal effect of chemotherapy is variable and depends on type, cumulative dose and duration of treatment and also potential interactions between various combination regimes. The negative effect on spermatogenesis is often permanent, but in some patients a few stem cells escape destruction resulting in recovery of fertility sometimes many years after ending treatment (Bramswig et al., 1990; Siimes and Rautonen, 1990; Meistrich et al., 1992; Heikens et al., 1996; Nurmi et al., 2009). Especially, patients treated with chemotherapeutic agents with alkylating properties as well as those patients receiving irradiation to the pelvic area and/or testes are at high risk of permanent infertility (Bramswig et al., 1990; Siimes and Rautonen, 1990; Meistrich et al., 1992; Heikens et al., 1996; Muller et al., 2000; Relander et al., 2000; Schmidt et al., 2004; Lahteenmaki et al., 2008).

Today semen cryopreservation should be offered to adults before potential gonadotoxic treatment. Cryopreservation can also be offered to boys and adolescents who are able to produce an ejaculate by masturbation. However, if the boy is unable to produce an ejaculate by masturbation due to psychological immaturity, shyness or stress after having received a cancer diagnosis, alternative methods to collect sperm for cryopreservation exist, such as stimulation by penile vibration or transrectal electrostimulation under general anesthesia (Schmiegelow et al., 1998; van den Berg et al., 2007). However, the experience with these procedures in adolescents is still limited.

Even if the quantity and quality of the semen sample is poor, there may still be chances for future fatherhood by use of assisted reproductive treatments (ART) such as intracytoplasmatic sperm injection (ICSI). This method has a 40% clinical pregnancy rate per ART cycle. There were 86 boys or adolescents referred to our department for cryopreservation of semen before initiation of gonadotoxic treatment, or other procedures that could potentially damage future fertility, during the period between December 1995 and May 2009. The patients were aged 12.2–17.9 years (median 16.2 years), and 80 were diagnosed with cancer. The patients had leukemia/non-Hodgkin’s lymphoma (L/NHL) n = 25, 16 acute lymphoblastic leukemia, 2 chronic myeloid leukemia, 1 myelodysplastic dysplasia, 6 NHL, HL (n = 19), testicular cancer (n = 16), solid tumors (n = 20) and other diagnoses (n = 6, 2 with aplastic anemia, 1 disseminated sclerosis, 1 histiocytosis, 1 varicocele and 1 Wegeners granulomatosis).

The clinical data were retrieved from the 86 patient record files and we recorded age at diagnosis, type of diagnosis, modalities of therapy, pubertal development according to Tanners criteria, testicular volume by Praders orchidometer, reproductive hormone levels and results from analysis of semen. We report data on 16 patients with testicular cancer, but excluded data from these subjects from correlation analyses as the testicular tumor itself influenced testicular volume as well as reproductive hormones (nine of the patients had hCG-producing testicular tumors and clearly suppressed gonadotrophin levels).

Clinical examination

Before treatment, the adolescents and parents received an explanation on the possible effects of the malignant disease and of the possible side effects of chemotherapy on their future fertility. Pubertal stages were assessed by clinical examination according to the methods by Marshall and Tanner (1970); i.e. genital stages (G1–G5) and pubic hair stages (PH1–PH6). Testicular volume was evaluated by Prader’s orchidometer (1) to the nearest 1 ml.

If puberty had begun (Tanner stage G2 or more) and if testicular volume was > 5 ml, the adolescents were interviewed in the absence of their parents/guardians (but with their consent) about their experience with masturbation, and if they believed masturbation was possible in the hospital setting. In case, the boys were unable to produce a semen sample by masturbation, they were offered electroejaculation or penile vibration under general anesthesia.

