# Sperm aneuploidy after testicular cancer treatment: data from a prospective multicenter study performed within the French Centre d'Étude et de Conservation des Oeufs et du Sperme network

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**Objective:** To study sperm aneuploidy in a population of testicular cancer (TC) patients treated with the use of either bleomycin-etoposide-cisplatin (BEP) chemotherapy or radiotherapy.
**Design:** Multicenter prospective longitudinal study of TC patients analyzed before treatment and after 3, 6, 12, and 24 months (T3–T24).
**Patient(s):** Fifty-four TC patients and a control group of 10 fertile sperm donors.
**Setting:** University hospital laboratories.
**Intervention(s):** Routine semen analyses; sperm aneuploidy and diploidy.
**Main Outcome Measure(s):** Comparison of sperm characteristics and sperm chromosome abnormalities during TC patient follow-up.
**Result(s):** Semen characteristics recovered pretreatment values 12 months after radiotherapy and 24 months after more than two BEP cycles. A significant increase in sperm disomy YY and XX was observed in the TC group before treatment compared with the control group. After more than two BEP cycles, the mean sperm aneuploidy rate increased significantly at T12 and reached the pretreatment value at T24. After radiotherapy, the mean sperm aneuploidy returned to the pretreatment value at T12. At T24, nearly 40% of TC patients did not recover their pretreatment sperm aneuploidy rate.
**Conclusion(s):** Genetic counseling of TC patients should include information on the potential elevated risk of aneuploid conceptus from sperm recovered after treatment and the necessity to postpone conception up to $\geq$12 months after radiotherapy and $\geq$24 months after more than two BEP chemotherapy cycles. However, few men receiving one or two BEP cycles and some dropouts are the main limitations of this study. (Fertil Steril® 2017;107:580–8. ©2016 by American Society for Reproductive Medicine.)
**Key Words:** Aneuploidy, chemotherapy, radiotherapy, spermatozoa, testicular cancer

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Over the past four decades, there has been an increase in the incidence of testicular cancer (TC) in men aged 20–35 years in most industrialized countries (1). Advances in medical therapy have considerably improved the long-term survival rate. TC is mainly treated by means of orchiectomy via the inguinal pathway followed by either chemotherapy or radiotherapy according to histologic type and disease stage (2). Antineoplastic therapy has adverse side-effects on the germinal epithelium, depending on the regimen or the cumulative dosage of treatment, pretreatment sperm production, or possible individual susceptibility to treatment toxicity (3, 4). Toxicity on germinal epithelium may be reversible, and after recovery conception can occur spontaneously or after assisted reproduction procedure (5). Antineoplastic treatments have potential genotoxic effects on male germ cells (6) and may theoretically induce chromosome abnormalities in mature spermatozoa, such as aneuploidy, chromosome structural rearrangements, sister chromatid exchanges, simple or double-strand breaks, mutations, and micronucleus formation (7–9). The studies that have evaluated the impact of antineoplastic treatment on sperm aneuploidy in TC patients are very heterogeneous, using different techniques, some including a low number of patients, often retrospective (10–16), exceptionally prospective (17–20), and with different regimens of treatment analyzed at different time periods (Table 1).

Published data are contradictory, and most studies only involved patients with TC treated with chemotherapy (10–16). The main objective of the present prospective study was to assess sperm aneuploidy before and after treatment in TC patients treated with the use of either chemotherapy or radiotherapy to further address counseling for safe use of spermatozoa and spontaneous conception after cancer therapy.

## PATIENTS AND METHODS

### Population and Study Design

This study was part of the collaborative and prospective research project "GAMATOX," conducted in eight Centre d'Étude et de Conservation des Oeufs et du Sperme (CECOS) fertility preservation sites (i.e., Caen, Clermont-Ferrand, Grenoble, Marseille, Paris Cochin, Paris Tenon, Rouen, and Toulouse). The study was proposed to every patient consulting for sperm banking in case of TC in the eight CECOS sites and initially enrolled 129 patients who were referred for sperm banking before TC treatment from January 2003 to December 2008. However, for the present study, the selection was performed on the basis of highest availability of sperm samples for fluorescence in situ hybridization (FISH) analyses and lowest dropout at the different time points. Thus, 54 patients (42% of the initial population) were finally enrolled: From the initial eligible population of 129 TC patients, 75 were excluded from the present study because of dropout at 24 months (T24; n = 34), azoospermia at more than two time points (n = 22), or the unavailability of straws that were preferentially used for the previous published experiments (n = 19; Supplemental Fig. 1 [available online at www.fertstert.org) (4). Data on conventional sperm characteristics and sperm chromatin damages have been published previously (4). This study was approved by the Institutional Ethics Review Board (CCPPRB Toulouse Sud-Ouest II), and all patients gave their written informed consents.

### Procedures

Patients provided a semen sample before treatment initiation (T0), at 3 (T3), 6 (T6), 12 (T12), and 24 (T24) months after treatment ending. Patients had been treated either with two to four bleomycin-etoposide-cisplatin (BEP) cycles or with standard radiotherapy consisting of irradiation administered to infradiaphragmatic, para-aortic, and ipsilateral iliac lymph nodes, usually in 15 fractions with a mean total dose of 25 Gy. Contralateral testis shielding was performed. Age, tobacco exposure, febrile episodes, and andrologic and reproductive histories were recorded. At each time point, participants completed a standard questionnaire on any unusual medical or nonmedical events since the last visit to the laboratory. Tumor histologic type was classified as pure seminoma or nonseminoma tumor. Because sperm aneuploidy does not vary significantly among fertile men with normal semen parameters (21), ten healthy men of proven fertility, aged 24–43 years, sperm donor volunteers, with no history of chronic illness or toxic exposure, who consulted for sperm donation during the period of the study, and who gave their informed consents for research, constituted the control group. Their semen characteristics and constitutional karyotypes were normal.

