Sperm DNA integrity in testicular cancer patients

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BACKGROUND: We evaluated the impact of testicular germ cell cancer (TGCC), its treatment and length of follow-up on sperm DNA integrity. METHODS: In 96 TGCC patients, semen was collected at specific intervals until 5 years after treatment. Sperm DNA integrity was assessed by the sperm chromatin structure assay (SCSA, n = 193) and by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL, n = 159) assay. Results were expressed as DNA fragmentation index (DFI). Controls comprised of 278 military conscripts. RESULTS: Post-surgery testicular cancer (TC) patients did not differ from controls. Compared with pretreatment values, radiotherapy induced a transient increase in SCSA_{DFI} (medians: 12 versus 19%; P = 0.03), normalizing after 3–5 years. One year or more after therapy, 5/13 (38%) of normozoospermic, irradiated patients had SCSA_{DFI} >27% compared with 7% of normozoospermic controls (P = 0.002). More than two cycles of chemotherapy decreased DFI 3–5 years post-therapy (median SCSA_{DFI}: 12 versus 9.1%, P = 0.02; median TUNEL_{DFI}: 11 versus 7.5%, P = 0.03). CONCLUSION: Irradiation increases sperm DNA damage 1–2 years after treatment, and 38% of irradiated patients with normozoospermia had high (>27%) DNA damage, which may affect the sperm-fertilizing ability. TC per se is not associated with an increase of DFI, and DFI is reduced by three or more cycles of chemotherapy.

Key words: chemotherapy/radiotherapy/SCSA/sperm DNA/testicular cancer/TUNEL

Introduction

Testicular germ cell cancer (TGCC) is the most frequent malignant disease in young men. With adequate treatment, 90–95% of the patients are cured (Schmoll et al., 2004). The excellent cure rate puts focus on long-term effects of the treatment, such as fertility preservation.

Sperm concentration is negatively affected by cancer treatment, the extent depending on both type of therapy and dosage (Eberhard et al., 2004). However, with standard TGCC treatment, the impairment is transient and pretreatment values are regained within 5 years.

Cancer treatment is potentially mutagenic, and animal studies have shown sperm DNA injury following exposure to chemotherapy or radiotherapy (Witt and Bishop, 1996; Brinkworth, 2000). Less is known regarding the effect of cancer treatment on human spermatozoa. An increased proportion of aneuploid spermatozoa following chemotherapy were reported, but the effect seems to be transient (Martin et al., 1997; Frias et al., 2003; Thomas et al., 2004). However, it cannot be excluded that more discrete changes in the sperm genome can be more persistent. Such phenomenon could have serious implications. Despite the recovery of spermatogenesis, the fertilizing capacity of the spermatozoa might be seriously impaired. Furthermore, new powerful assisted reproduction techniques (ART) such as intracytoplasmic sperm injection (ICSI) reduce the demands of sperm quality in terms of concentration, motility, morphology and DNA integrity (Morris, 2002). A potential worry with ICSI is that by surpassing normal biological control mechanisms in fertilization, there is a risk of transmitting defect paternal DNA to the offspring. Fertility in TGCC patients is reduced even before treatment (Petersen et al., 1998). Hence, these patients can be expected to benefit from ICSI, thereby being at risk of transmitting therapy-induced DNA damage to the offspring.

Studies have shown that the fertilizing capacity of the spermatozoa is also dependent on the integrity of their DNA (Larson et al., 2001; Carrell et al., 2003; Bungum et al., 2004). Several methods for assessment of sperm DNA breaks exist, and the sperm chromatin structure assay (SCSA) is the method mostly used for clinical purposes. Studies indicated a serious impairment of fertilization in vivo when the SCSA DNA fragmentation index (DFI) exceeds the level of 27–30% (Larson et al., 2001; Bungum et al., 2004). Nevertheless, SCSA has been regarded as an indirect method for the assessment of sperm DNA fragmentation as it relies on the assumption that DNA denaturability mirrors the presence of DNA strand breaks. There are other more direct tests available to measure the level
of sperm DNA breaks, such as terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL).

