Impact of chemotherapy and radiotherapy for testicular germ cell tumors on spermatogenesis and sperm DNA: a multicenter prospective study from the CECOS network

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Objective: To determine the consequences of adjuvant testicular germ cell tumor treatment (TGCT) on sperm characteristics and sperm DNA, and to evaluate the predictors of sperm recovery.

Design: Multicenter prospective longitudinal study of patients analyzed before treatment and after 3, 6, 12, and 24 months.

Setting: University hospitals.

Patient(s): One hundred twenty-nine volunteer TGCT patients and a control group of 257 fertile men.

Intervention(s): Routine semen analyses, sperm DNA, and chromatin assessments.

Main Outcome Measure(s): Comparisons of mean sperm characteristics before and after treatment, with sperm recovery analyzed by the Kaplan-Meier method.

Result(s): The quantitative and qualitative sperm characteristics decreased after treatment, with lowest values at 3 and 6 months and with variations according to treatment type. The mean total sperm count recovered to pretreatment values at 12 months after treatment after two or fewer bleomycin, etoposide, and cisplatin (BEP) cycles, but not after radiotherapy or more than two BEP cycles. Only the treatment modalities and pretreatment sperm production were related to recovery of the World Health Organization reference sperm values. An increased proportion of patients had elevated high sperm DNA stainability at 6 months after radiotherapy.

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Conclusion(s): Adjuvant treatments for testicular germ cell tumor have drastic effects on spermatogenesis and sperm chromatin quality. These new data on both the recovery period according to treatment modalities and the post-treatment chromatin status of sperm are useful tools for counseling patients wishing to conceive. (Fertil Steril® 2013; ). ©2013 by American Society for Reproductive Medicine.)

Key Words: Adverse effects, BEP chemotherapy, radiotherapy, spermatogenesis, sperm chromatin

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**MATERIALS AND METHODS**

**Patients**

This prospective study enrolled 129 patients who had been directed to the Centres d’Études et de Conservation des Oeufs et du Sperme humain (CECOS) for sperm banking before TGCT treatment. Eight CECOS sites participated: Caen, Clermont-Ferrand, Grenoble, Marseille, Paris Cochin, Paris Tenon, Rouen, and Toulouse. The sperm analyses were subject to external quality control in all centers. This study was supported by a national research grant (PHRC no. 20030222, GAMATOX project) and was approved by the institutional ethics review board. All patients gave written informed consent.

Usually, two to three semen samples were collected for sperm banking. Patients taking part in the study provided an additional semen sample before treatment (T0). In this prospective study, patients were asked to provide additional semen samples at 3, 6, 12, and 24 months after the end of treatment (Supplemental Fig. 1, available online). Age, andrologic and reproductive histories, tobacco exposure, and febrile episodes were recorded, and at each visit the participants completed a standard questionnaire about any unusual events since the last visit to the laboratory (disease, febrile episodes, and any change in lifestyle habits). To evaluate sperm alterations before treatment, the pretreatment sperm characteristics were compared with those of a control group of 257 fertile men. Fifty-one of these fertile men also underwent sperm DNA evaluation (DNA control group).

**Semen Analyses**

Semen samples were collected by masturbation after a recommended 3 to 5 days of sexual abstinence. The semen analysis was performed according to the World Health Organization (WHO) guidelines [22] with similar methodology in the eight laboratories. The characteristics considered were sexual abstinence (days), ejaculate volume (mL) and pH, sperm concentration (SC, 10⁶ spermatozoa/mL), round cell concentration (RC, 10⁶/mL), spermatozoa vitality (V, %) and forward motility (M, a + b: %), total sperm count (product of volume by SC = TSC, 10⁶ spermatozoa/ ejaculate), and total motile sperm count (TMSC = TSC × M, 10⁶/ ejaculate). The remaining semen sample was mixed with a cryoprotectant, frozen in straws, and stored in liquid nitrogen until used for further analyses, which were performed in the Toulouse CECOS by a single technician.