### Semen Analyses

Semen samples were analyzed in the eight CECOS sites with the use of standardized methods for semen analysis (22), and semen characteristics were interpreted according to World Health Organization guidelines (23). The remaining semen sample was frozen with cryoprotectant in straws with the use of standardized freezing protocol and stored in liquid nitrogen. Sperm aneuploidy was assessed by a single technician in the CECOS at Rouen University Hospital with the use of two straws of each semen sample that were thawed and fixed in fresh methanol–acetic acid (3:1, v/v). Data were analyzed blindly by coding the slides after sperm nuclei preparation. A three-color FISH procedure was performed with the use of $\alpha$-satellite centromeric probes for chromosome X (CEP X Spectrum Green, Abbott), chromosome Y (CEP Y Sat III Spectrum Orange, Abbott), and chromosome 18 (CEP 18 Spectrum Aqua, Abbott). Centromeric probes were used to obtain a higher hybridization rate compared with locus-specific probes. With the use of sex chromosome probes, we were also able to more specifically differentiate nondisjunctions occurring during the first or second meiotic divisions. Trisomy 18 and sex aneuploidies are compatible with survival, and sex chromosome aneuploidies are the most common aneuploidies observed at birth. Finally with chromosome 18, we examined nondisjunction for autosome (24). We explored the evolution of sperm aneuploidy during the study period and we did not try to quantify precisely the whole chromosome aneuploidy. Thereafter, slides were examined at $\times$1,000 magnification (25). A minimum of 5,000 spermatozoa were

## TABLE 1

**Summary of human sperm chromosome aneuploidy studies of testicular cancer patients who received either chemotherapy or radiotherapy.**

| Study | Type of study; histology (n) | No. of patients (no. of control subjects) | Follow-up (n) | Treatment (n) | Analysis | Chromosomes studied by FISH | No. of nuclei scored per patient | Time after treatment | Increased numeric chromosome abnormalities |
|---|---|---|---|---|---|---|---|---|---|
| Genesca et al. (10) | Retrospective; NS (2) | 2 | Post-T | Ch (BEP or BVP) (2) | Karyotype | – | Post-T: 100–118 | 2–5 y | No |
| Jenderny et al. (11) | Retrospective; S (1) | 1 (8) | Post-T | Ch (BVP) | Karyotype | – | Post-T: 63; C: nd | 9 mo | No |
| Martin et al. (12) | Retrospective; NS (4) | 4 (nd) | Pre-T, post-T | Ch (BEP) | Karyotype | – | Pre-T: 236; Post-T: 552; C: nd | 2–13 y | No |
| Alvarez et al. (22) | Retrospective; S (2), NS (2) | 4 (3) | Post-orchidectomy | Orchidectomy | Karyotype | – | Post-T: 340; C: 320 | 1–26 mo | No |
| Martin et al. (13) | Retrospective; NS (4) | 4 (10) | Pre-T, post-T | Ch (BEP) | FISH | X, Y, 1, 12 | Pre-T: 80,445; Post-T: 80,642; C: 161,097 | 2–13 y | No |
| Martin et al. (17) | Prospective; NS (1) | 1 | Pre-T, T, post-T | Ch (4× BEP) | FISH | X, Y, 1, 12 | Pre-T: 20,004; T: 20,005; Post-T: 20,391 | 59 d after T initiation, 12 mo post-T | Yes |
| De Mas et al. (15) | Retrospective; S (1), NS (4) | 5 (5) | Post-T | Ch | FISH | X, Y, 7, 16, 18 | Post-T: 100,000; C: 100,000 | 6–17 mo | Yes |
| Thomas et al. (16) | Retrospective; S (10), NS (4) | 14 (12) | Post-T | Ch (>2× BEP or EP) (5), Ra (8), Ch (EP) +Ra (1) | FISH | X, Y, 13, 18, 21 | Post-T: 111,378; C: 98,711 | 19 mo–5 y | No (except 4 patients with 2× BEP or 2× EP) |
| Tempest et al. (18) | Prospective; TGCT (5) | 5 (10) | Pre-T, T, post-T | Ch (2–4× BEP) (5) | FISH | X, Y, 13, 21 | Pre-T: 50,067; After T initiation: 99,578; C: 400,560 | 6, 12, 18, 24 mo after T initiation | Yes at 6 mo |
| Burello et al. (19) | Prospective; S (10), NS (4) | 11 (18) | Pre-T, post-T | Ch (2–3× BEP) (4), Ra (7), Ch (BEP) + Ra (3) | FISH | X, Y, 8, 12, 18 | Pre-T, post-T: 205,670; Pre-T: ~25,707; Post-T: ~179,949; C: 70,197 | 3, 6, 9, 12, 18, 24, 36 mo post-T | Yes at 6 mo, No after 6 mo |
| Ghezzi et al. (20) | Prospective | 154 | Pre-T, post-T | Ch (1–4× BEP or 1 Ca) | | X, Y, 18 | Pre-T: 2,500/patient; Post-T: 2,500/patient | After orchidectomy, 12 and 24 mo post-T | Yes at 12 and 24 mo |
| Present study | Prospective; S (26), NS (28) | 54 (10) | Pre-T, post-T | Ch (2× BEP) (5), Ch (3–4× BEP) (23), Ra (26) | FISH | X, Y, 18 | Pre-T: 289,972; Post-T: 709,147; C: 54,506 | 3, 6, 12, 24 mo post-T | Ra: Yes at 6 mo, no at 12 mo; Ch (>2× BEP): Yes at 6 and 12 mo, no at 24 mo |

*Note:* B = bleomycin; C = control subjects; Ca = carboplatin; Ch = chemotherapy; E = etoposide; FISH = fluorescent in situ hybridization; nd = not determined; NS = nonseminoma; P = cisplatin; Ra = radiotherapy; S = seminoma; T = treatment; TGCT = testicular germ cell tumor; V = vinblastin.

