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REVIEW ARTICLE

# Anticancer chemotherapeutic agents and testicular dysfunction

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**Abstract** The improvement of the survival rates of various cancer patients has resulted in increased focus on the long-term complications of treatment. Most anticancer chemotherapeutic agents are gonadotoxic, and sterility is therefore one of the most common complications for cancer survivors. The degree of gonadal dysfunction induced by anticancer chemotherapeutic agents seems to be drug specific and dose related. Following the development of new chemotherapeutic agents that have high benefit-to-risk ratios, sufficient sperm can be acquired by collection of ejaculated semen after the treatment in relatively many cases, and assisted reproductive techniques enable conceptions with even severe spermatogenesis dysfunction. However, anticancer chemotherapeutic agents have consistently exhibited the potential to induce permanent azoospermia. Cryopreservation of semen, which is currently the only proven successful option for future fertility preservation in male cancer patients, should certainly be recommended before cancer therapy. However, to date, no established effective methods have shown the capability to protect gonadal function from anticancer treatment in prepubertal cancer patients.

**Keywords** Chemotherapy · Gonadal dysfunction · Hematological malignancy · Spermatogenesis · Testicular cancer

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## Introduction

In recent decades, many novel anticancer chemotherapeutic agents have been developed. Various combinations of chemotherapy using many effective new drugs and radiotherapy or operation have dramatically improved the survival rates of cancer patients, especially those with testicular cancer (TC) or hematological malignancies, such as Hodgkin's lymphoma (HL). The 5-year survival rate of TC patients at low risk has reached over 90% [1]. Meanwhile, 80–90% of patients with HL are treatable [2–4]. The high survival rates and the young age of these cancer patients have resulted in increased focus on the long-term complications of treatment. It is well known that many antineoplastic agents are gonadotoxic. Gonadal dysfunction is therefore one of the most common complications for cancer survivors [5].

Spermatogenesis of patients with malignancies may be originally impaired in the pretreatment stage. It has been previously described that over half of all patients with TC had oligozoospermia or azoospermia at the time of cancer diagnosis [6–13]. The risk of TC is 20-fold higher in men with infertility and abnormal semen analysis than in age-matched controls. The relationship between TC and impairment of spermatogenesis can be partially explained by hormone production by the tumor, presence of antisperm antibodies, in situ carcinoma, and cryptorchidism [14]. Patients with TC are at the highest risk of having poor semen quality before cancer treatment [12]. Concerning other malignancies, Sabanegh and Ragheb [15] have described that hematological malignancies and tumors of the central nervous system might directly impair the hypothalamus and the pituitary gland by tumor cell invasion. Malignancies might also result in malnutrition, with deficiencies in various nutrients needed for the maintenance

of adequate gonadal function. Some malignancies are accompanied by periods of fever that negatively influence spermatogenesis. Moreover, gonadal dysfunction might be induced by various cytokines released by tumors. Administration of various anticancer chemotherapeutic agents for treatment additionally induces gonadal dysfunction, and it can result in more deteriorated fertility status [12, 16–18]. Spermatogenesis is a highly complex process regulated by various molecules formed by the hypothalamus, pituitary gland, and various testicular cells, such as germ cells, Sertoli cells (SC), Leydig cells (LC), and peritubular cells [19]. Germ cells, and in particular differentiating spermatogonia, are extremely susceptible to antineoplastic agents because of their proliferation. Although non-proliferative SC and LC usually survive, it has been revealed that acute exposure to antineoplastic agents results in elevated germ cell apoptotic rates and dysfunction of SC and LC [20–23]. In addition, the loss of germ cells has secondary effects on the hypothalamic-pituitary-gonadal axis [24].

Although conflicting results have been published concerning the degree of gonadal dysfunction induced by anticancer chemotherapeutic agents, it seems to be drug specific and dose related [25–35]. The common types that induce spermatogenesis dysfunction are shown in Table 1. The chance of recovery of spermatogenesis and the extent and speed of recovery are also related to the agents used for treatment and the dose received. Moreover, Rowley et al. [36] have suggested that the germinal epithelium of adult testis was more susceptible to damage than that of prepubertal testis. It has been implied that patient age or maturation of the testis at the time of treatment might influence the degree of damage.