Semen analysis

A minimal routine analysis was performed on each sample before cryopreservation. Semen volume was estimated by weighing the collection tube with the semen sample and subtracting the predetermined weight of the empty tube, assuming that 1 ml = 1 g. Phase-contrast microscopy (positive phase-contrast optics) was used for the examination of fresh semen. For sperm motility assessment, 10 μl of well-mixed semen was placed on a clean glass slide that had been kept at 37°C and covered with a 22 × 22 mm coverslip. The preparation was placed on the heating stage of a microscope at 37°C and immediately examined at ×400 magnification. Microscopic fields were assessed in a systematic way to classify 200 spermatozoa. The motility of each spermatozoa was graded as either fast progressive motile (WHO class A), slow progressive motile (class B), nonprogressive motile (class C) or immotile (class D) (WHO guidelines). The motility counting was repeated on a second aliquot of 10 μl well-mixed semen. If the difference between these two counts exceeded the acceptable value on the chart provided by the WHO (1992), new preparations were made for a renewed evaluation. For the assessment of the sperm concentration, each semen sample was thoroughly mixed for at least 10 min in a rotation device. An aliquot of the sample was put into the diluent using a positive displacement pipette and mixed further for 10 min. The diluent consisted of 50 g NaHCO3, 10 ml 40% formaldehyde and distilled water up to 1 l. The sperm concentration was subsequently assessed using a Bürker–Türk hemocytometer. One drop of the diluted specimen was transferred to each chamber of the hemocytometer, which was allowed to stand for 5 min in a humid chamber before the cells were counted at a total microscope magnification of ×400. Concentration counts were only done on one dilution of the sample in order to secure a sufficient amount of the samples for cryopreservation. Only spermatozoa with tails were counted (Jørgensen et al., 2002).
Semen cryopreservation
The samples were frozen and stored in liquid nitrogen. In brief, an equal amount of cryopreservation medium (purchased from Bie-Berntsen LAB, Denmark) was added to the raw semen samples and gently mixed. Aliquots of this mixture were transferred into 0.5 or 0.25 ml straws. These were then placed in a ‘Planer-plc, Kryo 360-1.7°C’, which gradually freeze the samples from room temperature to –80°C during a 45 min period. Following this, the straws were transferred to cryotanks with liquid nitrogen for storage. Any sample containing motile spermatozoa was cryopreserved.

Electroejaculation and penile vibration
Electroejaculation was performed under general anesthesia by inserting a transrectal probe with the electrodes in contact with the rectal mucosa in the area of the prostate and the seminal vesicles. A pulsatile pattern with a total of 25 stimulations (maximum 2.5 V/600 mA) was given, producing an antegrade ejaculate. A vibrator (Ferti Care person- neal, Multicest Aps, Rungsted, Denmark) was placed at the frenulum with a peak-to-peak amplitude of 1.5 mm at a frequency of 100 Hz. Penile vibration was performed in one subject during general anesthesia, which resulted in an antegrade ejaculation.

Blood sampling
Blood samples were obtained at the time of semen collection. Non-fasting samples were drawn from an antecubital vein. Serum follicle-stimulating hormone (FSH), testosterone, inhibin B, luteinizing hormone (LH) and sex-hormone binding globulin (SHBG) were determined by the same validated assays in our laboratory during the study period (Andersson et al., 1997), and were available for 73, 73, 72 and 69 patients, respectively.

Hormone assays
Serum FSH and LH were measured by time-resolved immunofluoro metric assays (Delfia; PerkinElmer, Boston, MA, USA) with detection limits of 0.06–0.05 IU/l for FSH and LH, respectively. Intra- and inter-assay coefficients of variation (CV) were less than 5% in both gonadotrophin assays. Testosterone was measured with the DPC Coat-A-Count RIA kit (Diagnostic Products, CA, USA). The detection limit was 0.23 nmol/l and the intra- and inter-assay CV were 7.6–8.6%, respectively. Inhibin B was determined by a specific two-sided ELISA (Serotec, UK). The intra- and inter-assay CVs were 15–18%, respectively. SHBG was determined by a time-resolved immunofluorescence assay (Delfia, Wallac Oy, Turku, Finland) with a detection limit of 0.20 nmol/l. Intra- and inter-assay CV were 5.8–6.4%, respectively.

Statistics
All statistical analyses were performed using the statistical software SPSS version 16.0 for Microsoft Windows XP. Data are presented as medians (ranges). Associations between variables were compared by Pearson’s correlation analyses and presented by R values and values of $P < 0.05$ were considered statistically significant. In our analyses, we divided the patients into three groups based on their semen quality: ≥ 20 million/ml, < 20 million/ml and azoospermia (0 million/ml).

Ethics
The study was a retrospective study with an evaluation of data collected as part of routine clinical care, and obtained from patient record files. In all included cases, both patients and parents gave informed consent to the procedures.