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## TABLE 2

**Sperm characteristics before and after treatment and during follow-up observation in chemotherapy and radiotherapy groups.**

| | | After treatment | | | |
|---|---|---|---|---|---|
| **Sperm characteristic** | **Before treatment** | **3 mo** | **6 mo** | **12 mo** | **24 mo** |
| Chemotherapy (≤2 cycles) | (n = 5) | (n = 4) | (n = 5) | (n = 3) | (n = 3) |
| Volume (mL) | 3.88 ± 1.51 | 4.52 ± 0.92 | 3.92 ± 1.71 | 3.83 ± 0.65 | 3.33 ± 1.30 |
| pH | 8.15 ± 0.25 | 8.00 ± 0.12 | 7.78 ± 0.39 | 7.50 ± 0.17 | 7.83 ± 0.12 |
| Sperm count ($10^6$/mL) | 19.00 ± 11.85 | 0.78 ± 0.65 | 5.10 ± 2.28 | 17.00 ± 5.00 | 34.3 ± 17.67 |
| Round cells ($10^6$/mL) | 1.16 ± 2.17 | 0.30 ± 0.48 | 0.45 ± 0.53 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Vitality (%) | 64.60 ± 11.74 | 67.00 ± 32.53 | 63.00 ± 15.72 | 61.00 ± 1.00 | 62.67 ± 12.50 |
| Motility (%) | 44.20 ± 17.98 | 28.75 ± 35.21 | 36.20 ± 17.88 | 36.67 ± 11.55 | 33.33 ± 5.77 |
| Total sperm count ($10^6$/ejaculate) | 74.06 ± 52.59 | 3.49 ± 3.27 | 21.15 ± 17.88 | 64.17 ± 16.37 | 112.47 ± 77.16 |
| Total motile sperm count ($10^6$/ejaculate) | 37.51 ± 34.26 | 0.89 ± 0.69 | 9.93 ± 13.73 | 24.35 ± 12.60 | 36.81 ± 22.53 |
| Chemotherapy (>2 cycles) | (n = 24) | (n = 19) | (n = 24) | (n = 19) | (n = 17) |
| Volume (mL) | 3.51 ± 1.43 | 3.61 ± 1.51 | 3.87 ± 1.75 | 3.36 ± 1.45 | 3.98 ± 1.31 |
| pH | 7.98 ± 0.23 | 7.86 ± 0.30 | 7.83 ± 0.30[a] | 7.87 ± 0.34 | 7.85 ± 0.38 |
| Sperm count ($10^6$/mL) | 25.30 ± 21.76 | 0.18 ± 0.38[a] | 6.02 ± 18.88[a] | 9.35 ± 12.76[a] | 35.72 ± 34.37 |
| Round cells ($10^6$/mL) | 1.35 ± 2.26 | 0.26 ± 0.34[a] | 0.27 ± 0.38[a] | 0.67 ± 1.21 | 0.72 ± 1.03 |
| Vitality (%) | 65.33 ± 12.30 | 49.00 ± 29.88 | 50.69 ± 31.69 | 69.93 ± 10.51 | 68.00 ± 14.90[a] |
| Motility (%) | 40.54 ± 13.39 | 12.65 ± 18.47[a] | 26.52 ± 20.54[a] | 31.67 ± 19.38 | 35.24 ± 11.59 |
| Total sperm count ($10^6$/ejaculate) | 97.43 ± 107.69 | 0.57 ± 1.09[a] | 21.54 ± 59.76[a] | 32.00 ± 48.22[a] | 141.47 ± 144.50 |
| Total motile sperm count ($10^6$/ejaculate) | 44.50 ± 55.93 | 0.18 ± 0.48[a] | 5.47 ± 9.81[a] | 14.80 ± 22.76[a] | 47.73 ± 49.56 |
| Radiotherapy | (n = 25) | (n = 24) | (n = 25) | (n = 25) | (n = 24) |
| Volume (mL) | 4.13 ± 1.65 | 3.87 ± 1.42 | 3.97 ± 1.48 | 4.62 ± 1.90 | 4.05 ± 1.75 |
| pH | 8.05 ± 0.28 | 8.10 ± 0.36 | 8.00 ± 0.36 | 8.10 ± 0.30 | 7.98 ± 0.40 |
| Sperm count ($10^6$/mL) | 42.24 ± 45.08 | 18.43 ± 17.57[a] | 21.32 ± 38.52[a] | 30.97 ± 26.14 | 44.20 ± 39.86 |
| Round cells ($10^6$/mL) | 1.12 ± 1.80 | 0.96 ± 1.13 | 0.98 ± 1.94 | 0.67 ± 0.65 | 0.9 ± 0.74 |
| Vitality (%) | 67.48 ± 10.56 | 65.04 ± 12.15 | 68.00 ± 13.43 | 66.67 ± 13.65 | 68.38 ± 11.82 |
| Motility (%) | 46.00 ± 9.76 | 40.75 ± 16.42 | 40.36 ± 15.53 | 43.03 ± 16.07 | 44.17 ± 15.84 |
| Total sperm count ($10^6$/ejaculate) | 188.95 ± 238.11 | 71.14 ± 82.69[a] | 95.22 ± 213.29[a] | 131.48 ± 133.76 | 169·43 ± 164.97 |
| Total motile sperm count ($10^6$/ejaculate) | 88.43 ± 106.61 | 32.93 ± 41.38[a] | 46.27 ± 107.41[a] | 63.06 ± 78.47 | 77·06 ± 91.57 |

[a] $P<.05$: difference between before treatment and after treatment (3, 6, 12, and 24 months).

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analyzed for each chromosome probe in each patient at each time point and in each control.