**Sperm Chromatin Structure Assay**

The sperm chromatin structure assay (SCSA) to evaluate sperm chromatin integrity [23] was performed as previously described elsewhere [24] in the routine manner of our laboratory [25, 26]. The extent of DNA denaturation was expressed as detected fluorescence intensity (DFI), which is the ratio of red to total (red plus green) fluorescence intensity. We also calculated the fraction of sperm with high DNA stainability (HDS), which represents sperm with immature chromatin. One aliquot of the quality control sperm was analyzed in pooled samples (results not shown). The analytic coefficient of variation was <5%, as calculated from the values obtained from aliquots of a semen sample.
Sperm DNA strand breaks were detected by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL assay) as previously described elsewhere (25). Sperm DNA fragmentation and propidium iodide labeling were measured on a FACScan flow cytometer. Each analysis included a minimum of 10,000 stained spermatozoa. The FL1 signals (green fluorescence from fluorescein isothiocyanate conjugate) were detected through a 525 ± 25 nm band pass filter and FL2 signals (red fluorescence from propidium iodide) through a 575 ± 25 nm filter. The TUNEL analysis consisted of subtracting control (no TdT enzyme) green fluorescence histograms from TdT-positive green fluorescence histograms, yielding the percentage of cells showing DNA strand breaks.

**DNA Fragmentation**

**Data Collection and Statistical Analyses**

All data were reported on centralized case report forms by Web access and the data files were verified by the coordinating center in Toulouse. Data were compared between the control group (fertile men) and the TGCT group using the nonparametric Mann-Whitney test. Linear regression was used to adjust the data on sexual abstinence and patient age because both parameters may influence sperm characteristics.

The sperm characteristics of TGCT patients were compared before and after treatment at T3, T6, T12, and T24 by the Wilcoxon signed rank-sum test. The treatment regimens were compared using the Mann-Whitney test. The Kaplan-Meier method was used to estimate the cumulative rates of successful semen recovery and the 95% confidence interval (CI). Censors were defined as patients who did not succeed in semen recovery—that is, the patients with TSC < 39 ± 10^6 spermatozoa/ejaculate (27) at their last visit. Statistically significant differences between the groups were calculated by the log-rank test. Univariate and multivariate Cox models were performed to describe factors associated with successful or unsuccessful semen recovery using crude and adjusted hazard ratios (HR) and 95% CIs. The statistical analysis was performed using SAS software (9.0, SAS Institute), and P < .05 was considered statistically significant.

**RESULTS**

**Patients**

The study enrolled 129 patients aged 20 to 44 years (mean age ± standard deviation [SD]: 30.9 ± 4.9). Seventy patients (54%) had pure seminoma, and 59 (46%) had mixed or nonseminoma tumors. Thirteen patients (10%) had a history of cryptorchidism. Sixty-seven patients with pure seminoma were treated by radiotherapy, and the other 62 patients underwent chemotherapy with the BEP regimen (bleomycin, etoposide, and cisplatin). 17 patients received one or two chemotherapy cycles, and 45 patients had more than two cycles (three or four).

**Semen Characteristics before Cancer Treatment**

The frequency of the risk factors that could influence sperm characteristics was not statistically significantly different between the controls and the TGCT group (P > .05, results not shown). Orchiectomy was performed before semen collection in 83 patients (85%), but no statistically significant difference was observed between the sperm characteristics collected before or after surgery (P > .05, data not shown).

Compared with the fertile group, the TGCT patients had a higher pH and lower ejaculate volume, SC, TSC, and TMSC (P < .05) (Table 1). Moreover, statistically significant decreases of round cell concentration and percentage of motility and

**TABLE 1**

Sperm characteristics in the control group (fertile men) and the testicular germ cell tumor (TGCT) group.

