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The Effects of Chemotherapy and Radiotherapy on Spermatogenesis in Humans

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Abstract

Treatment of cancer with chemo- or radiotherapy causes reduction of sperm counts often to azoospermic levels which may persist for several years or be permanent. The time course of declines in sperm count can be predicted by the sensitivity of germ cells, with differentiating spermatogonia being most sensitive, and the known kinetics of recovery. Recovery from oligo- or azoospermia is more variable and depends on whether there is killing of stem cells and alteration of the somatic environment that normally supports differentiation of stem cells. Of the cytotoxic therapeutic agents, radiation and most alkylating drugs are the most potent at producing long-term azoospermia. Most of the newer biological targeted therapies, except those used to target radioisotopes or toxins to cells, seem to have only modest effects, mostly on the endocrine aspects of the male reproductive system, however their effects when used in combination with cytotoxic agents have not been well studied.

Keywords

Cancer; chemotherapy; radiotherapy; biological targeted therapy; spermatogenesis

Chemotherapy and radiotherapy used in cancer treatment may result in temporary, long-term, or permanent gonadal toxicity in male patients. These cytotoxic treatments have a significant impact on a patient's ability to have his own biological offspring, which is of particular concern to cancer patients within child-bearing ages. In many cases, the ability to achieve a pregnancy may be temporarily diminished, forcing the patient to delay parenthood. In some cases sterility may be permanent. Loss of fertility potential can be devastating to a patient, especially in light of other physical and emotional turmoil entailed in cancer treatment.

This review will cover the basic principles explaining time course of reduction in sperm counts after initiation of chemo- or radiation therapy, the variable time course of recovery of sperm count, and the differential effects and effectiveness of different doses of radiation and different chemotherapeutic and biological targeted agents. Sperm count data from the author's laboratory were collected under protocol LAB92-028 approved by the IRB of M.D.

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Anderson Cancer Center. Studies in rodents will be included since they have elucidated some basic patterns that help us understand the kinetics of recovery of spermatogenesis in human males and they provide guidance as to the possible effects that new agents might have on the human testis. However, there are differences in spermatogenesis between rodents and primates, particularly in stem spermatogonial populations (1), so that results obtained in rodents may or may not be translatable to humans.

Time Course of Sperm Count Reduction

The time course of the loss of sperm production is based on the fact that the rapidly dividing differentiating spermatogonia are much more sensitive to killing by radiation (Fig. 1) and nearly all cytotoxic chemotherapeutic drugs than are the later stage germ cells (2–4), and that the kinetics of spermatogenesis is fixed and unchanged after cytotoxic treatments (5). Thus the surviving later stage germ cells progress along their differentiation pathway, but are not replaced by new cells that would have been derived from the differentiating spermatogonia that had been killed. Hence there is a progressive loss of the more mature differentiating cells in a process called maturation depletion.

As noted in Fig. 1 radiation markedly reduces the numbers of spermatocytes up to the leptotene (L) stage at 2 weeks after exposure; marked depletion of pachytene spermatocytes (P) occurs as expected by 25 days after irradiation (3). Dramatic declines in ejaculated sperm counts occur at about 10 weeks after irradiation (6), as predicted considering the epididymal and vas transit time of about 12 days. However, azoospermia is not achieved until 18 weeks (7), possibly due to the survival of a few differentiating spermatogonia and epididymal sperm storage.

Many combination chemotherapies used in the treatment of cancer also produce declines with similar time courses. Because of some toxicity to later stage germ cells, 10–100-fold declines in sperm count can occur within 1 to 2 months, but azoospermia usually does not occur until after 2 months when the sperm would be derived from differentiating spermatogonia (Fig. 2) (8, 9). Although sperm are being produced for several months after the start of cytotoxic therapies, pregnancies should be avoided during this period because of a higher risk of genetic damage to the sperm as will be discussed elsewhere (Brannigan, this issue, 2013)