## Statistical Analysis

All data were reported on centralized case report forms via the internet and were verified by the coordinating center in Toulouse. Data were compared between the control and TC groups with the use of the nonparametric Mann-Whitney test. Sperm aneuploidy data of TC patients were compared before and after treatment with the use of the Wilcoxon signed rank-sum test. Statistical analysis was performed with the use of SAS software (9.0; SAS Institute), and $P<.05$ was considered to be statistically significant.

## RESULTS

The study enrolled 54 patients with a mean age of 30.3 years (range 22–43 y). FISH analysis was not possible at T0 for one patient owing to repeated hybridization failure; this patient was also excluded thereafter during the follow-up (Supplemental Fig. 1).

At T0, 18 patients (33%) had fathered, four (7%) reported history of cryptorchism, one (2%) had scrotal injury, ten (19%) had genital infectious, and one (2%) had varicocele. A total of 45 patients (84%) were teetotalers or low consumers of alcohol. Twenty-one patients (40%) were tobacco smokers with fewer than 20 cigarettes per day, and six patients (11%) were regular consumers of recreational drugs, at least once per week. None of them were exposed to professional or occupational pesticides or xenobiotics. Hyperthermia of <3 months was reported in 15 patients (29%), four patients (7%) had regular hot baths, and seven patients (13%) had a professional exposure to high temperatures. Twenty-six patients with pure seminoma (48%) received radiotherapy, 28 with nonseminoma (52%) underwent BEP chemotherapy: Five patients received one or two BEP cycles, and 23 patients had three or four cycles.

At T0, semen parameters were not different between seminoma and nonseminoma patients. Normozoospermia was identified in 32 patients (59%) and oligozoospermia in 22 (41%). Sperm count, total sperm count, and total motile sperm count decreased significantly after chemotherapy with more than two BEP cycles or radiotherapy, with the lowest values observed at T3 and T6 (Table 2). The sperm count also decreased at T3 and T6 when one or two BEP cycles were given. The percentage of progressive motile sperm

decreased significantly at T3 ($P$=.00003) and T6 ($P$=.013) after more than two BEP cycles. Semen characteristics returned to pretreatment values 12 months after radiotherapy and 24 months after more than two BEP cycles, except for the percentage of progressive motile sperm, which remained lower than prechemotherapy values. Total sperm count and total motile sperm count were significantly lower at T3, T6, and T12 after chemotherapy compared with radiotherapy ($P$<.05). At T24, 100% of patients with one or two chemotherapy cycles, 76% of patients with more than two cycles, and 88% in the radiotherapy group recovered sperm production to $\geq 39 \times 10^6$/ejaculate ($P$>.05).

Sperm aneuploidy data are reported in Table 3 for control subjects and for patients before treatment, and in Table 4 during follow-up for patients who received either chemotherapy or radiotherapy. A mean of 5,451 (±155.2), 5,178 (±520.24) and 11,836 (±5,127.7) spermatozoa was scored for each control subject and for each patient before and after treatment, respectively.

At T0, disomy Y ($P$=.006) and disomy X ($P$=.047) were higher in the TC group compared with control subjects. Disomy Y was higher in patients with seminoma or nonseminoma compared with control subjects ($P$=.032 and $P$=.003, respectively). However, sperm aneuploidy and total chromosome abnormalities were not different between seminoma and nonseminoma patients (Table 3).

After chemotherapy (Supplemental Fig. 1; Table 4), sperm aneuploidy was not assessed at T3 in most patients who had received chemotherapy (n = 28) owing to patient dropout at this time point (n = 6), azoospermia (n = 6), or severe oligozoospermia (n = 14) that did not provide sufficient sperm for FISH analysis (Supplemental Fig. 1). For most patients who had received chemotherapy, FISH analysis was first assessed at T6 (n = 14). Hyperhaploidy XY ($P$=.024), diploidy ($P$=.029), aneuploidy ($P$=.041), and total chromosome abnormality ($P$=.024) rates increased significantly at T6. Chromosome nondisjunctions increased during the first ($P$=.041) and the second ($P$=.024) meiotic divisions. The rates of aneuploidy and total chromosome abnormalities remained significantly higher at T12 ($P$=.013 and $P$=.021) and reached pretreatment values at T24 after more than two BEP cycles. Nevertheless, frequencies of chromosome aneuploidy did not vary significantly before and after treatment in patients receiving one or two BEP cycles. In contrast, in the group of patients receiving more than two BEP cycles, aneuploidy ($P$=.013) and total chromosome abnormalities ($P$=.021) increased significantly from T0 to T12. Meiotic nondisjunctions occurred preferentially during the first meiotic division. Diploidy rates ($P$=.039) were significantly higher at T6 compared with pretreatment values. At T6, even if aneuploidy and total chromosome abnormality rates were close to those observed at T12, the values did not reach significance. At T24, only hyperhaploid XY spermatozoa ($P$=.047) remained numerous and did not reach pretreatment value.

After radiotherapy (Supplemental Fig. 1; Table 4), sperm aneuploidy was not assessed at T3 and T6 in three patients who had received radiotherapy owing to patient dropout (one patient), severe oligozoospermia (one patient), and hybridization failure (one patient). Hyperhaploidy XY, diploidy, aneuploidy, and total chromosome abnormality frequencies were higher at T3 ($P$=.005, $P$=.008, $P$=.006, and $P$=.001 respectively) and T6 ($P$=.001, $P$=.002, $P$=.0001, and $P$=.001) compared with pretreatment values. Disomy X was higher at T3 compared with T0 values ($P$=.032). At T6, chromosome nondisjunctions increased significantly during meiosis I ($P$=.04) and meiosis II ($P$=.02). Aneuploidy rates returned to pretreatment values at T12 and remained stable at T24.