<table>
<thead>
<tr>
<th>Sperm characteristic</th>
<th>Control group (n = 257)</th>
<th>TGCT group (n = 129)</th>
<th>Without cryptorchidism (n = 67)</th>
<th>With cryptorchidism (n = 3)</th>
<th>Without cryptorchidism (n = 49)</th>
<th>With cryptorchidism (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>3.95 ± 1.90</td>
<td>3.60 ± 1.65*</td>
<td>3.41 ± 1.57**</td>
<td>5.73 ± 3.22</td>
<td>3.89 ± 1.69</td>
<td>3.26 ± 1.07</td>
</tr>
<tr>
<td>pH</td>
<td>7.94 ± 0.28</td>
<td>8.08 ± 0.33***</td>
<td>8.01 ± 0.28*</td>
<td>8.17 ± 0.31</td>
<td>8.17 ± 0.39**</td>
<td>8.03 ± 0.23*</td>
</tr>
<tr>
<td>Sperm count (10^6/mL)</td>
<td>98.67 ± 90.91</td>
<td>31.26 ± 31.80**</td>
<td>36.05 ± 36.94***</td>
<td>7.83 ± 4.25*</td>
<td>29.08 ± 25.15***</td>
<td>16.84 ± 19.67***</td>
</tr>
<tr>
<td>Round cells (10^6/mL)</td>
<td>2.10 ± 2.62</td>
<td>1.16 ± 1.61***</td>
<td>1.11 ± 1.42***</td>
<td>0.57 ± 0.51</td>
<td>1.29 ± 1.89***</td>
<td>1.04 ± 1.59*</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>69.87 ± 13.44</td>
<td>67.38 ± 13.83**</td>
<td>67.14 ± 15.38**</td>
<td>63.33 ± 15.28</td>
<td>67.96 ± 11.40</td>
<td>67.30 ± 15.43</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>43.05 ± 13.53</td>
<td>42.08 ± 13.84**</td>
<td>42.94 ± 14.72</td>
<td>30.00 ± 17.32</td>
<td>43.67 ± 12.19</td>
<td>32.10 ± 9.89**</td>
</tr>
<tr>
<td>Total sperm count (10^9/jugulate)</td>
<td>361.95 ± 343.25</td>
<td>115.16 ± 142.48**</td>
<td>124.86 ± 153.93***</td>
<td>47.45 ± 47.31*</td>
<td>118.87 ± 139.99**</td>
<td>52.29 ± 56.78**</td>
</tr>
<tr>
<td>Total motile sperm count (10^9/jugulate)</td>
<td>150.49 ± 126.37</td>
<td>53.93 ± 69.08***</td>
<td>59.45 ± 72.85***</td>
<td>16.71 ± 21.00*</td>
<td>55.60 ± 70.10***</td>
<td>19.95 ± 25.57***</td>
</tr>
</tbody>
</table>

Note: Values are mean ± standard deviation.  
* P < .05, difference between control group and TGCT group, seminoma group without cryptorchidism, seminoma group with cryptorchidism, nonseminoma group without cryptorchidism, and nonseminoma group with cryptorchidism.  
** P < .05, adjusted for abstinence duration and age, difference between control group and TGCT group, seminoma group without cryptorchidism, seminoma group with cryptorchidism, nonseminoma group without cryptorchidism, and nonseminoma group with cryptorchidism.

vitality were also observed (P<.05). The TSC was lower in non-seminoma patients with a history of cryptorchidism (P=.001).

### Change in Semen Parameters after Cancer Treatment

Sperm characteristics (SC, TSC, TMSC) decreased after treatment. The lowest values occurred at T3 and were particularly marked after chemotherapy with more than two cycles and after radiotherapy (Table 2, Fig. 1). For qualitative characteristics such as motility, the mean values decreased at T3 and T6 in patients treated with more than two cycles of chemotherapy or with radiotherapy. The mean ejaculate volume differed only at T3 in both treatment groups. The semen characteristics recovered to pretreatment values 12 months after chemotherapy after fewer than two cycles, but only at 24 months after other treatments. The percentage of men with azoospermia increased from 0 before treatment to 16% and 17% at T3 and T6, respectively, and remained high at T12 (6%) (P<.05) but decreased to 2% at T24 (P<.05).