We have previously reported preliminary results on the effect of different TGCC treatment modalities on sperm DNA integrity, as assessed by SCSA (Stahl et al., 2004). Radiotherapy induced a transient increase of SCSA_{DFI}, the values normalizing after 3–5 years. Chemotherapy induced a decrease in SCSA_{DFI} which, with more than two cycles of chemotherapy treatment, was observed throughout the 5 years of follow-up.

In the present study, we performed both SCSA and TUNEL analysis on an extended number of ejaculates to detect the level of sperm DNA fragmentation with two independent sperm DNA integrity approaches.

Our aim was to investigate cancer therapy-induced changes in sperm DNA integrity to improve the prediction of fertility in TGCC patients. In addition, we aimed at assessing the potential risk of using sperm from men treated for cancer for ART.

**Patients and methods**

**Patients**

This study is a part of a longitudinal survey of reproductive function in males treated for TGCC, initiated in 2001. All men with TGCC, below the age of 50, diagnosed <5 years before inclusion, were eligible for the study. Fixed time intervals for delivery of semen samples were defined: T_{0}—after orchidectomy but before further therapy; T_{6}, T_{12}, T_{24}, T_{36} and T_{60}—6 to 60 months, respectively, after completed treatment. Patients entered the study at any time between T_{0} and T_{60} and delivered samples at the remaining intervals.

In November 2004, 178 eligible patients had passed through the Department of Oncology, Lund University Hospital, Lund, Sweden. Twenty-five patients, not differing from the included patients in the distribution of age, histological subtype or tumor stage, refused to participate, whereas 25 patients were excluded for various reasons (bilateral disease, psychological, psychiatric reasons, hepatitis C or linguistic problems). Of the 128 patients included in the survey, one patient declined to participate, whereas 25 patients were excluded for various reasons (bilateral disease, psychological, psychiatric reasons, hepatitis C or linguistic problems). Of the 128 patients included in the survey, one patient declined to participate, whereas 25 patients were excluded for various reasons (bilateral disease, psychological, psychiatric reasons, hepatitis C or linguistic problems). Of the 128 patients included in the survey, one patient declined to participate, whereas 25 patients were excluded for various reasons (bilateral disease, psychological, psychiatric reasons, hepatitis C or linguistic problems). Of the 128 patients included in the survey, one patient declined to participate, whereas 25 patients were excluded for various reasons (bilateral disease, psychological, psychiatric reasons, hepatitis C or linguistic problems).

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**Semen analysis**

Fresh semen samples were collected, and within an hour post-ejaculation, sperm concentration was assessed according to WHO guidelines 1999 (World Health Organization, 1999), and the results in both TGCC patients and controls were in accordance with previous reports.

An aliquot was stored at −80°C for the subsequent SCSA and TUNEL analysis.

**SCSA**

The SCSA analysis is based on the phenomenon that chromatin with abundant DNA strand breaks has a tendency to denature when exposed to acid detergent, whereas normal chromatin remains stable. Acidine Orange stains the native double-stranded DNA and the single-stranded nucleic acids, and in excitation of blue light, the intact DNA emits green fluorescence, whereas the denatured DNA emits red fluorescence. The extent of DNA denaturability is expressed as the DFI, being the ratio of red to total (red plus green) fluorescence intensity. DFI hereby expresses the proportion of cells containing denatured DNA (Evenson et al., 2002). Five thousand cells were analysed by FACSort (Becton Dickinson, San Jose, CA, USA). The analysis was performed as previously described (Stahl et al., 2004). An intra-laboratory coefficient of variation (CV) of 4.5% was found.