Time Course of Recovery

The eventual recovery of sperm production depends on the survival of the spermatogonial stem cells, the regeneration of their numbers, and their ability to differentiate. In rodents, after treatment with chemotherapeutic agents that do not kill stem cells, there is only a transient decline in sperm production resulting from killing the differentiating spermatogonia followed by a full recovery of sperm production to control levels corresponding to the time required for stem cells to become sperm (10), which in mice is about 45 days. In mice even if some of the stem cells are killed, the surviving spermatogonial stem cells not only regenerate their numbers by self-renewal, but they rapidly enter the differentiation pathway resulting in some sperm being produced by 8 weeks. The subsequent recovery to a maximal level of sperm production, which is often below control levels, is gradual and requires 20–40 weeks after exposure (11, 12). In contrast in rats after treatment with cytotoxic agents that produce stem cell killing, there also appears to be a dose-dependent block in differentiation of the surviving stem spermatogonia (13). This block is a result of alteration of the somatic environment as the prevention of spermatogonial differentiation depends on the action of testosterone and FSH on somatic cells (14) and the failure of the cytotoxic-agent treated adult rat testis to support differentiation of normal transplanted spermatogonia (15).

In humans as in rodents, following treatment with chemotherapy agents that do not kill stem spermatogonia, there is usually a return of normal sperm count (Fig. 2A) and potential fertility in many individuals within 12 weeks after the cessation of chemotherapy (9, 16).

However, many combination antineoplastic regimens include treatment with radiation or chemotherapy drugs (e.g. alkylating agents) that kill stem cells. Even moderate doses of these agents corresponding to partial stem cell killing will produce azoospermia that lasts much longer than the 12 weeks predicted by Fig. 1. In many cases there is an eventual recovery of sperm production. Since the underlying testicular events leading to this sequence of events have been best studied after single doses of radiation, that toxicant will be discussed in detail, but the principles apply to chemotherapy as well.

Although radiation usually acts by killing cells immediately by apoptosis or when they attempt proliferation or division, the reduction of the numbers of type A spermatogonia to their minimum levels after single radiation doses between 0.2 and 4 Gy does not occur rapidly but rather progressively over about 21 weeks (3, 6, 7). The reasons for this gradual decline are not known but it may be speculated in part that some of the non-cycling A stem spermatogonial population only express the lethal damage when they are recruited into cycle. Also it has been noted that the differentiation of spermatogonia to spermatocytes is reduced during this time (17), similar to the phenomenon observed in rats and may indicate somatic damage or at least altered signaling from somatic cells.

The numbers of type A spermatogonia begin to increase after about 21 weeks indicating that self-renewal exceeds cell loss. The ability to differentiate to spermatocytes and later stages increases after this time. The initiation of sperm count recovery depends on the dose of radiation and it begins at 7 months after irradiation with a single dose of 1 Gy but takes 24 months after irradiation with 6 Gy (3). Sperm count progressively recovers but it requires about 2 years to reach pre-irradiation levels after a single dose of 1 Gy (17) and longer after higher doses of irradiation (7).

High doses of radiation can result in permanent azoospermia, likely by killing all of the spermatogonial stem cells. For example, after single doses of about 10 Gy, only about 15% of patients recover sperm count or fertility, although these results may be affected by the fact that these patients also received cyclophosphamide, which has some gonadal toxicity as well (18, 19).

It should be noted that the responses to doses given above are for single doses of radiation, which has been the best studied. However the fractionated radiation used in the treatment of cancer (often given in doses over 3–4 weeks) causes greater delays in spermatogenic recovery and lower total doses are required to cause permanent azoospermia (17). For example, a total gonadal dose of more than 2.5 Gy of fractionated radiation generally produces permanent azoospermia (20), whereas doses >6 Gy given as a single exposure are required.

The kinetics of recovery of spermatogenesis after modest doses of radiation that kills some stem cells (Fig. 3A) is much more delayed than that after the chemotherapy regimen that does not kill stem cells (Fig. 2A). Recovery is not noted until 9 months after the end of radiotherapy with gonadal doses in the 0.5–0.8 dose range. Even greater delays in recovery are observed after a dose of about 1.7 Gy (Fig. 3B), in which case recovery is not initiated until between 14–26 months after treatment. The absolute azoospermia for 14 months in this patient receiving 1.7 Gy, even though there must be surviving stem cells as evidenced by the subsequent recovery of spermatogenesis, could be due to the proposed concept that the type A spermatogonia do not reinitiate differentiation until their population is regenerated (7) and/or to the fact that when there are few sperm being produced in the testes, those sperm do

not survive epididymal transport and consequently do not reach the ejaculate (21). It is also possible that there is some damage to or alteration in signaling from the somatic cells that limits the spermatogonial differentiation, as had been observed in the rat, and in human it is slowly reversible.