### Predictors of Sperm Recovery after Treatment

Stratified according to treatments, the Kaplan-Meier estimates showed that the cumulative rates of recovery of sperm production $\geq 39 \times 10^6$/ejaculate reached 92% (95% CI, 70–99) in patients with two or fewer chemotherapy cycles, 63% (95% CI, 48–79) in patients with more than two cycles, and 86% (95% CI, 76–94) for the radiotherapy group (P<.05). A statistically significant difference in recovery was also observed according to history of cryptorchidism—that is, 81% (95% CI, 73–89) for patients without a history of cryptorchidism versus 55% (95% CI, 30–84) for those with cryptorchidism—and according to pretreatment TSC value—that is, 89% (95% CI, 80–96) for patients with TSC $\geq 39 \times 10^6$ versus 61% (95% CI, 46–76) for TSC < $39 \times 10^6$. However, no difference was observed according to TGCT type (Fig. 2).

Adjusted for patient age, history of cryptorchidism, smoking status, and type of TGCT in a multivariate Cox model, only the type of treatment (chemotherapy >2 cycles vs. chemotherapy $\leq$2 cycles, HR = 0.44 [95% CI, 0.22–0.90], and radiotherapy vs. chemotherapy $\leq$2 cycles, HR = 0.77 [95% CI, 0.29–2.00]) and TSC before treatment (TSC $< 39 \times 10^6$ versus TSC $\geq 39 \times 10^6$, HR = 1.88 [95% CI, 1.16–3.05]) remained statistically significant.

### DNA Damage

To compare the results of the cancer patients versus the controls, we defined the reference thresholds as the 90th percentiles