Chromosome nondisjunctions occurred preferentially during the first meiotic division whatever the time points and the treatment. Sperm chromosome aneuploidy ($P$=.04) and total chromosome abnormalities ($P$=.04) were significantly higher at T24 after chemotherapy compared with radiotherapy. At T24, even though the mean sperm aneuploidy rate reached pretreatment values in the two groups of patients, nine patients out of 16 (56%) after chemotherapy, who all received more than two BEP cycles, and nine patients out of 23 (39%) after radiotherapy presented a sperm aneuploidy rate that exceeded the 95% confidence interval

## TABLE 3

**Frequencies (%) of chromosome abnormalities in control group and testicular cancer group before treatment.**

| Chromosome abnormality | Control group (n = 10) | Testicular cancer group (n = 53) | Seminoma | Nonseminoma |
|---|---|---|---|---|
| Haploidy | 99.35 ± 0.28 | 99.25 ± 0.39 | 99.24 ± 0.42 | 99.27 ± 0.35 |
| Hyperhaploidy XY | 0.41 ± 0.21 | 0.37 ± 0.21 | 0.39 ± 0.24 | 0.34 ± 0.18 |
| Disomy Y | 0.01 ± 0.01 | 0.04 ± 0.04[a] | 0.03 ± 0.04[a] | 0.03 ± 0.04[a] |
| Disomy X | 0.02 ± 0.02 | 0.04 ± 0.04[a] | 0.04 ± 0.04 | 0.04 ± 0.04 |
| Disomy18 | 0.03 ± 0.04 | 0.05 ± 0.05 | 0.05 ± 0.05 | 0.04 ± 0.04 |
| Diploidy | 0.19 ± 0.10 | 0.26 ± 0.20 | 0.25 ± 0.21 | 0.27 ± 0.18 |
| Aneuploidy | 0.47 ± 0.24 | 0.49 ± 0.27 | 0.51 ± 0.30 | 0.46 ± 0.24 |
| Total chromosome abnormalities | 0.65 ± 0.28 | 0.75 ± 0.39 | 0.76 ± 0.42 | 0.73 ± 0.35 |
| Meiosis I | 0.14 ± 0.08 | 0.21 ± 0.18 | 0.20 ± 0.20 | 0.21 ± 0.16 |
| Meiosis II | 0.05 ± 0.03 | 0.06 ± 0.05 | 0.06 ± 0.05 | 0.07 ± 0.05 |

*Note:* Haploidy: sum of frequencies of presumed 23,X and 23,Y spermatozoa. Diploidy: sum of frequencies of presumed 46,XX, 46,YY, and 46,XY diploid spermatozoa. Aneuploidy: sum of frequencies of presumed disomic X, Y, and 18 and hyperhaploid XY spermatozoa. Total chromosome abnormalities: sum of diploidy and aneuploidy. Meiosis I: sum of frequencies of presumed hyperhaploid XY and 46,XY diploid spermatozoa. Meiosis II: sum of presumed disomic X and Y and 46,XX and 46,YY diploid spermatozoa.

[a] Difference between control group and testicular cancer group, $P$<.05

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## TABLE 4

**Frequencies (%) of chromosome abnormalities before and after treatment and during follow-up observation in chemotherapy and radiotherapy groups.**