After treatment with chemotherapy agents that kill stem cells, the same phenomena are observed. Patients may be azoospermic for several years and then sometimes by recover spermatogenesis. Examples of such recovery in 5 lymphoma patients, who were azoospermic for 2 to 5 years, are shown in Figure 4A. The recovery is almost always progressive and significant subsequent declines in sperm counts are rarely observed. Many recover to normospermic levels, although some may reach a plateau at oligospermia. Plots of the time course and extent of recovery for groups of patients (Fig. 4B) reveal that for this regimen when the dose of cyclophosphamide is low, recovery to normospermia begins at about 1 year but 5 years are required for 70% of patients to recover. With higher cyclophosphamide doses recovery is rare; most of the patients who do not recover to normospermia are in fact permanently azoospermic. Various studies have shown the probability of recovery is highest within the first 2 years after treatment, but is still possible to 5 years. After that recovery is rare, although there is a case report of recovery after 20 years (25).

However, even when a patient's ejaculate is azoospermic after cytotoxic cancer therapy, it is possible that some sperm are being produced in the testis. Spermatozoa were retrieved from the testes by microdissection testicular sperm extraction (TESE) in 37% of patients who were azoospermic after chemotherapy (28). Studies have shown that when the human testis contains fewer than 3–4 million sperm, these sperm do not survive epididymal transit and do not reach the ejaculate (21) (P.N. Schlegel, personal communication). The probability of retrieving sperm by TESE is related to the presence of residual hypospermatogenesis in the testis and is more likely when patients were not treated with alkylating agents (successful in 46% of patients) than when they were treated with these agents (success rate of only 21%), which are toxic to the stem cells and/or somatic environment.

Effects of Different Classes of Cytotoxic Agents

In addition to radiation, certain chemotherapeutic agents are toxic to stem cells and produce prolonged azoospermia as individual agents or as the main active agent in combinations. These drugs are limited to most alkylating agents, and cisplatin, which acts similarly to alkylating agents in its ability to cross-link DNA (Table 1). The nitrosoureas (BCNU, CCNU), which also alkylate the DNA, are primarily used in treatment of pediatric tumors and also cause prolonged or permanent azoospermia. Among the alkylating agents that have only been evaluated in combinations, it is very likely that busulfan is most highly sterilizing, as it is very effective as a single agent in killing spermatogonial stem cells in rodents and monkeys (29).

The duration and permanence of the induced azoospermia depends on the dose of the cytotoxic agent and additive effects of different agents. For example when cyclophosphamide is given as a single agent, doses of 19 g/M² are required for prolonged azoospermia (8). However, in the CY(V)ADIC combination, doses >7.5 g/M² result in permanent azoospermia in nearly all patients (Fig. 4B). When the cyclophosphamide dose in the CY(V)ADIC regimen is less than <7.5 g/M², sperm production recovers in 70% of patients. Comparison of different regimens have shown that it is most likely the Adriamycin (median dose of 77 mg/M² in these patients) that produced the additive toxicity.

Some agents, including the anthracyclines (e.g. Adriamycin), microtubule inhibitors (e.g. vinblastine), and select antimetabolites (e.g. cytarabine) do not produce prolonged

azoospermia if not given with more highly gonadotoxic agents listed above, but can have additive effects when given with these agents (31). In addition, many other chemotherapeutic drugs only cause temporary reductions in sperm counts, as most of them kill differentiating spermatogonia, but do not appreciably affect the stem cells or their subsequent differentiation. Such agents include topoisomerase inhibitors (amsacrine, daunorubicin, mitoxantrone), nucleoside analogs (thioguanine, fludarabine, 6-mercaptopurine), metabolic and synthesis inhibitors (methotrexate, and 5-fluorouracil), microtubule-targeted drugs (vincristine, taxanes), inducers of single strand DNA breaks (bleomycin), corticosteroids (prednisone), and one alkylating agent (dacarbazine). Currently there is no evidence that they have significant additive effects in conjunction with highly sterilizing agents.

Biological Targeted Therapies

Biological or targeted therapies that often involve small molecule inhibitors or monoclonal antibodies have recently gained wide usage in the treatment of cancer. These include of tyrosine kinase growth factor receptor inhibitors, antiangiogenic agents that bind VEGF or block its receptor, mTOR (involved in growth signaling) inhibitors, histone deacetylase (HDAC) inhibitors, retinoids, proteasome inhibitors, immunomodulating (suppressive or stimulatory) agents, and tumor-specific monoclonal antibodies delivering toxins. Two main differences between the cytotoxic therapies and many of the biological targeted therapies is that the former are designed to ideally kill all the tumor cells during intense courses that are given over several months whereas the latter are often just cytostatic and must be given continuously, even for many years. Studies of the effects of these agents on male reproductive system have been limited.