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**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment type and sperm characteristic</th>
<th>Before treatment</th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>24 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemotherapy, ≤2 cycles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>3.67 ± 1.33</td>
<td>3.79 ± 1.09</td>
<td>4.26 ± 1.50</td>
<td>3.74 ± 0.79</td>
<td>3.76 ± 1.25</td>
</tr>
<tr>
<td>pH</td>
<td>8.19 ± 0.30</td>
<td>7.99 ± 0.40*</td>
<td>7.98 ± 0.41*</td>
<td>7.90 ± 0.37*</td>
<td>8.02 ± 0.29</td>
</tr>
<tr>
<td>Sperm count (10^6/mL)</td>
<td>32.01 ± 29.51</td>
<td>4.10 ± 5.95*</td>
<td>13.98 ± 23.40*</td>
<td>30.88 ± 41.77</td>
<td>32.48 ± 25.03</td>
</tr>
<tr>
<td>Round cells (10^6/mL)</td>
<td>1.31 ± 1.70</td>
<td>2.09 ± 6.72*</td>
<td>0.45 ± 0.48*</td>
<td>0.55 ± 0.64</td>
<td>1.58 ± 3.49</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>68.07 ± 16.73</td>
<td>61.73 ± 25.18</td>
<td>66.08 ± 12.66</td>
<td>65.91 ± 20.20</td>
<td>66.50 ± 15.94</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>43.73 ± 14.75</td>
<td>31.29 ± 25.51</td>
<td>39.23 ± 16.01</td>
<td>43.27 ± 20.32</td>
<td>48.50 ± 24.27</td>
</tr>
<tr>
<td>Total sperm count (10^6/ejaculate)</td>
<td>105.94 ± 90.96</td>
<td>12.73 ± 15.05*</td>
<td>63.83 ± 135.95*</td>
<td>100.34 ± 106.28</td>
<td>122.29 ± 102.58</td>
</tr>
<tr>
<td>Total motile sperm count (10^6/ejaculate)</td>
<td>48.78 ± 38.37</td>
<td>5.01 ± 7.39*</td>
<td>37.93 ± 96.62*</td>
<td>55.39 ± 75.21</td>
<td>73.73 ± 83.86</td>
</tr>
<tr>
<td><strong>Chemotherapy, &gt;2 cycles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>3.46 ± 1.61</td>
<td>3.17 ± 1.48*</td>
<td>3.53 ± 1.85</td>
<td>3.32 ± 1.97</td>
<td>3.66 ± 1.71</td>
</tr>
<tr>
<td>pH</td>
<td>8.09 ± 0.40</td>
<td>8.04 ± 0.37</td>
<td>7.97 ± 0.43</td>
<td>8.05 ± 0.48</td>
<td>8.03 ± 0.39</td>
</tr>
<tr>
<td>Sperm count (10^6/mL)</td>
<td>23.46 ± 21.37</td>
<td>2.04 ± 0.59*</td>
<td>4.40 ± 14.97*</td>
<td>10.01 ± 15.24*</td>
<td>30.99 ± 29.08</td>
</tr>
<tr>
<td>Round cells (10^6/mL)</td>
<td>1.16 ± 1.87</td>
<td>0.48 ± 0.93*</td>
<td>0.28 ± 0.43*</td>
<td>0.56 ± 0.98*</td>
<td>0.65 ± 0.86</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>67.62 ± 11.77</td>
<td>47.78 ± 30.78</td>
<td>45.89 ± 30.80*</td>
<td>62.32 ± 21.63</td>
<td>65.53 ± 15.07</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>39.33 ± 13.65</td>
<td>12.48 ± 16.51*</td>
<td>25.45 ± 20.15*</td>
<td>30.76 ± 18.70*</td>
<td>38.93 ± 13.76</td>
</tr>
<tr>
<td>Total sperm count (10^6/ejaculate)</td>
<td>88.08 ± 106.20</td>
<td>0.76 ± 1.52*</td>
<td>16.02 ± 47.98*</td>
<td>31.46 ± 49.53*</td>
<td>114.77 ± 127.45</td>
</tr>
<tr>
<td>Total motile sperm count (10^6/ejaculate)</td>
<td>41.20 ± 60.62</td>
<td>0.24 ± 0.46*</td>
<td>5.29 ± 9.67*</td>
<td>13.62 ± 20.84*</td>
<td>46.99 ± 46.67</td>
</tr>
<tr>
<td><strong>Radiotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>3.68 ± 1.77</td>
<td>3.42 ± 1.59*</td>
<td>3.53 ± 1.64</td>
<td>4.08 ± 1.90</td>
<td>3.69 ± 1.69</td>
</tr>
<tr>
<td>pH</td>
<td>8.05 ± 0.29</td>
<td>8.11 ± 0.41</td>
<td>8.05 ± 0.34</td>
<td>8.03 ± 0.35</td>
<td>8.00 ± 0.36</td>
</tr>
<tr>
<td>Sperm count (10^6/mL)</td>
<td>36.48 ± 37.37</td>
<td>11.14 ± 15.59*</td>
<td>12.13 ± 27.65*</td>
<td>22.01 ± 22.45*</td>
<td>37.36 ± 36.90</td>
</tr>
<tr>
<td>Round cells (10^6/mL)</td>
<td>1.12 ± 1.43</td>
<td>0.92 ± 1.04</td>
<td>0.66 ± 1.36*</td>
<td>0.88 ± 1.44*</td>
<td>0.98 ± 0.96</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>67.17 ± 14.79</td>
<td>63.10 ± 15.45</td>
<td>62.00 ± 21.83</td>
<td>67.04 ± 17.43</td>
<td>67.73 ± 12.84</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>43.69 ± 13.80</td>
<td>35.58 ± 17.25*</td>
<td>33.41 ± 19.51*</td>
<td>42.61 ± 16.97</td>
<td>49.02 ± 15.40</td>
</tr>
<tr>
<td>Total sperm count (10^6/ejaculate)</td>
<td>135.69 ± 170.33</td>
<td>41.68 ± 70.37*</td>
<td>48.24 ± 143.91*</td>
<td>84.82 ± 107.36*</td>
<td>135.82 ± 156.73</td>
</tr>
<tr>
<td>Total motile sperm count (10^6/ejaculate)</td>
<td>63.91 ± 78.99</td>
<td>18.52 ± 32.53*</td>
<td>24.50 ± 74.44*</td>
<td>41.60 ± 61.34*</td>
<td>61.42 ± 87.75</td>
</tr>
</tbody>
</table>