| | | After treatment | | | |
|---|---|---|---|---|---|
| **Chromosome abnormality** | **Before treatment** | **3 mo** | **6 mo** | **12 mo** | **24 mo** |
| Chemotherapy (≤2 cycles) | (n = 5) | (n = 1) | (n = 5) | (n = 3) | (n = 2) |
| Haploidy | 99.6 ± 0.34 | 99.59 ± – | 99.30 ± 0.33 | 99.57 ± 0.30 | 99.59 ± 0.15 |
| Hyperhaploidy XY | 0.29 ± 0.21 | 0.10 ± – | 0.43 ± 0.30 | 0.24 ± 0.28 | 0.22 ± 0.13 |
| Disomy Y | 0.04 ± 0.04 | 0.00 ± – | 0.02 ± 0.02 | 0.03 ± 0.03 | 0.02 ± 0.00 |
| Disomy X | 0.02 ± 0.02 | 0.00 ± – | 0.03 ± 0.02 | 0.05 ± 0.02 | 0.01 ± 0.01 |
| Disomy18 | 0.02 ± 0.01 | 0.10 ± – | 0.02 ± 0.03 | 0.02 ± 0.02 | 0.02 ± 0.00 |
| Diploidy | 0.17 ± 0.11 | 0.20 ± – | 0.21 ± 0.08 | 0.09 ± 0.07 | 0.15 ± 0.00 |
| Aneuploidy | 0.37 ± 0.26 | 0.20 ± – | 0.50 ± 0.30 | 0.33 ± 0.23 | 0.27 ± 0.15 |
| Total chromosome abnormalities | 0.54 ± 0.34 | 0.41 ± – | 0.70 ± 0.33 | 0.43 ± 0.30 | 0.41 ± 0.15 |
| Meiosis I | 0.15 ± 0.10 | 0.01 ± – | 0.16 ± 0.06 | 0.05 ± 0.06 | 0.13 ± 0.02 |
| Meiosis II | 0.02 ± 0.02 | 0.20 ± – | 0.05 ± 0.03 | 0.05 ± 0.05 | 0.02 ± 0.03 |
| Chemotherapy (>2 cycles) | (n = 23) | (n = 1) | (n = 9) | (n = 13) | (n = 14) |
| Haploidy | 99.26 ± 0.33 | 96.79 ± – | 98.83 ± 0.69 | 99.06 ± 0.52[a] | 99.05 ± 0.49 |
| Hyperhaploidy XY | 0.33 ± 0.18 | 1.96 ± – | 0.50 ± 0.25 | 0.41 ± 0.25 | 0.52 ± 0.27[a] |
| Disomy Y | 0.04 ± 0.03 | 0.53 ± – | 0.06 ± 0.06 | 0.06 ± 0.07 | 0.03 ± 0.02 |
| Disomy X | 0.05 ± 0.02 | 0.27 ± – | 0.05 ± 0.05 | 0.06 ± 0.06 | 0.03 ± 0.02[a] |
| Disomy18 | 0.05 ± 0.04 | 0.09 ± – | 0.09 ± 0.08 | 0.09 ± 0.14 | 0.04 ± 0.05 |
| Diploidy | 0.27 ± 0.19 | 0.36 ± – | 0.47 ± 0.37[a] | 0.31 ± 0.33 | 0.32 ± 0.24 |
| Aneuploidy | 0.47 ± 0.25 | 2.85 ± – | 0.70 ± 0.35 | 0.63 ± 0.32[a] | 0.63 ± 0.29 |
| Total chromosome abnormalities | 0.74 ± 0.33 | 3.21 ± – | 1.17 ± 0.69 | 0.94 ± 0.52[a] | 0.95 ± 0.49 |
| Meiosis I | 0.21 ± 0.16 | 0.40 ± – | 0.34 ± 0.29[a] | 0.23 ± 0.27 | 0.25 ± 0.18 |
| Meiosis II | 0.07 ± 0.05 | 0.08 ± – | 0.14 ± 0.11 | 0.10 ± 0.08 | 0.08 ± 0.07 |
| Chemotherapy (all cycles) | (n = 28) | (n = 2) | (n = 14) | (n = 16) | (n = 16) |
| Haploidy | 99.30 ± 0.34 | 98.19 ± 1.98 | 98.99 ± 0.62[a] | 99.16 ± 0.52[a] | 99.12 ± 0.49 |
| Hyperhaploidy XY | 0.32 ± 0.18 | 1.03 ± 1.31 | 0.48 ± 0.26[a] | 0.38 ± 0.25 | 0.48 ± 0.28 |
| Disomy Y | 0.04 ± 0.04 | 0.27 ± 0.38 | 0.05 ± 0.06 | 0.06 ± 0.07 | 0.03 ± 0.02 |
| Disomy X | 0.04 ± 0.04 | 0.13 ± 0.19 | 0.04 ± 0.04 | 0.06 ± 0.06 | 0.03 ± 0.02[a] |
| Disomy18 | 0.05 ± 0.04 | 0.10 ± 0.01 | 0.06 ± 0.07 | 0.08 ± 0.13 | 0.04 ± 0.05 |
| Diploidy | 0.25 ± 0.18 | 0.28 ± 0.11 | 0.38 ± 0.32[a] | 0.27 ± 0.31 | 0.30 ± 0.23 |
| Aneuploidy | 0.45 ± 0.25 | 1.53 ± 1.87 | 0.63 ± 0.33[a] | 0.57 ± 0.32[a] | 0.58 ± 0.30 |
| Total chromosome abnormalities | 0.70 ± 0.34 | 1.81 ± 1.98 | 1.01 ± 062[a] | 0.84 ± 0.52[a] | 0.88 ± 0.49 |
| Meiosis I | 0.20 ± 0.15 | 0.20 ± 0.27 | 0.28 ± 0.25[a] | 0.19 ± 0.25 | 0.24 ± 0.17 |
| Meiosis II | 0.06 ± 0.05 | 0.14 ± 0.09 | 0.11 ± 0.10[a] | 0.09 ± 0.08 | 0.07 ± 0.07 |
| Radiotherapy | (n = 25) | (n = 22) | (n = 22) | (n = 24) | (n = 23) |
| Haploidy | 99.20 ± 0.43 | 98.88 ± 0.51[a] | 98.74 ± 0.80[a] | 99.13 ± 0.44 | 99.33 ± 0.32 |
| Hyperhaploidy XY | 0.42 ± 0.23 | 0.58 ± 0.38[a] | 0.71 ± 0.70[a] | 0.44 ± 0.31 | 0.38 ± 0.17 |
| Disomy Y | 0.03 ± 0.04 | 0.04 ± 0.04 | 0.05 ± 0.06 | 0.02 ± 0.02 | 0.02 ± 0.02 |
| Disomy X | 0.04 ± 0.03 | 0.05 ± 0.06[a] | 0.05 ± 0.05 | 0.04 ± 0.04 | 0.02 ± 0.02[a] |
| Disomy18 | 0.04 ± 0.05 | 0.06 ± 0.04 | 0.06 ± 0.06 | 0.04 ± 0.04 | 0.02 ± 0.03 |
| Diploidy | 0.27 ± 0.22 | 0.38 ± 0.24[a] | 0.40 ± 0.23[a] | 0.33 ± 0.24 | 0.23 ± 0.16 |
| Aneuploidy | 0.53 ± 0.30 | 0.74 ± 0.42[a] | 0.87 ± 0.73[a] | 0.54 ± 0.33 | 0.44 ± 0.20 |
| Total chromosome abnormalities | 0.80 ± 0.43 | 1.12 ± 0.51[a] | 1.26 ± 0.80[a] | 0.87 ± 0.44 | 0.67 ± 0.32 |
| Meiosis I | 0.22 ± 0.21 | 0.28 ± 0.19[a] | 0.29 ± 0.17[a] | 0.26 ± 0.18 | 0.20 ± 0.14 |
| Meiosis II | 0.06 ± 0.05 | 0.11 ± 0.09[a] | 0.12 ± 0.12[a] | 0.08 ± 0.08 | 0.04 ± 0.03 |

*Note:* Haploidy: sum of frequencies of presumed 23,X and 23,Y spermatozoa. Diploidy: sum of frequencies of presumed 46,XX, 46,YY, and 46,XY diploid spermatozoa. Aneuploidy: sum of frequencies of presumed disomic X, Y, and 18 and hyperhaploid XY spermatozoa. Total chromosome abnormalities: sum of diploidy and aneuploidy. Meiosis I: sum of frequencies of presumed hyperhaploid XY and 46,XY diploid spermatozoa. Meiosis II: sum of presumed disomic X and Y and 46,XX and 46,YY diploid spermatozoa.