Among these agents, imatinib (Gleevec®), a successfully-used tyrosine kinase inhibitor of the Bcr-Abl oncogene, KIT-receptor, and PDGF-receptor, has been most studied for its action on the male reproductive system. Effects on spermatogenesis could be expected since PDGFR has an essential role in gonocyte migration and Leydig cell development, whereas KIT-receptor has important roles in spermatogonial differentiation and Leydig cell function. However, imatinib treatment of adult rodents has only shown modest or no effects on spermatogenesis or male fertility (32, 33). In neonatal rodents, imatinib partially inhibits the migration of gonocytes to the basement membrane of tubules to form the stem spermatogonia (34, 35). Nevertheless, the stem spermatogonia that do form eventually repopulate the testis so that there are only marginal decreases in adult testis weights and fertility. Similarly in humans, men on treatment with imatinib do produce normal pregnancies and offspring (36, 37), although there may be some effects on spermatogenesis. One study reported a decline in sperm count to <1 million/ml (38) although another study reported normal sperm counts (39). Endocrine functions are indeed affected by imatinib as some patients develop gynecomastia associated with low levels of testosterone, likely caused by decreased Leydig cell function (40). Imatinib given during puberty has more severe effects as this is the time the adult stem spermatogonia and Leydig cells are being formed; indeed gynecomastia, low sperm count, and increased inhibin B/FSH ratio, also indicative of spermatogenic failure, have been observed during imatinib treatment during puberty (41, 42). Other tyrosine kinase inhibitors, dasatinib and sunitinib, have also been reported to induce gynecomastia.

The reproductive effects of mTOR (mammalian target of rapamycin) inhibitors currently used in cancer therapy have not yet been studied. Multiple studies of the prototype drug, rapamycin (sirolimus) in humans have shown that it decreases testosterone levels with elevated LH (43). FSH was generally elevated, indicating spermatogenic dysfunction; sperm counts on one patient did show oligospermia (<1 million/ml) which was reversed within 6

months of cessation of rapamycin treatment (44). This might be a result of inhibiting the KIT/PI3-kinase/mTOR pathway, which is involved in spermatogonial proliferation.

Reports of the reproductive effects of HDAC inhibitors have been limited to a few animal studies. Treatment of rats with a clinically used inhibitor, vorinostat, had no effect on spermatogenesis or male fertility (45), but another inhibitor, trichostatin A, not currently used in cancer therapy, produced spermatocyte apoptosis in mice (46).

Although immunomodulating agents are not known to directly affect spermatogenesis the target on lymphocytes of the monoclonal alemtuzumab (Campath®), CD52, is also produced by the male reproductive tract and localizes to the surface of sperm (47). Campath can agglutinate and immobilize human sperm and this might occur while a patient (male or female) is receiving Campath, and could potentially inhibit fertilization. But since a course of Campath is limited to 12 weeks, this effect should be reversible.

Although now it has been largely replaced by targeted therapies, the cytokine, interferon, had been used to treat hematological malignancies. Generally, chronic treatment of adult male cancer patients with interferon-alpha did not affect sperm counts, hormone levels, or sexual function (27, 48), although acute treatment produced a transient drop in testosterone levels (49) and there is one report of azoospermia after interferon therapy (50).

The consequences of monoclonal antibodies delivering toxins in cancer therapy is like the cytotoxic agents in that they are often given in single doses and attempt to be curative by selective cytotoxicity to tumor cells. Data to predict the reproductive toxicity from radiosimetry to the testis are only available for radiolabeled monoclonal antibodies to CD20, used in treatment of non-Hodgkin's lymphoma. The median absorbed testicular dose from treatment with ¹³¹I-tositumomab was 22 Gy (51), which is much higher than that for completely eliminating spermatogonial stem cells and is also above the 20 Gy limit, above which there is loss of Leydig cell function (52). In fact in the 42% of patients with testicular doses higher 25 Gy, there was a significant reduction in testosterone levels. Other studies with ⁹⁰Y-ibritumomab tiuxetan (Zevalin) have indicated median doses to the testis of 10–20 Gy, but with variability so that some patients received >20 Gy (53, 54).