*Note: Values are mean ± standard deviation.  
* P <.05, difference between values before and after treatment (3, 6, 12, and 24 months).  
in controls: DFI = 20%, HDS = 7.5%, DFI + HDS = 26.1%, TUNEL = 16.7%. After adjusting for age and sexual abstinence, before chemotherapy or radiotherapy the TGCT patients had statistically significantly higher mean values than the controls for DFI (17.1 ± 9.8 vs. 11.4 ± 7.6%, P < .05) and DFI + HDS (22.7 ± 10.3 vs. 16.9 ± 8.1%, P < .05) but not for DNA fragmentation (TUNEL assay). Before chemotherapy or radiotherapy, the DFI and TUNEL values were above normal in 30% and 11% of the TGCT patients, respectively. The proportion of patients with abnormal HDS increased at T6 (Supplemental Table 1).

**DISCUSSION**

To our knowledge, ours is the first prospective study based on a standardized protocol, involving the largest TGCT population to date who were serially assessed after TGCT treatment. Before treatment, TGCT patients have altered sperm characteristics compared with fertile men (28). Several explanations have been proposed for sperm alteration before cancer treatment. Cryptorchidism is a well-documented risk factor for TGCT (29) and decreased semen quality (30). However, we found sperm alterations in cancer patients who had no cryptorchidism. Orchidectomy could explain sperm alterations (28), but like Fraietta et al. (31) we found no difference in sperm production before or after orchidectomy. Other causes could be suggested, such as tumor-associated secreted factors or stress. Moreover, it is postulated that TGCT is part of the testicular dysgenesis syndrome defined by Skakkebaek et al. (32), which also leads to defective spermatogenesis.

The TSC values were lowest 3 months after the end of treatment, particularly when more than two BEP cycles were performed. Decreased TSC was reported at 3 months in one study (19), but an analysis according to the number of BEP cycles was not performed. Radiotherapy induced a TSC decrease at 3 months which persisted at 6 months, whereas two BEP cycles had little effect at that time; the TSC values began to increase after more than two cycles. Gandini et al. (19) reported that decreased sperm production was of longer duration after radiotherapy (6 months) than after chemotherapy (3 months). This could reflect the different impacts of these treatments on spermatogenesis and sperm matura-

It is generally accepted that 74 days are required for one complete spermatogenic cycle and that around 12 days are necessary for spermatozoa maturation in the epididymis. Therefore, 3 months after treatment, one cycle of spermatogenesis and sperm epididymal transit have been completed. The sperm production changes observed at 3, 6, and 12 months reflect treatment-induced germ-cell injury and probably also the impact of treatment on Sertoli-germ cell interactions. These hypothetical effects on the microenvironment of spermatogenesis could explain the long-lasting damage.

To identify predictors of recovery of sperm production ≥39 × 10⁶/ejaculate, we used a Cox model integrating the
different parameters that could modify sperm characteristics. To the best of our knowledge, this is the first time such analysis has been done. Treatment modalities and sperm production before cancer treatment were the only factors linked with sperm recovery in the multiple variable analyses.

It is noteworthy that during the first year, while mean total sperm count was decreased, some men had sperm characteristics compatible with natural fertility. Therefore, gamete quality and potential risk for progeny were particularly relevant questions. We used SCSA to explore chromatin compaction, and the TUNEL assay to evaluate DNA sperm fragmentation.

After treatment, we did not find a higher proportion of men with increased sperm DNA fragmentation. However, more men had chromatin defects 6 months after treatment.