[a] $P<.05$: difference between before treatment and after treatment (3, 6, 12, and 24 months).

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observed in the healthy donor control population. However, among these patients (n = 18), 72.2% (n = 13) had a normal sperm concentration, varying from 29 × $10^6$/mL and 158 × $10^6$/mL, and 89% (n = 16) had a normal total sperm count. At T24, 15 patients out of 39 (38.5%: eight after more than two BEP cycles and seven after radiotherapy) did not recover their pretreatment sperm aneuploidy rates. Considering each group of patients separately, no relationship was established between the sperm aneuploidy rate and the total sperm count, except at T24 for patients who had received more than two BEP cycles, and a positive correlation was established between these parameters ($r = 0.54$; $P=.04$).

## DISCUSSION

Our study demonstrates that TC patients present elevated frequencies of aneuploid sperm depending on the treatment received and the time after end of treatment. Patients who

had received radiotherapy recovered their pretreatment sperm aneuploidy rate earlier than patients treated with more than two BEP cycles. However, nearly 40% of TC patients did not recover their pretreatment values after 24 months. Furthermore, BEP chemotherapy maintained higher rates of sperm aneuploidy at T24 compared with radiotherapy.

To the best of our knowledge, we report here the first prospective analysis based on a standardized protocol that enrolled the largest TC population in which sperm aneuploidy was followed serially in the same patients before and after treatment. The time points of follow-up were precisely defined, and semen characteristics were systematically evaluated. Semen parameters followed evolution before and after chemotherapy with more than two BEP cycles similarly as reported in the overall TC population published previously, with the recovery of pretreatment values at T24. However, our subcohort of patients treated with the use of radiotherapy recovered their pretreatment total sperm count at T12 rather than at T24. However, the percentage of patients who recovered sperm production of $\geq 39 \times 10^6$/ejaculate, considering the different types of treatment, is close to previously published data reported in the whole population of TC patients (4). We selected this subcohort of patients considering the availability of sperm samples at the different time points and with the lowest rate of dropout during the follow-up and probably with a higher total sperm count at the different time points compared with the whole population (4). To date, only four prospective studies have explored sperm aneuploidy with the use of FISH after TC treatment (17–20), and none of the studies performed with the hamster oocyte–human sperm fusion assay were prospective (10–12, 26). The main strength of the present study is that it is the first in which sperm aneuploidy was determined according to the type of treatment and the number of chemotherapy cycles. Of the 181 TC patients previously reported in the literature (Table 1), most had received chemotherapy, only 15 patients had been treated with radiotherapy alone, and four patients had received both. None of the studies compared sperm aneuploidy between patients treated by chemotherapy versus radiotherapy (16, 19).

The present study shows a significantly elevated rate of sperm aneuploidy at T0 in accordance with the locoregional or systemic toxicity of the cancer itself able to perturb the meiotic process, resulting in diminished sperm production and increased number of aneuploid spermatozoa (4, 18, 19). Our data do not agree with the recent report that concludes that semen samples dedicated for cryopreservation before TC treatment initiation do not carry an elevated risk for numeric chromosome aberrations (27). In addition, our study confirms a significantly higher frequency of sperm chromosome abnormalities at T6 (18, 19), due to chromosome nondisjunctions occurring during the two meiotic divisions. Another strength of our study is that it demonstrates similar evolution of sperm aneuploidy at T6 whatever the type of treatment. Furthermore, an earlier return to pretreatment values was observed after radiotherapy compared with chemotherapy: T12 versus T24, respectively. Nevertheless, the frequencies of aneuploidy did not vary significantly before and after treatment in patients receiving one or two BEP cycles even if a similar tendency of increase was detected compared with the other treatment regimens. However, the number of patients included in this subgroup remains very low to make conclusions.

This study specifically demonstrates that the type of treatment may induce variable heritable consequences (18–20). During spermatogenesis, the actively dividing spermatogonia are the cells that are the most sensitive to the toxicity of radiation and chemotherapeutic agents, followed by spermatocytes and stem spermatogonia (6, 28). Antineoplastic treatment may transiently or durably impair normal chromosome pairing and segregation, resulting in sperm aneuploidy (26). Ionizing radiation is known to generate free radical–induced alterations in DNA responsible for germ cell death by apoptosis or chromatid breaks (6). Bleomycin provokes genetic damage via generation of oxygen radicals as well as through direct DNA intercalation and binding preferentially to differentiating spermatogonia and to a lesser extent to spermatocytes (28). Etoposide, an inhibitor of topoisomerase II, may affect meiotic germ cells and dividing and stem spermatogonia by stabilizing its binding to the DNA-topoisomerase II (29). Cisplatin has a preferential clastogenic effect on mice spermatocytes and differentiating spermatogonia (9). Clastogenicity induced by radiation, cisplatin, or etoposide is concentration and time dependent with persistent or permanent chromosomal lesions that may affect stem spermatogonia and induce long-term sperm aneuploidy (6, 28–31). It is more difficult to assess the toxicity of these drugs in combination but it appears that TC treatment preferentially alters the differentiating germ cells and to less extent stem spermatogonia, because sperm aneuploidy returned to pretreatment values by 12 months after radiotherapy and 24 months after chemotherapy. The persistence of an increased rate of aneuploid spermatozoa 24 months after treatment in nearly 40% of patients is in agreement with induced mutations in stem spermatogonia (6, 28). Our data support the observation that the offspring of TC survivors are more likely to have congenital abnormalities than the offspring of fathers with no history of cancer if the conception occurs within 2 years of their father's cancer diagnosis (32).