**TUNEL**

The TUNEL assay quantifies the incorporation of fluorescently labelled dUTP at breaks in double-stranded DNA, utilizing a reaction catalysed by terminal deoxynucleotidyl transferase. TUNEL positivity in somatic cells reflects apoptosis, but the origin of sperm DNA strand breaks detected by TUNEL remains unclear (Sakkas et al., 2002; Perreault et al., 2003). The TUNEL analysis was performed according to the manufacturer’s (Roche Diagnostics GmbH, Mannheim, Germany)
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protocol with slight modifications as previously described (Erenpreiss et al., 2004). A total of 10 000 events were accumulated for each measurement and analysed by FACSort (Becton Dickinson) flow cytometer; the same was used for SCSA. The intra-laboratory CV of 8.6% was found.

Statistical analysis

Statistical analysis was performed using the SPSS 11.0 software (SPSS, Chicago, IL, USA). To obtain sufficient numbers of individuals, the results of samples collected at T12 and T24 were combined into one time category T12–24, and samples collected at T36 and T60 were combined into T36–60. If two samples were delivered by a patient in one time category, the mean value was used in the analysis. The group descriptive values were expressed as medians and ranges. The data were treated in a cross-sectional manner (Mann–Whitney U-test). SO (post-surgery) values were compared with those of controls in regard to both DFI and TUNEL. For each therapy, group comparisons between SO and the three different time categories were made, a total of nine comparisons for DFI and TUNEL, respectively.

The proportion of men at T12 – T60 with SCSA DFI ≥27% was calculated, and for normozoospermic (sperm concentration ≥20 × 10⁶/ml) men, the odds ratio for such high SCSA DFI, as compared with controls, was calculated for each therapy group by means of binary logistic regression analysis. If one patient had delivered more than one ejaculate, the first sample was used for analysis.

Spearman’s rho was calculated to correlate the results of SCSA and TUNEL. P < 0.05 was considered statistically significant.

Results

Surgery only

Semen from TGCC patients before post-surgery treatment, SO patients, did not differ from that of controls in regard to sperm DNA integrity (Tables II and III and Figures 2 and 3).

Chemotherapy

ACT

Patients receiving ACT did not differ from SO at any time-point, neither in SCSA DFI nor in TUNEL DFI (Tables II and III and Figures 2 and 3).

HCT

Between 1 and 2 years after therapy, TUNEL DFI was significantly lower than in the SO group. SCSA DFI at T12–24 was also lower compared to SO, but without reaching statistical significance.

Between 3 and 5 years after therapy, both TUNEL DFI and SCSA DFI were significantly lower than in the SO group (Tables II and III and Figures 2 and 3).

RT

At T12–24, SCSA DFI was significantly higher in patients receiving RT than in SO. TUNEL DFI at T12–24 was also higher compared with SO, without reaching statistical significance. At T36–60, neither SCSA DFI nor TUNEL DFI differed between RT and SO (Tables II and III and Figures 2 and 3).

The data for patients receiving combined therapy were too few to analyse.

Proportion of patients with SCSA DFI >27%

Among the normozoospermic controls, 6.9% had SCSA DFI ≥27%, and in normozoospermic TGCC patients treated by

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### Table II. TUNELDFI and sperm concentration in testicular germ cell cancer patients according to treatment and follow-up time

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>SO</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
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<th>HCT</th>
<th>RT</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>24</td>
<td>19</td>
<td>12</td>
<td>22</td>
<td>19</td>
<td>3</td>
<td>10</td>
<td>17</td>
<td>4</td>
<td>4</td>
<td>13</td>
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<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
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<tr>
<td>Median sperm concentration (10^6/ml) (range)</td>
<td>57 (13–320)</td>
<td>21^a (2.6–86)</td>
<td>21 (5.3–38)</td>
<td>31 (1.0–66)</td>
<td>32 (2.5–104)</td>
<td>3 (0.5–1.4)</td>
<td>7.1 (3.5–58)</td>
<td>23 (5.4–92)</td>
<td>4.2 (3.1–13)</td>
<td>25 (5.5–112)</td>
<td>39 (7.5–174)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUNELDFI median (range)</td>
<td>11 (2.5–31)</td>
<td>11 (2.7–28)</td>
<td>9.1 (1.7–23)</td>
<td>9.6 (4.1–30)</td>
<td>11 (6.3–22)</td>
<td>3.1 (2.9–17)</td>
<td>7.5^b (1.2–19)</td>
<td>7.5^c (4.1–27)</td>
<td>18 (4.5–52)</td>
<td>18 (2.4–44)</td>
<td>11 (4.5–33)</td>
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</table>