Other studies using SCSA (21, 33) or a CMA3 assay and TUNEL assay (34) did not find increased DNA/chromatin alteration after TGCT treatment. However, using the COMET assay, O’Flaherty et al. (20) demonstrated increased sperm DNA damage after chemotherapy, which remained elevated until 24 months despite sperm production recovery. Our radiotherapy patients had significantly a increased mean HDS 6 months after treatment, reflecting defects in chromatin condensation. Smit et al. (21) also found higher DFI levels in radiotherapy than in chemotherapy patients.

Discrepancies in the published studies may arise from differences in treatments, populations studied, end points assessed, or the assessment methods, and particularly from different follow-up protocols. Nevertheless, radiotherapy or...
chemotherapy could induce genome alterations not assessed by the methods used. We previously reported increased aneuploidy during the 17 months after BEP chemotherapy in cancer patients (35), which was confirmed by some studies in the first 2 years after treatment (36, 37) but not by others (38). It is interesting that in rats exposed to a BEP regimen similar to that used in humans, increased preimplantation loss was noted when these males were mated with healthy females although normal sperm characteristics were recovered (39). Other animal studies have demonstrated that BEP affects spermatozoa quality (40, 41), germ-cell gene expression (42), and spermatozoa methylation patterns (43). Furthermore, radiation induced transgenerational genome instability and DNA damage in recent animal studies (44).

It is still debated whether cancer treatments have such effects on humans. Studies of children of fathers treated for cancer have not shown evidence of more frequent abnormalities in offspring. However, it seems that these studies did not have sufficient power to detect relative risks of <3–5 (45), and the interval between the end of cancer treatment and conception was not precisely analyzed. Moreover, it seems particularly important to note the paradigm difference (the “fundamental mystery”) (46) between the results from animal and from human studies, underlining the need to conduct further human studies using new technologies.

CONCLUSION

We have demonstrated drastic deleterious effects of chemotherapy or radiotherapy on spermatogenesis of TGCT patients, with possible recovery 2 years after treatment. Treatment modalities and pretreatment sperm production are predictive of recovery of sperm production ≥ 39 x 10⁶/ejaculate. Radiotherapy induced slight chromatinn changes 6 months after treatment. In view of differences in published data, the drastic effects on spermatogenesis during follow-up evaluations and the results of animal studies, we believe that other prospective studies are necessary, using new methods of genome and epigenome exploration in humans to evaluate the effects of the treatment on spermatozoa quality and the possible risk for progeny (male-mediated developmental toxicity) (47).

Currently, couples are advised to use contraception for 1 to 2 years after TGCT treatment (20, 36).

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REFERENCES


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SUPPLEMENTAL FIGURE 1

Design of the prospective study.

## SUPPLEMENTAL TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFI</td>
<td>15.3 (30)</td>
<td>15.0 (38)</td>
<td>17.9 (33)</td>
<td>15.4 (33)</td>
<td>14.7 (29)</td>
</tr>
<tr>
<td>HDS</td>
<td>5.3 (9)</td>
<td>5.3 (15)</td>
<td>5.8 (28)</td>
<td>5.3 (8)</td>
<td>5.3 (15)</td>
</tr>
<tr>
<td>DFI + HDS</td>
<td>20.9 (32)</td>
<td>20.5 (35)</td>
<td>23.1 (33)*</td>
<td>21.6 (33)</td>
<td>19.2 (32)</td>
</tr>
<tr>
<td>TUNEL</td>
<td>9.4 (11)</td>
<td>8.2 (5)</td>
<td>7.9 (14)</td>
<td>10.1 (14)</td>
<td>9.7 (15)</td>
</tr>
</tbody>
</table>

Note: Values are median (% of number of patients >P90 of control group). For detected fluorescence intensity (DFI), P90 = 20%; for high DNA stainability (HDS), P90 = 7.5%; for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), P90 = 16.7%.

* P=.05 compared with pretreatment values.