Results
Results from clinical examinations, as well as biochemical and semen analyses according to diagnostic groups are presented in Table I. Reproductive hormone levels (Fig. 1) and sperm concentrations (Fig. 2) are illustrated according to age.

Among the 86 boys, semen samples were obtained by masturbation in 74 patients (86%), whereas the remaining 12 boys where unable to collect semen by masturbation for various reasons (primarily psycholog-ical reasons). These boys were offered penile vibration (n = 1) or electroejaculation (n = 11) under general anesthesia, which resulted in a semen sample in all 12 boys who could not deliver a semen sample by masturbation. However, from four of the boys only 0.1–0.3 ml semen was obtained, and no sperm were present in these samples. Another two samples contained only immotile sperm that were not cryopreserved. Thus, 6 out of these 12 semen samples from adolescent boys were cryopreserved (Table II).

Of the 86 included boys, 76 (88.4%) had visible spermatozoa in their ejaculate by microscopy. Of these, 33 (38.4%) had a sperm concentration of at least 20 million/ml, 43 patients (50.0%) had spermatozoa in their ejaculate but less than 20 million/ml, whereas 10 (11.6%) had azoospermia. Of the 76 patients in whom a semen sample was obtained, 71 had motile spermatozoa in the ejaculate that could be cryopreserved (while a few immotile sperm were seen in 5 patients). In all patients, the median sperm concentration was 9.6 (range 0–284) million/ml, and the median percentage of motile spermatozoa was 45.5% (range 0–86%).

Out of all patients, the youngest boy with an ejaculate containing motile spermatozoa was 12.2 years old, and the smallest testicular volume observed in a boy with successful outcome was 6–7 ml.

Chronological age
Age correlated with testicular volume ($R = 0.389, P = 0.003$) and with the ejaculated semen volume ($R = 0.339, P = 0.004$), the number of spermatozoa ($R = 0.288, P = 0.023$) and the percentage of motile spermatozoa ($R = 0.289, P = 0.023$). There was a significant correlation between age and sperm concentration ($R = 0.251, P = 0.049$), but the correlation between age and the total number of motile spermatozoa was not quite significant ($R = 0.246, P = 0.054$).

Testicular volume
The testicular volume correlated strongly with sperm concentration ($R = 0.283, P = 0.046$) and with the percentage of motile spermatozoa ($R = 0.410, P = 0.003$), but not with the total number of spermatozoa or total number of motile spermatozoa.

Reproductive hormones
FSH correlated negatively with inhibin-B ($R = -0.577, P < 0.0001$) and positively with SHBG ($R = 0.318, P = 0.020$) and LH ($R = 0.414, P = 0.001$) concentrations. Testosterone correlated positively...
Table I  Descriptive reproductive characteristics of 86 boys referred for cryopreservation of semen.