ACT, 1–2 cycles of cisplatin-based chemotherapy; HCT, more than two cycles of chemotherapy; RT, adjuvant radiotherapy; SO, surgery only; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling.

^aP = 0.001 in comparison to controls.

^bP = 0.03 in comparison to SO.

^cP = 0.02 in comparison to SO.

### Table III. SCSA<sub>20F</sub> and sperm concentration in testicular germ cell cancer patients according to treatment and follow-up time

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>SO</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
<th>ACT</th>
<th>HCT</th>
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<th>RT</th>
<th>ACT</th>
<th>HCT</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>278</td>
<td>25</td>
<td>15</td>
<td>24</td>
<td>21</td>
<td>3</td>
<td>12</td>
<td>17</td>
<td>8</td>
<td>16</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Median sperm concentration (10^6/ml) (range)</td>
<td>52 (0.5–390)</td>
<td>23^a (0.6–86)</td>
<td>15 (3.0–38)</td>
<td>29 (1.0–66)</td>
<td>30 (2.5–104)</td>
<td>3 (0.5–1.4)</td>
<td>7.8 (3.5–58)</td>
<td>23 (5.4–92)</td>
<td>2.7 (0.1–13)</td>
<td>14 (0.7–112)</td>
<td>34 (7.5–174)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SCSA&lt;sub&gt;20F&lt;/sub&gt; median (range)</td>
<td>11 (1.7–62)</td>
<td>12 (7.1–72)</td>
<td>10 (5.4–28)</td>
<td>13 (8.0–45)</td>
<td>14 (4.1–29)</td>
<td>18 (16–31)</td>
<td>9.7^b (4.7–33)</td>
<td>9.1^c (4.0–34)</td>
<td>16 (8.0–47)</td>
<td>19^d (7.8–45)</td>
<td>14 (9.7–64)</td>
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</tr>
</tbody>
</table>

ACT, 1–2 cycles of cisplatin-based chemotherapy; HCT, more than two cycles of chemotherapy; RT, adjuvant radiotherapy; SCSA, sperm chromatin structure assay; SO, surgery only; SO, surgery only.

^aP < 0.001 in comparison to controls.

^bP = 0.09 in comparison to SO.

^cP = 0.02 in comparison to SO.

^dP = 0.03 in comparison to SO.
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RT, this proportion was 38%. The odds ratio for SCADFI ≥27 was significantly increased only in the RT group (Table IV).

<table>
<thead>
<tr>
<th>Table IV.</th>
<th>Number of men with SCADFI &gt;27% in relation to total number of subjects investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion of men with normozoospermia (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>16/233 (6.9)</td>
</tr>
<tr>
<td>ACT</td>
<td>3/24 (12)</td>
</tr>
<tr>
<td>HCT</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>RT</td>
<td>5/13* (38)</td>
</tr>
</tbody>
</table>

ACT, 1–2 cycles of cisplatin-based chemotherapy; HCT, more than two cycles of chemotherapy; RT, adjuvant radiotherapy; SCASA, sperm chromatin structure assay; SO, surgery only.

For the controls and the patients, the calculations were only made for those having normozoospermia (sperm concentration ≥20 × 10^6/ml). Odds ratios (95% CI) between the different treatment groups and controls are given.

*P = 0.002 in comparison to controls.

SCASA–TUNEL correlation

SCASA DFI and TUNEL DFI correlated significantly (Spearman’s rho = 0.41; P = 0.01).