Thus, except for the targeted radionuclides, data in patients obtained so far with most of these biological targeted therapies are compatible with the men being either naturally fertile or have some sperm for use to achieve pregnancies by assisted reproductive techniques. However, most targeted therapies are new and newer targets and therapies are constantly under development but have not been evaluated in humans for reproductive effects. Furthermore, targeted and cytotoxic therapies are often used in combination and information as to how the biological modifiers affect gonadal cytotoxicity are lacking. Lessons from past studies of gene knockouts in mice in which numerous gene knockouts had surprising and specific detrimental effects on male fertility should warn us that complete inhibition of the gene products with targeted therapies might affect male fertility with minimal toxicity to other normal systems. Therefore, it is important to test the biomarkers of male fertility, preferably sperm count and quality, but alternatively at least hormonal indicators, in patients receiving these new agents to ascertain their effects on male reproductive function.

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References

1. Hermann BP, Sukhwani M, Hansel MC, Orwig KE. Spermatogonial stem cells in higher primates: are there differences from those in rodents? *Reproduction*. 2010; 139:479–93. [PubMed: 19880674]
2. Oakberg EF. Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse. *Radiat Res*. 1955; 2:369–91. [PubMed: 14385033]
3. 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. [PubMed: 4428015]
4. Meistrich ML, Finch M, da Cunha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Res*. 1982; 42:122–31. [PubMed: 7198505]
5. Edwards RG, Sirlin JL. The effect of 200 R of x-rays on the rate of spermatogenesis and spermiogenesis in the mouse. *Exp Cell Res*. 1958; 15:522–8. [PubMed: 13609630]
6. Clifton DK, Bremner WJ. The effect of testicular X-irradiation on spermatogenesis in man. A comparison with the mouse. *J Androl*. 1983; 4:387–92. [PubMed: 6654753]
7. Paulsen, CA. U.S. Department of Energy. 1973. The study of radiation effects on the human testis: including histologic, chromosomal and hormonal aspects. Final progress report of AEC contract AT(45-1)-2225, Task Agreement 6. RLO-2225-2; p. 1-36.
8. Meistrich ML, Wilson G, Brown BW, da Cunha MF, Lipshultz LI. Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing's and soft tissue sarcomas. *Cancer*. 1992; 70:2703–12. [PubMed: 1423201]
9. Meistrich ML, Wilson G, Mathur K, Fuller LM, Rodriguez MA, McLaughlin P, et al. Rapid recovery of spermatogenesis after mitoxantrone, vincristine, vinblastine, and prednisone chemotherapy for Hodgkin's disease. *J Clin Oncol*. 1997; 15:3488–95. [PubMed: 9396402]
10. Lu CC, Meistrich ML. Cytotoxic effects of chemotherapeutic drugs on mouse testis cells. *Cancer Res*. 1979; 39:3575–82. [PubMed: 476683]
11. Meistrich ML, Hunter N, Suzuki N, Trostle PK, Withers HR. Gradual regeneration of mouse testicular stem cells after ionizing radiation. *Radiat Res*. 1978; 74:349–62. [PubMed: 149333]
12. Meistrich ML. Quantitative correlation between testicular stem cell survival, sperm production, and fertility in the mouse after treatment with different cytotoxic agents. *J Androl*. 1982; 3:58–68.
13. Shuttlesworth GA, de Rooij DG, Huhtaniemi I, Reissmann T, Russell LD, Shetty G, et al. Enhancement of A spermatogonial proliferation and differentiation in irradiated rats by GnRH antagonist administration. *Endocrinology*. 2000; 141:37–49. [PubMed: 10614621]
14. Shetty G, Weng CC, Meachem SJ, Bolden-Tiller OU, Zhang Z, Pakarinen P, et al. Both testosterone and FSH independently inhibit spermatogonial differentiation in irradiated rats. *Endocrinology*. 2006; 147:472–82. [PubMed: 16210366]
15. Zhang Z, Shao S, Meistrich M. The radiation-induced block in spermatogonial differentiation is due to damage to the somatic environment, not the germ cells. *J Cell Physiol*. 2007; 211:149–58. [PubMed: 17167785]
16. da Cunha MF, Meistrich ML, Haq MM, Gordon LA, Wyrobek A. Temporary effects of AMSA [4'(9-acridinylamino) methanesulfon-m-anisidine] chemotherapy on spermatogenesis. *Cancer*. 1982; 49:2459–62. [PubMed: 7074561]
17. Meistrich ML, van Beek MEAB. Radiation sensitivity of the human testis. *Adv Radiat Biol*. 1990; 14:227–68.
18. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood*. 1996; 87:3045–52. [PubMed: 8639928]
19. Jacob A, Barker H, Goodman A, Holmes J. Recovery of spermatogenesis following bone marrow transplantation. *Bone Marrow Transplant*. 1998; 22:277–9. [PubMed: 9720742]
20. Sandeman TF. The effects of X irradiation on male human fertility. *Br J Radiol*. 1966; 39:901–7. [PubMed: 5954090]
21. Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure. *Hum Reprod*. 1997; 12:2422–8. [PubMed: 9436677]