Routine semen analysis is not sufficient to detect sperm genetic defects after TC treatment. Even if it has been suggested that there may be an increased incidence of chromosome abnormalities in sperm from infertile men with an abnormal semen analysis (33), no relationship was established in our study between low sperm count and sperm aneuploidy. Surprisingly, chromosome abnormalities were more frequently observed at T24 in the spermatozoa of patients with the highest total sperm count. These data confirmed a primarily genetic defect of stem spermatogonia responsible specifically for meiotic nondisjunctions and sperm aneuploidy. However, the increased rate of sperm aneuploidy was not observed in all patients, confirming that the toxicity of the treatment also depends on the individual ability of stem cells to repair. Specific polymorphisms may be associated with decreased or increased toxicity to stem spermatogonia, as reported with alkylating agents for sperm concentration

in childhood cancer survivors (34). Damage of the somatic microenvironment of germ cells could also affect durably spermatogenesis (6, 28). Indeed, in rodents, cisplatin has broad toxicity targeting not only germ cells but also Leydig and Sertoli cells (35).

The present study has several limitations. We did not have a sufficient number of patients treated with one or two BEP cycles to confirm the lowest toxicity of such regimen and dose-dependent BEP toxicity. Owing to severe spermatogenesis impairment at 3 and 6 months after more than two BEP cycles, the number of patients explored was lower compared with TC patients treated with the use of radiotherapy. Another limitation was the high number of dropouts after enrollment (six at T3, seven at T12, and ten at T24) owing to patient refusal or nonresponse to the appointment for semen analysis. Our sample size was not sufficient to consider the potential effect of lifestyle factors or professional exposure on meiotic disturbances. Continued follow-up after 24 months is necessary to test whether an increase in sperm aneuploidy persists in nearly 40% of patients. In addition, other DNA biomarkers should be evaluated to detect the long-lasting reproductive effects of TC treatment.

## CONCLUSION

It is critical to understand the potential long-term effects on the genetic integrity of germ cells following antineoplastic treatment specifically for each regimen of treatment. Thus, our findings have clinical relevance for the counseling and management of TC patients. Clinicians should discuss the potential genotoxic effects of TC therapy, preferably before treatment, and propose sperm cryopreservation. TC survivors may be at a significantly higher risk of abnormal reproductive outcomes throughout their reproductive life. Our findings also raise the question of preferentially using sperm cryopreserved before treatment to conceive if a higher rate of sperm aneuploidy remains after treatment.

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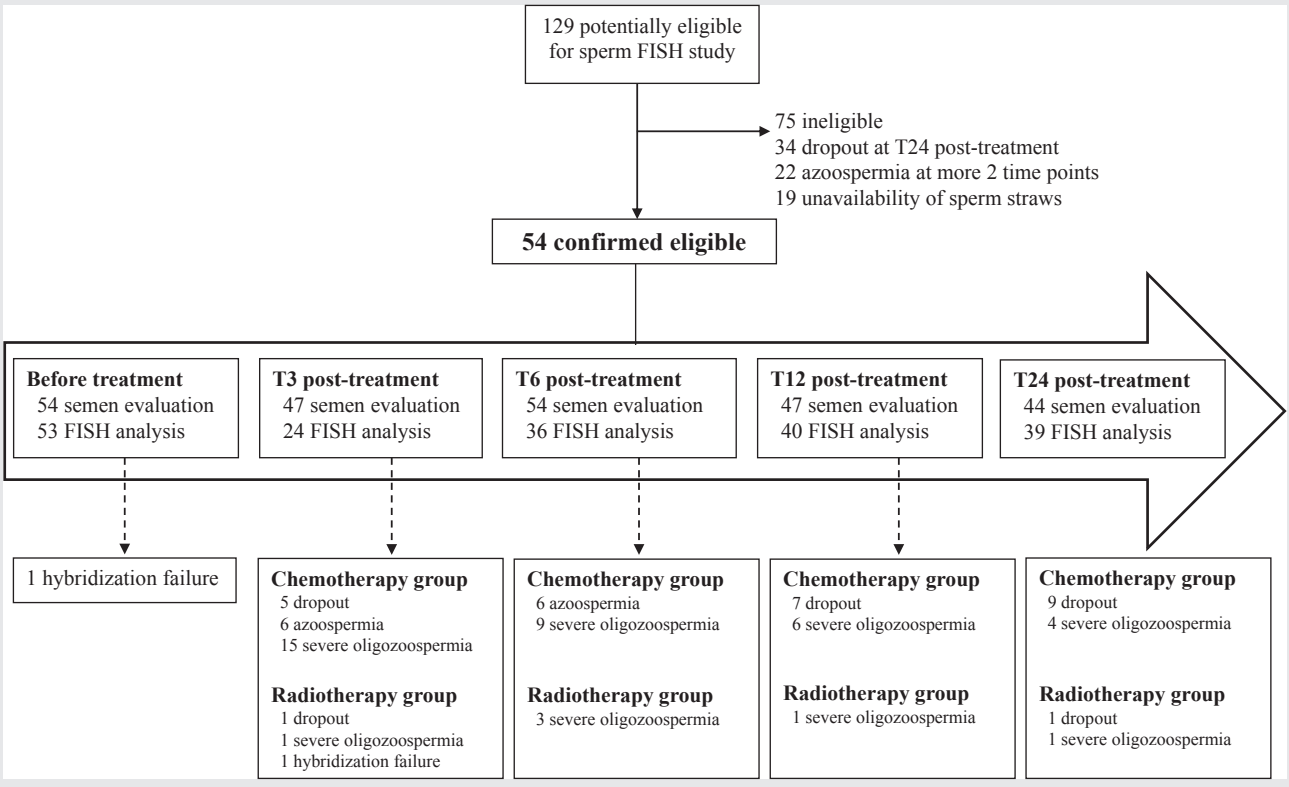
## SUPPLEMENTAL FIGURE 1




An overview of the selection criteria and number of patients enrolled in the study. FISH = fluorescence in situ hybridization; T3, T6, T12, T24 = 3, 6, 12, and 24 months after end of treatment.

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