Discussion

In the present prospective study, sperm DNA integrity was investigated in 96 TGCC patients and correlated with treatment and time of follow-up. TGCC patients, before post-surgical treatment, had no increase of defective sperm DNA compared with controls. Adjuvant abdominal radiotherapy induced a transient increase in the proportion of sperm with DNA strand breaks. The normozoospermic RT patients had 8.5 times increased odds ratio for SCADFI ≥27% compared with controls, indicating a therapy-induced decrease of fertility in vivo despite normal sperm concentration.

Three or more courses of chemotherapy induced a permanent decrease of DFI.

This is the largest study on sperm DNA integrity in cancer patients, and two methods—SCASA and TUNEL—were applied. In samples analysed with both methods, a moderate correlation between the results of the two analyses was found. However, when comparing different treatment groups, identical trends were found regardless of method.

Sperm DNA integrity has been receiving increasing attention. Discrete DNA injuries have been demonstrated in human spermatozoa and shown to affect the fertilization ability in vivo, and possibly even in vitro, regardless of standard semen parameters (Larson et al., 2000; Bungum et al., 2004). Furthermore, it is known that defective paternal genome can be transmitted to the offspring (Cram et al., 2000), but the significance of iatrogenic DNA damage, induced by cancer treatment, is unknown. Large follow-up studies on the offspring of cancer survivors have not shown any adverse effects of cancer treatment. However, these studies were based on children born after natural conception (Blatt, 1999; Meistrich and Byrne, 2002), and there is a fear that the ICSI procedure, surpassing the biological control system of natural conception, imposes a risk of transmitting defect DNA.

In contrast to our study results, previous studies indicated that TGCC per se was associated with impairment of sperm...
DNA integrity. Three studies used proven fertile men as controls (Fosså et al., 1997; Kobayashi et al., 2001; O’Donovan, 2005), and because fertility is associated with a low level of sperm DNA damage, our approach of using a group of unselected males as controls might explain the divergent results. Finally, Gandini et al. (2000), comparing sperm DNA integrity, assessed with TUNEL, in an unselected healthy population with Mb Hodgkin and TGCC patients found a statistically significant higher TUNEL\textsubscript{DFI} in both cancer groups. However, when comparing the Gandini study with ours, the differing conclusions are explained with the difference in controls (TUNEL\textsubscript{DFI} 2.5 versus 11%) and not in cancer patients. In the present study, the median TUNEL\textsubscript{DFI} in controls was at the same level as their median SCSA\textsubscript{DFI}. The finding of the same DFI in SO patients and in controls cannot be explained by age difference between the two groups. TGCC men were older than controls, which should rather lead to a higher DFI among these men because this sperm characteristic is known to increase by age (Spano et al., 1998).

In clinical terms, a normal level of sperm DNA damage before therapy in TGCC patients indicates that the use of cryopreserved semen constitutes no increased risk of transmitting damaged DNA compared with non-TGCC ICSI-candidates.

The genetic risks of paternal exposure to irradiation are unclear. Whereas animal models have demonstrated both the transmission of radiotherapy-induced genetic damage and a subsequent increase in both early embryonic loss and malformations (Brinkworth, 2000), the potential hazards for humans are less evident. Following oncological treatment, no such risk has been detected (Meistrich and Byrne, 2002; Tawn et al., 2005). Observations from the Chernobyl accident are inconsistent regarding a possible increase in the rate of malformations or genetic diseases, but data suggested an increase in germline mutations (Dubrova, 2003).

Our study demonstrated the significant sensitivity of spermatozoa to radiotherapy. An estimated total dose of <0.5 Gy in 14 fractions was enough to induce long-standing, although not permanent, sperm DNA damage. The proportion of spermatozoa with DNA strand breaks was significantly higher in RT patients the first 2 years after therapy. One year or more after RT, patients with a normal sperm count had 8.5 times higher odds ratio of SCSA\textsubscript{DFI} ≥27% compared with controls, which may indicate in vivo infertility, which cannot be foreseen by judging the sperm concentration only. Although there are still some controversies regarding the impact of high DFI on fertility, the available data indicate that SCSA\textsubscript{DFI} ≥27% reduces the probability of in vivo fertilization, either by natural conception or by intrauterine insemination, almost to zero with ICSI being the most efficient way of achieving pregnancy (Bungum et al., 2004).