**Table 1** The gonadotoxic risk of common anticancer chemotherapeutic agents

| Chemotherapy agent | Type of agent | Risk category |
|---|---|---|
| Cyclophosphamide | Alkylating agents | High risk |
| Ifosfamide | | |
| Procarbazine | | |
| Busulfan | | |
| Melphalan | | |
| Chlorambucil | | |
| Cisplatin | Platinum analogs | Medium risk |
| Carboplatin | | |
| Doxorubicin | Antibiotics | Medium risk |
| Vincristine | Vinca alkaloids | Low risk |
| Vinblastine | | |
| Methotrexate | Antimetabolites | Low risk |
| Mercaptopurine | | |
| Dactinomycin | Antibiotics | Low risk |
| Bleomycin | | |

The ultimate assessment of germinal cell function should be the achievement of fatherhood; however, there are many confounding factors, such as female-related factors, and most studies have therefore focused on semen analysis and biochemical markers of fertility, such as serum FSH levels for exocrine gonadal function or testosterone (T) and LH levels for endocrine function.

## Testicular cancer

Testicular cancer is the most common cancer diagnosis in men between the ages of 15 and 35 years old, with approximately 8,000 cases detected every year in the United States [37]. In the past 15 years, the number of young men diagnosed with TC has almost doubled in Western Europe [38]. The treatment for this malignancy, which is becoming increasingly common, has progressed remarkably, and the cure rates have increased from 25% in the mid-1970s to nearly 80% today [39]. Not only the efficacy but also adverse effects of the treatment for TC have been widely investigated. Cisplatin, a DNA cross-linking and commonly used platinum antineoplastic agent, is distributed evenly throughout the body and can pass through the blood-testis barrier to enter individual cells, where it is hydrolyzed to its reactive form, which binds to intracellular macromolecules, including DNA [40]. Such an effect causes not only cancer cell death, but also impairs spermatogenesis and subsequently leads to azoospermia [41, 42]. Nearly 100% of patients become azoospermic during and immediately after cisplatin chemotherapy [12, 43]. However, cisplatin- or carboplatin-based multi-drug regimens applied in TC have a relatively low risk of causing permanent infertility. Lampe et al. [43] analyzed data concerning 170 patients with TCs who underwent treatment with either cisplatin- or carboplatin-based chemotherapy. Although only 64% of those patients who were normospermic before the chemotherapy remained normospermic in a median of 30 months after completion of the therapy, recovery continued for more than 2 years, with the calculated chance of spermatogenesis at 2 years being 48% and at 5 years 80%. The probability of recovery to a normal sperm count was found to be higher for those men with a normal pretreatment sperm count, in those who received carboplatin- rather than cisplatin-based therapy, and in those treated with fewer than five cycles of chemotherapy. Hansen et al. [17] and Petersen et al. [44] have reported that the risk of post-therapeutic azoospermia increased when the cumulative cisplatin dose was $>600$ mg/m$^2$. However, Ishikawa et al. [30] analyzed long-term gonadal function after high-dose chemotherapy (HDC), and they found no correlation of the dose of drugs with the fertility status after chemotherapy. Ten patients with TC underwent

one or two cycles of HDC (1,250 mg/m$^2$ carboplatin, 15,00 mg/m$^2$ etoposide, and 7.5 g/m$^2$ ifosfamide) after two to four cycles of cisplatin, etoposide, and bleomycin chemotherapy. Spermatogenesis recovered after cessation of HDC in 50% of these patients. Semen analysis in these patients showed the mean sperm concentration and motility at 42.4 ± 10.4 million/ml and 67.2 ± 17.0%, respectively. Two of the patients fathered children 19 and 44 months after HDC.

Kim et al. [45] reported the germinal cell function after treatment by the achievement or not of fatherhood, although, as previously noted, there are few similar studies. They analyzed the patients enrolled in the US Servicemen's Testicular Tumor Environment and Endocrine Determinants (STEED) study and controls using a self-administered questionnaire. Despite expressing greater fertility distress, higher likelihood of fertility testing, and difficulty fathering children, patients who received chemotherapy were more likely to father children than controls (OR 2.24; 95% CI 1.14–4.41). Three of 29 patients who received chemotherapy fathered children via banked sperm. Results did not change significantly when men fathering children through banked sperm were excluded from analysis. They concluded that it is possible that treatment for TC does not permanently affect fertility or, alternatively, that TC survivors attempt to father children with greater persistence or at younger ages than other men.