<table>
<thead>
<tr>
<th>Hodgkins lymphoma</th>
<th>C.Testis</th>
<th>NHL/Leukemia</th>
<th>Solid tumor</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>19</td>
<td>16</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.4 (12.7–17.9)</td>
<td>16.9 (15.6–17.9)</td>
<td>16.1 (13.9–17.9)</td>
<td>15.2 (12.2–17.8)</td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>1.7 (0.1–5.9)</td>
<td>3.0 (0.5–6.9)</td>
<td>1.8 (0.1–6.2)</td>
<td>1.5 (0.03–6.1)</td>
</tr>
<tr>
<td>Sperm concentration (million/ml)</td>
<td>8.5 (0–86)</td>
<td>2.9 (0–59)</td>
<td>36 (0–191)</td>
<td>21 (0.1–103)</td>
</tr>
<tr>
<td>Total sperm count (millions)</td>
<td>7 (0–243)</td>
<td>8.7 (0.03–216)</td>
<td>46.8 (0.04–611)</td>
<td>34.1 (0.15–210)</td>
</tr>
<tr>
<td>Motile spermatozoa (%)</td>
<td>35 (0–86)</td>
<td>43.5 (1–86)</td>
<td>57 (0–80)</td>
<td>43 (0–86)</td>
</tr>
<tr>
<td>Total no. of motile (millions)</td>
<td>3.2 (0–186.3)</td>
<td>3.4 (0–136.1)</td>
<td>28.1 (0–385.1)</td>
<td>12.4 (0–180.6)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>3.5 (0.69–5.45)</td>
<td>0.01 (0.01–19.1)</td>
<td>3.87 (1.0–41.7)</td>
<td>3.2 (0.53–18.7)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>2.2 (0.8–7.9)</td>
<td>1.1 (0.01–45)</td>
<td>3.3 (0.7–14)</td>
<td>3.3 (1.1–13.2)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>9.4 (0.1–25)</td>
<td>17.9 (10.8–42.4)</td>
<td>12.7 (2.9–23.3)</td>
<td>11.7 (1.2–20.8)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>29 (16–62)</td>
<td>27.5 (11–42)</td>
<td>35 (19–76)</td>
<td>26.5 (12–65)</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>190 (72–329)</td>
<td>92 (27–211)</td>
<td>196 (1–419)</td>
<td>220 (28–352)</td>
</tr>
<tr>
<td>Semen sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;20 million/ml</td>
<td>5/19 (26.3)</td>
<td>4/16 (25)</td>
<td>12/25 (48)</td>
<td>9/20 (45)</td>
</tr>
<tr>
<td>&lt;20 million/ml</td>
<td>13/19 (68.4)</td>
<td>10/16 (62.5)</td>
<td>9/25 (36)</td>
<td>9/20 (45)</td>
</tr>
<tr>
<td>0 million/ml</td>
<td>1/19 (5.3)</td>
<td>2/16 (12.5)</td>
<td>4/25 (16)</td>
<td>2/20 (10)</td>
</tr>
</tbody>
</table>

Results are presented as medians (range) or % of subjects (semen analysis).
with both age ($R = 0.382$, $P = 0.003$) and semen volume ($R = 0.294$, $P = 0.026$). FSH and inhibin-B did not correlate with sperm concentration, motility, total sperm number nor total number of motile spermatozoa.

Discussion

In this single-center study, we evaluated the success of semen collection in 86 boys and adolescents, aged 12.2–17.9 years, prior to the start of cytotoxic treatment or other procedures that could impair future fertility. Cryopreservation of semen was possible in 71 adolescents, with testicular size as the best parameter to predict a successful semen collection.

A successful semen collection was obtained following masturbation in a 12.2-year-old boy (testicular volume 20 ml) as well as in a 13.4-year-old patient, who had testicular volumes of only 6–7 ml, respectively. Thus, even at such a young age and at small testicular volumes, it is possible to obtain a semen sample that is eligible for cryopreservation, by masturbation.

Obviously, a certain pubertal stage is required before semen sampling is a realistic option. The median age for spermarche has been reported to be 13–14 years of age (Nielsen et al., 1986a, b; Kulin et al., 1989; Pedersen et al., 1993). The ages reported for spermarche correlated with the reported age based on interviews on age at first conscious ejaculation (Laron et al., 1980; Schaefer et al., 1990).

No normative data exist on sperm concentration in developing boys and adolescents. In our study, it is therefore not possible to evaluate whether or not semen quality in our adolescents is normal or, for example, influenced by cancer. In addition to a certain biological maturity, some degree of psychological maturity is needed before masturbation is possible, especially in a hospital setting after having received a life-threatening diagnosis. Furthermore, a successful outcome can be inhibited due to time constraints as well as parental anxiety (Bashore, 2007). Efforts for success in semen preservation should include private discussions between health-care professionals and the adolescents. Information on infertility needs to be given to families early in the diagnostic phase to provide them with an opportunity to ask questions and to arrange for alternative procedures such as electroejaculation if masturbation is not possible. It is our personal experience that the families, although maximally stressed at the time of providing this information, consider these discussions as positive even in the few cases where semen collection is not successful. This is possibly because it reassures them that everything possible has been done to preserve fertility.