22. Jacobsen KD, Olsen DR, Fossa K, Fossa S. External beam abdominal radiotherapy in patients with seminoma stage I: field type, testicular dose, and spermatogenesis. *Int J Radiat Oncol Biol Phys.* 1997; 38:95–102. [PubMed: 9212009]
23. May CA, Tamaki K, Neumann R, Wilson G, Zagars G, Pollack A, et al. Minisatellite mutation frequency in human sperm following radiotherapy. *Mutat Res.* 2000; 453:67–75. [PubMed: 11006413]
24. Dubey P, Wilson G, Mathur KK, Hagemeister FB, Fuller LM, Ha CS, et al. Recovery of sperm production following radiation therapy for Hodgkin's disease after induction chemotherapy with mitoxantrone, vincristine, vinblastine and prednisone (NOVP). *Int J Radiat Oncol Biol Phys.* 2000; 46:609–17. [PubMed: 10701740]
25. Marmor D, Grob-Menendez F, Duyck F, Delafontaine D. Very late return of spermatogenesis after chlorambucil therapy: Case reports. *Fertil Steril.* 1992; 58:845–6. [PubMed: 1426338]
26. Preti A, Hagemeister FB, McLaughlin P, Swan F, Redriguez A, Pesa P, et al. Hodgkin's disease with a mediastinal mass greater than 10 cm: Results of four different treatment approaches. *Ann Oncol.* 1994; 5(Suppl. 2):S97–S100.
27. Pryzant RM, Meistrich ML, Wilson E, 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. [PubMed: 8426200]
28. Hsiao W, Stahl PJ, Osterberg EC, Nejat E, Palermo GD, Rosenwaks Z, et al. Successful treatment of postchemotherapy azoospermia with microsurgical testicular sperm extraction: the Weill Cornell experience. *J Clin Oncol.* 2011; 29:1607–11. [PubMed: 21402606]
29. Hermann BP, Sukhwani M, Lin CC, Sheng Y, Tomko J, Rodriguez M, et al. Characterization, cryopreservation, and ablation of spermatogonial stem cells in adult rhesus macaques. *Stem Cells.* 2007; 25:2330–8. [PubMed: 17585169]
30. Meistrich, ML.; Vassilopoulou-Sellin, R.; Lipshultz, LI. Gonadal dysfunction. In: DeVita, VT.; Hellman, S.; Rosenberg, SA., editors. *Cancer: Principles and Practice of Oncology.* 7th ed.. Lippincott Williams & Wilkins; Philadelphia: 2005. p. 2560-74.
31. Meistrich ML, Chawla SP, da Cunha MF, Johnson SL, Plager C, Papadopoulos NE, et al. Recovery of sperm production after chemotherapy for osteosarcoma. *Cancer.* 1989; 63:2115–23. [PubMed: 2720562]
32. Apperley J. CML in pregnancy and childhood. *Best Pract Res Clin Haematol.* 2009; 22:455–74. [PubMed: 19959094]
33. Schultheis B, Nijmeijer BA, Yin H, Gosden RG, Melo JV. Imatinib mesylate at therapeutic doses has no impact on folliculogenesis or spermatogenesis in a leukaemic mouse model. *Leuk Res.* 2012; 36:271–4. [PubMed: 22018447]
34. Basciani S, De Luca G, Dolci S, Brama M, Arizzi M, Mariani S, et al. Platelet-derived growth factor receptor beta-subtype regulates proliferation and migration of gonocytes. *Endocrinology.* 2008; 149:6226–35. [PubMed: 18687785]
35. Nurmio M, Kallio J, Toppa J, Jahnukainen K. Adult reproductive functions after early postnatal inhibition by imatinib of the two receptor tyrosine kinases, c-kit and PDGFR, in the rat testis. *Reprod Toxicol.* 2008; 25:442–6. [PubMed: 18472395]
36. Ault P, Kantarjian H, O'Brien S, Faderl S, Beran M, Rios MB, et al. Pregnancy among patients with chronic myeloid leukemia treated with imatinib. *J Clin Oncol.* 2006; 24:1204–8. [PubMed: 16446320]
37. Shash E, Bassi S, Cocorocchio E, Colpi GM, Cinieri S, Peccatori FA. Fatherhood during imatinib. *Acta Oncol.* 2011; 50:734–5. [PubMed: 21517714]
38. Seshadri T, Seymour JF, McArthur GA. Oligospermia in a patient receiving imatinib therapy for the hypereosinophilic syndrome. *N Engl J Med.* 2004; 351:2134–5. [PubMed: 15537917]
39. Breccia M, Cannella L, Montefusco E, Frustaci A, Pacilli M, Alimena G. Male patients with chronic myeloid leukemia treated with imatinib involved in healthy pregnancies: report of five cases. *Leuk Res.* 2008; 32:519–20. [PubMed: 17804066]
40. Gambacorti-Passerini C, Tornaghi L, Cavagnini F, Rossi P, Pecori-Giraldi F, Mariani L, et al. Gynaecomastia in men with chronic myeloid leukaemia after imatinib. *Lancet.* 2003; 361:1954–6. [PubMed: 12801741]