## Malignant lymphoma and other malignancies

Chemotherapy regimens used for the treatment of HL, especially in advanced stages, are generally more gonadotoxic than those used for non-Hodgkin's lymphoma (NHL) [46, 47]. Concerning the gonadal function after chemotherapy for HL patients, most studies have focused on MVPP (mustine, vinblastine, procarbazine, and prednisolone) or MVPP-like chemotherapy regimes. Not only MVPP [48, 49], but also MVPP-like regimens, such as MOPP (similar to MVPP but with vinblastine replaced by vincristine) [50], ChlVPP (chlorambucil, vinblastine, prednisolone, procarbazine) [51], and COPP (cyclophosphamide, vincristine, procarbazine, prednisolone) [52], contain alkylating chemotherapeutic agents and induce severe gonadal dysfunction. These regimens have been replaced by newer regimens excluding alkylating agents. In patients receiving ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) chemotherapy, 90% returned to having normal sperm counts 12 months after the therapy [53]. In a larger study, Marleen et al. revealed that only 3–8% of patients treated with radiotherapy only or without alkylating chemotherapy [ABVD or EBVP (epirubicin, bleomycin, vinblastine, prednisone)] exhibited an elevated FSH level [54]. In contrast, this value was 60% after chemotherapy containing alkylating agents [MOPP, MOPP/ABV (adriamycin, bleomycin, vinblastine), BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisolone)]. After a median time of 19 months, recovery of fertility occurred in 82% of patients treated with ABVD or EBVP chemotherapy. This proportion was only 30% in those treated with alkylating chemotherapy. The median time to recovery was 27 months. Fosså and Magelssen [55] have revealed that ABVD or its derivatives preserved fertility in 90% of patients without being less effective. The 5-year failure-free survival rate for those receiving ABVD was 61% compared to 50% for those with MVPP [56]. In consideration of the risk and benefit of therapy for HL, alkylating chemotherapy regimens are no longer considered the best therapy for HL; ABVD is the current treatment standard for HL [15, 56, 57].

The effect on the gonadal function of chemotherapy used in the treatment of NHL has also been widely reported. All 71 patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-based chemotherapy temporarily became azoospermic during treatment, but by 5 years post-treatment, 67% had recovered to normospermic levels, with a further 5% being oligozoospermic [32]. Other regimens not containing procarbazine, which is one of the alkylating agents and has been frequently used for HL, have also been shown to be less gonadotoxic. VAPEC-B (vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin) [58], VACOP-B (vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide, and bleomycin), MACOP-B (mustine in place of vinblastine) [59], and VEEP (vincristine, etoposide, epirubicin, and prednisolone) [60] have all been associated with normal post-treatment fertility in the vast majority of men. Although these regimens also contain alkylating agents, less gonadotoxic effects of the chemotherapy for NHL compared with that for HL patients seem to be related to the absence of procarbazine in the regimens [61].

The gonadal function after chemotherapy for various other malignancies has also been analyzed. In patients with lung cancer, SHBG and FSH levels were significantly elevated after 6–9 weeks of chemotherapy consisting of cisplatin and etoposide in nine patients, or vincristine, adriamycin, and cyclophosphamide in three patients [62]. This result suggested that the therapy induced endocrine gonadal dysfunction at least for a short time. Raviv et al. [63] have demonstrated that intravesical therapy with BCG for bladder cancer can have potential adverse effects on spermatogenesis. Concerning the gonadal toxicity of HDC used as conditioning for bone marrow transplantation, severe gonadal dysfunction following the treatment has

been shown by Chatterjee et al. [64]. Thirteen post-pubertal male patients aged 17–25 years were assessed for pituitary–gonadal function 0–3 months prior to and 2–3 months post-bone marrow transplantation for hematological malignancy. Fifty percent had a reduction in testicular volume, and all had azoospermia 2–3 months post-transplantation. In a larger and longer follow-up study, however, among 155 postpubertal men who had received HDC with cyclophosphamide (200 mg/kg) or cyclophosphamide (200 mg/kg) and busulfan (16 mg/kg) for the preparation of bone marrow transplantation for aplastic anemia or hematological malignancies, 75 (48%) recovered gonadal function [65].