In our study, semen was obtained during general anesthesia using electroejaculation or penile vibration, in all 12 boys who could not deliver a semen sample for various reasons. However, in four of the
<table>
<thead>
<tr>
<th>No</th>
<th>Mode</th>
<th>Age years</th>
<th>Disease</th>
<th>Puberty Genital</th>
<th>Tvol L/ R (ml)</th>
<th>FSH IU/l</th>
<th>Inhibin B pg/ml</th>
<th>LH IU/l</th>
<th>SHBG nmol/l</th>
<th>T nmol/l</th>
<th>Volume ml</th>
<th>Conc million/ml</th>
<th>Total million</th>
<th>A%</th>
<th>B%</th>
<th>C%</th>
<th>D%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Electroejaculation</td>
<td>12.7</td>
<td>Hodgkin's lymphoma</td>
<td>G5</td>
<td>PH4</td>
<td>8/8</td>
<td>0.69</td>
<td>320</td>
<td>1</td>
<td>32</td>
<td>0.01</td>
<td>0.1</td>
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<td>NA</td>
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<td>NA</td>
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<td>2</td>
<td>Electroejaculation</td>
<td>12.9</td>
<td>Hodgkin's lymphoma</td>
<td>NA</td>
<td>PH3</td>
<td>15/15</td>
<td>1.8</td>
<td>279</td>
<td>2.5</td>
<td>62</td>
<td>8.04</td>
<td>1.3</td>
<td>5.5</td>
<td>7.15</td>
<td>4</td>
<td>11</td>
<td>16</td>
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<td>3</td>
<td>Electroejaculation</td>
<td>13.8</td>
<td>Ewing's sarcoma</td>
<td>G4</td>
<td>NA</td>
<td>0.53</td>
<td>307</td>
<td>1.1</td>
<td>28</td>
<td>4.08</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>4</td>
<td>Electroejaculation</td>
<td>13.9</td>
<td>Lymphoma</td>
<td>G3</td>
<td>PH4</td>
<td>8/8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>0.1</td>
<td>0</td>
<td>0</td>
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<td>5</td>
<td>Electroejaculation</td>
<td>14</td>
<td>Lymphoma</td>
<td>NA</td>
<td>PH5</td>
<td>20/20</td>
<td>1</td>
<td>277</td>
<td>0.7</td>
<td>NA</td>
<td>12.6</td>
<td>0.8</td>
<td>99</td>
<td>79.2</td>
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<td>Rhabdomyosarcoma</td>
<td>G4</td>
<td>NA</td>
<td>15/15</td>
<td>3.33</td>
<td>294</td>
<td>4.9</td>
<td>65</td>
<td>5.22</td>
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<td>0</td>
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<td>24</td>
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<td>14.4</td>
<td>Osteosarcoma</td>
<td>NA</td>
<td>PH4</td>
<td>10/10</td>
<td>1.73</td>
<td>277</td>
<td>2.3</td>
<td>28</td>
<td>13.12</td>
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<td>0.61</td>
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<td>Electroejaculation</td>
<td>14.5</td>
<td>Osteosarcoma</td>
<td>G3</td>
<td>PH3</td>
<td>10/10</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.03</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>9</td>
<td>Electroejaculation</td>
<td>15</td>
<td>Hodgkin's lymphoma</td>
<td>NA</td>
<td>NA</td>
<td>3.18</td>
<td>72</td>
<td>0.8</td>
<td>24</td>
<td>1.43</td>
<td>0.4</td>
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<td>0.1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>95</td>
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<td>10</td>
<td>Electroejaculation</td>
<td>15.3</td>
<td>Rhabdomyosarcoma</td>
<td>NA</td>
<td>PH5</td>
<td>15/15</td>
<td>1.27</td>
<td>310</td>
<td>1.85</td>
<td>12</td>
<td>1.64</td>
<td>2.6</td>
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<tr>
<td>11</td>
<td>Electroejaculation</td>
<td>17.3</td>
<td>C. testis</td>
<td>NA</td>
<td>PH6</td>
<td>8/8</td>
<td>0.01</td>
<td>56</td>
<td>0.01</td>
<td>21</td>
<td>42.43</td>
<td>1.8</td>
<td>0.1</td>
<td>0.18</td>
<td>0</td>
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<td>1</td>
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<tr>
<td>12</td>
<td>Penile Vibration</td>
<td>15.8</td>
<td>Lymphoma</td>
<td>NA</td>
<td>PH6</td>
<td>12/15</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.8</td>
<td>2.5</td>
<td>4.5</td>
<td>0</td>
<td>8</td>
<td>21</td>
<td>71</td>
</tr>
</tbody>
</table>

Genital and pubic hair stages are reported according to Tanner’s criteria. Tvol, testicular volume by orchidometry (L, left; R, right).