The median age of the RT patients was 36 years, when even a transient reduction of fertility can have consequences in terms of fatherhood. If these men present with infertility despite normal semen parameters, sperm DNA integrity should be assessed, and use of cryopreserved sperm might be indicated in cases of high level of sperm DNA damage.

The decrease in SCSA\textsubscript{DFI} following chemotherapy observed in our previous report (Stahl et al., 2004) was confirmed with the TUNEL analysis. This is inconsistent with several animal models in which chemotherapy induced sperm gene mutations and transmittable chromosome aberrations (Marchetti et al., 2001; Hales et al., 2005). Both cisplatin and etoposide were shown to induce sperm DNA injury and germ cell apoptosis (Sjoblom et al., 1998; Zhang et al., 2001; Cherry et al., 2004; Stump et al., 2004). However, mouse models and the human clinical setting differ in many aspects. Experimental studies are mostly monotherapeutical, using either a high single dosage or chronic low-dosage exposition (Sjoblom et al., 1998; Brinkworth, 2000; Hales et al., 2005). Most importantly, animal studies focus on the acute gonadotoxicity, which at least in humans differs completely from the long-term effects.

Few studies on humans address the issue of sperm chromatin in relation to anticancer therapy. The induction of sperm aneuploidy has been described after both radiotherapy and chemotherapy, but no permanent changes have been described (Martin et al., 1997; De Mas et al., 2001; Frias et al., 2003). Thomas et al. (2004) reported the absence of increase in sperm aneuploidy rates after anticancer therapy in 14 TGCC and 14 lymphoma patients, investigated between 7 months and 7 years after treatment with RT, CT or both.

The effect of cancer therapy on sperm DNA integrity is even less studied. A study on non-azoospermic adult childhood cancer survivors, compared with 66 proven fertile men, found no significant difference regarding sperm DNA integrity using the TUNEL assay (Thomson et al., 2002).

The decrease in the proportion of spermatozoa with DNA breaks seemed to occur after cessation of the spermatogenic arrest caused by CT. We, therefore, suggest that this effect may be exerted via the spermatogonial stem cells. One can hypothesize that spermatozoa with DNA strand breaks arise from stem cells with defective DNA repair mechanisms, which thereby make them more vulnerable to chemotherapy. Cisplatin was previously shown to increase apoptosis of germ cells (Cherry et al., 2004) and may add to the elimination of spermatogonia with DNA breaks. Whether sperm DNA is affected in the same way by other chemotherapy combinations remains to be investigated. The clinical application of our findings is restricted to patients receiving bleomycin, etoposide and cisplatin, for whom our study results indicated no increased risk in using post-therapy sperm for in vitro fertilization, including ICSI, and that normal fertility can be expected in those achieving full recovery of sperm count. However, semen cryopreservation should be performed before treatment, because neither the full extent of the therapy nor the degree of sperm recovery for the individual patient can be foreseen at the initiation of treatment.

In conclusion, irradiation induced an increase in the number of sperms with DNA damage, lasting for at least 1–2 years post-therapy, whereas more than two cycles of chemotherapy reduced the proportion of sperms with impaired DNA integrity. Further studies need to be performed for other patient groups, for example, those being treated for cancer in childhood and adolescence, because other treatment regimens as well as age at treatment might influence the effect on sperm DNA integrity.
Acknowledgements

We thank Anette Möller for her assistance in organizing the patient flow and the sampling procedures. We thank Dr Per Flodgren for his engagement in the study, helping with patient controls and study sampling. We thank Dr Jonas Richthoff for allowing us to use his material on military conscripts as control material.

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References


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