## Genetic damage

Cancer treatment has the potential to induce sperm DNA damage. It is well documented for male rodents that chemo- and radiotherapy before mating can cause genetic damage in the germ line [66, 67]. In addition, in humans, an increase in chromosomal abnormalities has been demonstrated several years later for TC patients [68]. Spermon et al. [69] have also revealed that an abnormally high percentage of DNA-damaged sperm was found in TC patients not only before chemotherapy, but also after the therapy, compared with that of controls. Furthermore, in patients with HL, aneuploid frequency in spermatozoa has been found to be increased after chemotherapy and radiotherapy [70]. These damaged, mutated sperm may enhance the risk of birth defects; however, in terms of genetic diseases or tumors among the offspring of cancer survivors, no significant increase in structural or functional abnormalities among children fathered by men who had undergone cytotoxic therapy before conception has been detected [71–74]. There are no differences in genetic birth defects, altered sex ratios, or birth weight effects in offspring of cancer survivors compared with those in the general population. No reported increased risk for congenital malformation was found [75]. On the evidence thus far, it is therefore reasonable to conclude that patients treated with cytotoxic chemotherapy who remain fertile are not at increased risk of fathering children with genetic abnormalities [74]. It has also been emphasized that, aside from hereditary genetic syndromes, there is no evidence that a history of cancer, cancer therapy, or fertility interventions increases the risk of congenital malformations in offspring, as described in the statement of the American Society of Clinical Oncology (ASCO) [76]. However, given the various biases, such as the limited number of investigated children born to treated patients and potential issues of modern reproductive techniques, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), which bypass natural sperm selection mechanisms, correct evaluation of reproductive risks remains unclear. The incidence of genetic diseases in offspring might increase with the use of IVF/ICSI using sperm from cancer patients treated with cytotoxic therapy. Therefore, long-term follow-up of these offspring must be continued in the future.

## Preservation of fertility

Many efforts have been made to preserve female gonadal function for women undergoing cytotoxic therapy. In the analysis of pharmacological interventions such as using GnRH analogs (GnRHa), among others, for fertility preservation during chemotherapy for female cancer or system lupus erythematosus (SLE) patients, Irit et al. revealed the possibility that GnRHa might be effective in reducing amenorrhea rates and in increasing pregnancy rates in the review [77]. GnRHa has also shown promising results in male rodents; however, no effective established pharmacological methods have shown the capability to protect gonadal function from anticancer treatment in a clinical situation in male patients. Even though, in most cases, sufficient sperm can be acquired by collection of ejaculated semen after the treatment and assisted reproductive techniques (ART) enable conceptions with even severe oligozoospermia, anticancer chemotherapeutic agents have consistently exhibited the potential to induce permanent azoospermia. Therefore, cryopreservation of semen, which is currently the only proven successful option for future fertility preservation in male cancer patients, should certainly be recommended before cancer therapy. In guidelines from the American Society of Reproductive Medicine (ASRM), it is emphasized that "physicians should inform cancer patients about options for fertility preservation and future reproduction prior to treatment" [78]. In previous data, only 47% of American oncologists were found to routinely refer cancer patients of childbearing age to a reproductive specialist [79], and the actual semen cryopreservation rate before therapy was only 21% in TC patients [80].

For prepubertal patients, semen cryopreservation is not available. At present, the only theoretical option for preservation of fertility in these boys is the preservation of the spermatogonial stem cells for autologous intratesticular stem cell transplantation [81]. There are many animal studies focused on this method, and successful spermatogonial stem cell transplantation has been reported in a rodent model [82]. To increase the efficacy of this transplantation technique, in vitro culture systems of spermatogonial stem cells have been reported [83, 84]. In vitro differentiation of these stem cells and the use of derived spermatids for ICSI

could also be an option to restore fertility [85]. Although these methods are promising, there are some problems, such as the contamination of malignant tumor cells in the transplanted testicular tissue. It is uncertain that these techniques will actually be put into practice in humans, and further investigations are necessary.