‘A’–D’ where A refers to fast progressive motile spermatozoa, B to slow progressive motile spermatozoa, C to non-progressive motile spermatozoa and D to immotile spermatozoa.

NA, not available.
boys only 0.1–0.3 ml semen was obtained, and no sperm were visible in these samples. Another two samples contained only immotile sperm that were not cryopreserved. Thus, 6 out of 12 semen samples obtained from adolescent boys by electroejaculation or vibration, were cryopreserved. It remains unknown if the procedure of electroejaculation may influence sperm motility. Preferably, this procedure should be combined with other procedures requiring general anesthesia such as insertion of an intravenous catheter, in order to minimize anesthesia. Initially, we reported on successful cryopreservation outcome from two pubertal boys (aged 14–15 years) by electroejaculation or penile vibration, respectively (Schmiegelow et al., 1998). In our present study, we confirmed that this methodology is suitable in adolescents who are not able to masturbate for various reasons.

There are several potential predictors of spermatogenic potential that co-varies with pubertal development and age, including size of testes and serum levels of pituitary-gonadal hormones such as LH, FSH, inhibin-B and testosterone (Nielsen et al., 1986b; Kulik et al., 1989; Andersson et al., 1997). Nonetheless, none of the biochemical variables correlated with sperm concentration in our study of adolescents.

The non-steroid hormone inhibin-B mediates a negative feedback loop from the Sertoli cells of the testes to pituitary-gonadal axis. Inhibin-B concentration correlates inversely with FSH that stimulates sperm production. A recent study in which 33 cancer survivors were compared with healthy adult controls recognized that serum inhibin-B levels reflected sperm concentration. In that study, serum inhibin-B was barely detectable in the azoospermic patients despite the preserved Sertoli cells (Thomson et al., 2002). The interaction between germ cells and Sertoli cells are required for inhibin-B production in adults (Andersson et al., 1998). Altogether, inhibin-B has a potential value as the non-invasive marker of spermatogenesis after cytotoxic treatment in adults (Thomson et al., 2002).

However, we did not observe this in our present study of adolescents. This could be due to the fact that the interaction between Sertoli cells and germ cells may not have matured yet in all of these 12–18-year-old boys.

Other studies have evaluated the success rate of semen sampling in adolescents. Bahadur et al. (2002) evaluated a total of 238 adolescent cancer sufferers who produced semen samples with a success rate of 86.1%. They all retrieved their sample by masturbation, and the youngest boy from whom a semen sample was retrieved was 12 years old. Similar results were reported in another study reporting on semen sampling from 156 adolescents with a success rate at 88.5% (Menon et al., 2009). Van Casteren et al. (2008) studied 80 boys with a median age of 16.6 years (ranging from 13.7 to 18.9 years), who were offered cryopreservation prior to gonadotoxic treatment. Of these boys, 53 had a semen quality adequate for cryopreservation, whereas 13 boys were unable to produce a sample, and 14 had immotile sperm in their ejaculate not eligible for cryopreservation. In another study, 27 adolescents aged 14–19 years and diagnosed with cancer, were offered cryopreservation prior to cancer treatment. In that study, 40 out of 62 successful ejaculation attempts resulted in a normal sperm count above 20 million/ml and these were used for cryopreservation. Altogether the authors reported that only 4 out of 62 produced a fully normal semen sample according to adult criteria (Postovsky et al., 2003), but it should be reinforced that only a few motile sperm may be sufficient due to ICSI.

## Conclusion

In conclusion, we demonstrated successful collection of semen for cryopreservation in 71 out of 86 boys or adolescents who were offered this option. Testicular volume was significantly associated with successful outcome of semen collection in adolescent boys aged 12.2–17.9 years, whereas reproductive hormones (FSH, inhibin-B, LH, testosterone) were not. We believe that adolescent boys who have entered puberty and have testicular volumes of more than 5 ml should be offered semen banking prior to gonadotoxic treatment or other procedures that could potentially damage future fertility.

## References


Andersson AM, Muller J, Skakkebaek NE. Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. J Clin Endocrinol Metab 1998;83:4451–4458.