## References


1. Mead GM, Stenning SP. The International Germ Cell Consensus Classification: a new prognostic factor-based staging classification for metastatic germ cell tumours. Clin Oncol (R Coll Radiol). 1997;9:207–9.
2. Sieber M, Rueffer U, Josting A, Diehl V. Treatment of Hodgkin's disease: current strategies of the German Hodgkin's Lymphoma Study Group. Ann Oncol. 1999;10:23–9.
3. Raemaekers J, Kluin-Nelemans H, Teodorovic I, Meerwaldt C, Noordijk E, Thomas J, Glabbeke M, Henry-Amar M, Carde P, The Achievements of the EORTC Lymphoma Group. European Organisation for Research and Treatment of Cancer. Eur J Cancer. 2002;38:S107–13.
4. Chen YT, Zheng T, Chou MC, Boyle P, Holford TR. The increase of Hodgkin's disease incidence among young adults. Experience in Connecticut, 1935–1992. Cancer. 1997;79:2209–18.
5. DeSantis M, Albrecht W, Höltl W, Pont J. Impact of cytotoxic treatment on long-term fertility in patients with germ-cell cancer. Int J Cancer. 1999;83:864–5.
6. Fosså SD, Aass N, Molne K. Is routine pre-treatment cryopreservation of semen worthwhile in the management of patients with testicular cancer? Br J Urol. 1989;64:524–9.
7. Scheiber K, Bartsch G. Exocrine and endocrine functions in patients with testicular tumors. Prog Clin Biol Res. 1985;203:715–24.
8. Carroll PR, Whitmore WF Jr, Herr HW, Morse MJ, Sogani PC, Bajorunas D, Fair WR, Chaganti RS. Endocrine and exocrine profiles of men with testicular tumors before orchiectomy. J Urol. 1987;137:420–3.
9. Horwich A, Nicholls EJ, Hendry WF. Seminal analysis after orchiectomy in stage I teratoma. Br J Urol. 1988;62:79–81.
10. Nijman JM, Schraffordt Koops H, Kremer J, Willemse PH, Sleijfer DT, Oldhoff J. Fertility and hormonal function in patients with a nonseminomatous tumor of the testis. Arch Androl. 1985;14:239–46.
11. Hansen PV, Trykker H, Andersen J, Helkjaer PE. Germ cell function and hormonal status in patients with testicular cancer. Cancer. 1989;64:956–61.
12. Drasga RE, Einhorn LH, Williams SD, Patel DN, Stevens EE. Fertility after chemotherapy for testicular cancer. J Clin Oncol. 1983;1:179–83.
13. Dearnaley DP, Horwich A, A'Hern R, Nicholls J, Jay G, Hendry WF, Peckham MJ. Combination chemotherapy with bleomycin, etoposide and cisplatin (BEP) for metastatic testicular teratoma: long-term follow-up. Eur J Cancer. 1991;27:684–91.
14. Raman JD, Nobert CF, Goldstein M. Increased incidence of testicular cancer in men presenting with infertility and abnormal semen analysis. J Urol. 2005;174:1819–22.
15. Sabanegh ES Jr, Ragheb AM. Male fertility after cancer. Urology. 2009;73:225–31.
16. Hendry WF, Stedronska J, Jones CR, Blackmore CA, Barrett A, Peckham MJ. Semen analysis in testicular cancer and Hodgkin's disease: pre- and post-treatment findings and implications for cryopreservation. Br J Urol. 1983;55:769–73.
17. Hansen SW, Berthelsen JG, von der Maase H. Long-term fertility and Leydig cell function in patients treated for germ cell cancer with cisplatin, vinblastine, and bleomycin versus surveillance. J Clin Oncol. 1990;8:1695–8.
18. Howell SJ, Radford JA, Ryder WD, Shalet SM. Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency. J Clin Oncol. 1999;17:1493–8.
19. Huleihel M, Lunenfeld E. Regulation of spermatogenesis by paracrine/autocrine testicular factors. Asian J Androl. 2004;6:259–68.
20. Brennemann W, Stoffel-Wagner B, Helmers A, Mezger J, Jäger N, Klingmüller D. Gonadal function of patients treated with cisplatin based chemotherapy for germ cell cancer. J Urol. 1997;158:844–50.
21. Monsees TK, Franz M, Gebhardt S, Winterstein U, Schill WB, Hayatpour J. Sertoli cells as a target for reproductive hazards. Andrologia. 2000;32:239–46.
22. Carreau S, Papadopoulos V, Boujrad N, Drosdowsky MA. Effects of cisplatin and bleomycin on mature rat Leydig cell testosterone production. J Steroid Biochem. 1988;30:449–51.
23. Stumpp T, Freymüller E, Miraglia SM. Sertoli cell function in albino rats treated with etoposide during prepubertal phase. Histochem Cell Biol. 2006;126:353–61.
24. Wang J, Galil KA, Setchell BP. Changes in testicular blood flow and testosterone production during aspermatogenesis after irradiation. J Endocrinol. 1983;98:35–46.
25. Amelar RD, Dubin L, Hotchkiss RS. Restoration of fertility following unilateral orchiectomy and radiation therapy for testicular tumors. J Urol. 1971;106:714–8.
26. Arai Y, Kawakita M, Okada Y, Yoshida O. Sexuality and fertility in long-term survivors of testicular cancer. J Clin Oncol. 1997;15:1444–8.
27. Hansen PV, Glavind K, Panduro J, Pedersen M. Paternity in patients with testicular germ cell cancer: pretreatment and post-treatment findings. Eur J Cancer. 1991;27:1385–9.
28. Herr HW, Bar-Chama N, O'Sullivan M, Sogani PC. Paternity in men with stage I testis tumors on surveillance. J Clin Oncol. 1998;16:733–4.
29. Böhlen D, Burkhard FC, Mills R, Sonntag RW, Studer UE. Fertility and sexual function following orchiectomy and 2 cycles of chemotherapy for stage I high risk nonseminomatous germ cell cancer. J Urol. 2001;165:441–4.
30. Ishikawa T, Kamidono S, Fujisawa M. Fertility after high-dose chemotherapy for testicular cancer. Urology. 2004;63:137–40.
31. Pont J, Albrecht W. Fertility after chemotherapy for testicular germ cell cancer. Fertil Steril. 1997;68:1–5.
32. Pryzant RM, Meistrich ML, Wilson G, Brown B, McLaughlin P. Long-term reduction in sperm count after chemotherapy with and without radiation therapy for non-Hodgkin's lymphomas. J Clin Oncol. 1993;11:239–47.
33. Meistrich ML, Chawla SP, Da Cunha MF, Johnson SL, Plager C, Papadopoulos NE, Lipshultz LI, Benjamin RS. Recovery of sperm production after chemotherapy for osteosarcoma. Cancer. 1989;63:2115–23.
34. da Cunha MF, Meistrich ML, Fuller LM, Cundiff JH, Hagemeister FB, Velasquez WS, McLaughlin P, Riggs SA, Cabanillas FF, Salvador PG. Recovery of spermatogenesis after treatment for Hodgkin's disease: limiting dose of MOPP chemotherapy. J Clin Oncol. 1984;2:571–7.
35. Watson AR, Rance CP, Bain J. Long term effects of cyclophosphamide on testicular function. Br Med J. 1985;291:1457–60.
36. Rowley MJ, Leach DR, Warner GA, Heller CG. Effect of graded doses of ionizing radiation on the human testis. Radiat Res. 1974;59:665–78.

37. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin. 2007;57:43–66.
38. Bray F, Richiardi L, Ekbom A, Pukkala E, Cuninkova M, Møller H. Trends in testicular cancer incidence and mortality in 22 European countries: continuing increases in incidence and declines in mortality. Int J Cancer. 2006;118:3099–111.
39. Einhorn LH, Donohue J. Cis-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med. 1977;87:293–8.
40. Leng M, Brabec V. DNA adducts of cisplatin, transplatin and platinum-intercalating drugs. IARC Sci Publ. 1994;339–48.
41. Schilsky RL, Lewis BJ, Sherins RJ, Young RC. Gonadal dysfunction in patients receiving chemotherapy for cancer. Ann Intern Med. 1980;93:109–14.
42. Loehrer PJ, Einhorn LH. Drugs five years later. Cisplatin. Ann Intern Med. 1984;100:704–13.
43. Lampe H, Horwich A, Norman A, Nicholls J, Dearnaley DP. Fertility after chemotherapy for testicular germ cell cancers. J Clin Oncol. 1997;15:239–45.
44. Petersen PM, Hansen SW, Giwercman A, Rørth M, Skakkebaek NE. Dose-dependent impairment of testicular function in patients treated with cisplatin-based chemotherapy for germ cell cancer. Ann Oncol. 1994;5:355–8.
45. Kim C, McGlynn KA, McCorkle R, Zheng T, Erickson RL, Niebuhr DW, Ma S, Zhang Y, Bai Y, Dai L, Graubard BI, Kilfoy B, Barry KH, Zhang Y. Fertility among testicular cancer survivors: a case–control study in the U.S. J Cancer Surviv. 2010;4:266–73.
46. Krawczuk-Rybak M, Solarz E, Gadomski J, Matysiak M, Wołczyński S. Spermato- and steroidogenesis in young men treated for non-Hodgkin's and Hodgkin's lymphoma during childhood. Med Wieku Rozwoj. 2006;10:623–30.
47. Kiserud CE, Fosså A, Bjøro T, Holte H, Cvancarova M, Fosså SD. Gonadal function in male patients after treatment for malignant lymphomas, with emphasis on chemotherapy. Br J Cancer. 2009;100:455–63.
48. Chapman RM, Sutcliffe SB, Rees LH, Edwards CR, Malpas JS. Cyclical combination chemotherapy and gonadal function. Retrospective study in males. Lancet. 1979;1:285–9.
49. Whitehead E, Shalet SM, Blackledge G, Todd I, Crowther D, Beardwell CG. The effects of Hodgkin's disease and combination chemotherapy on gonadal function in the adult male. Cancer. 1982;49:418–22.
50. Viviani S, Santoro A, Ragni G, Bonfante V, Bestetti O, Bonadonna G. Gonadal toxicity after combination chemotherapy for Hodgkin's disease. Comparative results of MOPP vs ABVD. Eur J Cancer Clin Oncol. 1985;21:601–5.
51. Mackie EJ, Radford M, Shalet SM. Gonadal function following chemotherapy for childhood Hodgkin's disease. Med Pediatr Oncol. 1996;27:74–8.
52. Charak BS, Gupta R, Mandrekar P, Sheth NA, Banavali SD, Saikia TK, Gopal R, Dinshaw KA, Advani SH. Testicular dysfunction after cyclophosphamide–vincristine–procarbazine–prednisolone chemotherapy for advanced Hodgkin's disease. A long-term follow-up study. Cancer. 1990;65:1903–6.
53. Tal R, Botchan A, Hauser R, Yogev L, Paz G, Yavetz H. Follow-up of sperm concentration and motility in patients with lymphoma. Hum Reprod. 2000;15:1985–8.
54. van der Kaaij MA, Heutte N, Le Stang N, Raemaekers JM, Simons AH, Carde P, Noordijk EM, Fermé C, Thomas J, Eghbali H, Kluin-Nelemans HC, Henry-Amar M, European Organisation for Research and Treatment of Cancer: EORTC Lymphoma Group, Groupe d'Etude des Lymphomes de l'Adulte. Gonadal function in males after chemotherapy for early-stage Hodgkin's lymphoma treated in four subsequent trials by the European Organisation for Research and Treatment of Cancer: EORTC Lymphoma Group and the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol. 2007;25:2825–32.
55. Fosså SD, Magelssen H. Fertility and reproduction after chemotherapy of adult cancer patients: malignant lymphoma and testicular cancer. Ann Oncol. 2004;15:259–65.
56. Canellos GP, Anderson JR, Propert KJ, Nissen N, Cooper MR, Henderson ES, Green MR, Gottlieb A, Peterson BA. Chemotherapy of advanced Hodgkin's disease with MOPP, ABVD, or MOPP alternating with ABVD. N Engl J Med. 1992;327:1478–84.
57. Duggan DB, Petroni GR, Johnson JL, Glick JH, Fisher RI, Connors JM, Canellos GP, Peterson BA. Randomized comparison of ABVD and MOPP/ABV hybrid for the treatment of advanced Hodgkin's disease: report of an intergroup trial. J Clin Oncol. 2003;21:607–14.
58. Radford JA, Clark S, Crowther D, Shalet SM. Male fertility after VAPEC-B chemotherapy for Hodgkin's disease and non-Hodgkin's lymphoma. Br J Cancer. 1994;69:379–81.
59. Müller U, Stahel RA. Gonadal function after MACOP-B or VACOP-B with or without dose intensification and ABMT in young patients with aggressive non-Hodgkin's lymphoma. Ann Oncol. 1993;4:399–402.
60. Hill M, Milan S, Cunningham D, Mansi J, Smith I, Catovsky D, Gore M, Zulian G, Selby P, Horwich A. Evaluation of the efficacy of the VEEP regimen in adult Hodgkin's disease with assessment of gonadal and cardiac toxicity. J Clin Oncol. 1995;13:387–95.
61. Bokemeyer C, Schmoll HJ, van Rhee J, Kuczyk M, Schuppert F, Poliwoda H. Long-term gonadal toxicity after therapy for Hodgkin's and non-Hodgkin's lymphoma. Ann Hematol. 1994;68:105–10.
62. Aasebø U, Slørdal L, Aanderud S, Aakvaag A. Chemotherapy and endocrine function in lung cancer. Acta Oncol. 1989;28:667–9.
63. Raviv G, Pinthus JH, Shefi S, Mor Y, Kaufman-Francis K, Levron J, Weissenberg R, Ramon J, Madgar I. Effects of intravesical chemotherapy and immunotherapy on semen analysis. Urology. 2005;65:765–7.
64. Chatterjee R, Mills W, Katz M, McGarrigle HH, Goldstone AH. Germ cell failure and Leydig cell insufficiency in post-pubertal males after autologous bone marrow transplantation with BEAM for lymphoma. Bone Marrow Transplant. 1994;13:519–22.
65. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, Doney K, Storb R, Sullivan K, Witherspoon R, Appelbaum FR. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87:3045–52.
66. Russell LB, Shelby MD. Tests for heritable genetic damage and for evidence of gonadal exposure in mammals. Mutat Res. 1985;154:69–84.
67. Lähdetie J. Induction and survival of micronuclei in rat spermatids. Comparison of two meiotic micronucleus techniques using cyclophosphamide. Mutat Res. 1988;203:47–53.
68. Genescà A, Benet J, Caballín MR, Miró R, Germà JR, Egozcue J. Significance of structural chromosome aberrations in human sperm: analysis of induced aberrations. Hum Genet. 1990;85:495–9.
69. Spermon JR, Ramos L, Wetzels AM, Sweep CG, Braat DD, Kiemeney LA, Witjes JA. Sperm integrity pre- and post-chemotherapy in men with testicular germ cell cancer. Hum Reprod. 2006;21:1781–6.
70. Monteil M, Rousseaux S, Chevret E, Pelletier R, Cozzi J, Sèle B. Increased aneuploid frequency in spermatozoa from a Hodgkin's disease patient after chemotherapy and radiotherapy. Cytogenet Cell Genet. 1997;76:134–8.
71. Meistrich ML, Byrne J. Genetic disease in offspring of long-term survivors of childhood and adolescent cancer treated with

potentially mutagenic therapies. Am J Hum Genet. 2002;70: 1069–71.
72. Chow EJ, Kamineni A, Daling JR, Fraser A, Wiggins CL, Mineau GP, Hamre MR, Severson RK, Drews-Botsch C, Mueller BA. Reproductive outcomes in male childhood cancer survivors: a linked cancer-birth registry analysis. Arch Pediatr Adolesc Med. 2009;163:887–94.
73. Robbins WA. Cytogenetic damage measured in human sperm following cancer chemotherapy. Mutat Res. 1996;355:235–52.
74. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. J Natl Cancer Inst Monogr. 2005;34:12–7.
75. Dohle GR. Male infertility in cancer patients: review of the literature. Int J Urol. 2010;17:327–31.
76. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, Beck LN, Brennan LV, Oktay K, American Society of Clinical Oncology. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24:2917–31.
77. Ben-Aharon I, Gafter-Gvili A, Leibovici L, Stemmer SM. Pharmacological interventions for fertility preservation during chemotherapy: a systematic review and meta-analysis. Breast Cancer Res Treat. 2010;122:803–11.
78. Ethics Committee of the American Society for Reproductive Medicine. Fertility preservation and reproduction in cancer patients. Fertil Steril. 2005;83:1622–8.
79. Quinn GP, Vadaparampil ST, Lee JH, Jacobsen PB, Bepler G, Lancaster J, Keefe DL, Albrecht TL. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. J Clin Oncol. 2009;27:5952–7.
80. Bastit P, Bisson JP. Autoconservation before sterilising treatment in the CECOS from 1973 to 1988. Contracept Fertil Sex. 1989;17:617–8.
81. Geens M, Goossens E, De Block G, Ning L, Van Saen D, Tournaye H. Autologous spermatogonial stem cell transplantation in man: current obstacles for a future clinical application. Hum Reprod Update. 2008;14:121–30.
82. Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. Proc Natl Acad Sci USA. 1994;91:11303–7.
83. Kanatsu-Shinohara M, Ogonuki N, Inoue K, Miki H, Ogura A, Toyokuni S, Shinohara T. Long-term proliferation in culture and germline transmission of mouse male germline stem cells. Biol Reprod. 2003;69:612–6.
84. Kubota H, Avarbock MR, Brinster RL. Culture conditions and single growth factors affect fate determination of mouse spermatogonial stem cells. Biol Reprod. 2004;71:722–31.
85. Feng LX, Chen Y, Dettin L, Pera RA, Herr JC, Goldberg E, Dym M. Generation and in vitro differentiation of a spermatogonial cell line. Science. 2002;297:392–5.