41. Mariani S, Giona F, Basciani S, Brama M, Gnessi L. Low bone density and decreased inhibin-B/FSH ratio in a boy treated with imatinib during puberty. *Lancet*. 2008; 372:111–2. [PubMed: 18620939]
42. Mariani S, Basciani S, Fabbri A, Agati L, Ulisse S, Lubrano C, et al. Severe oligozoospermia in a young man with chronic myeloid leukemia on long-term treatment with imatinib started before puberty. *Fertil Steril*. 2011; 95:1120, e15–e17. [PubMed: 20888557]
43. Huyghe E, Zairi A, Nohra J, Kamar N, Plante P, Rostaing L. Gonadal impact of target of rapamycin inhibitors (sirolimus and everolimus) in male patients: an overview. *Transpl Int*. 2007; 20:305–11. [PubMed: 17326771]
44. Bererhi L, Flamant M, Martinez F, Karras A, Thervet E, Legendre C. Rapamycin-induced oligospermia. *Transplantation*. 2003; 76:885–6. [PubMed: 14501876]
45. Wise LD, Spence S, Saldutti LP, Kerr JS. Assessment of female and male fertility in Sprague-Dawley rats administered vorinostat, a histone deacetylase inhibitor. *Birth Defects Res B Dev Reprod Toxicol*. 2008; 83:19–26. [PubMed: 18092367]
46. Fenic I, Hossain HM, Sonnack V, Tchatalbachev S, Thierer F, Trapp J, et al. In vivo application of histone deacetylase inhibitor trichostatin-a impairs murine male meiosis. *J Androl*. 2008; 29:172–85. [PubMed: 18046049]
47. Focarelli R, Francavilla S, Francavilla F, Della Giovampaola C, Santucci A, Rosati F. A sialoglycoprotein, gp20, of the human capacitated sperm surface is a homologue of the leukocyte CD52 antigen: analysis of the effect of anti-CD52 monoclonal antibody (CAMPATH-1) on capacitated spermatozoa. *Mol Hum Reprod*. 1999; 5:46–51. [PubMed: 10050661]
48. Schilsky RL, Davidson HS, Magid D, Daiter S, Golomb HM. Gonadal and sexual function in male patients with hairy cell leukemia: Lack of adverse effects of recombinant $\alpha 2$ -interferon treatment. *Cancer Treat Rep*. 1987; 71:179–81. [PubMed: 3100036]
49. Corssmit EP, Endert E, Sauerwein HP, Romijn JA. Acute effects of interferon-alpha administration on testosterone concentrations in healthy men. *Eur J Endocrinol*. 2000; 143:371–4. [PubMed: 11022179]
50. Longo I, Sanchez-Mateos P, Lazaro P, Longo N. Azoospermia in a patient receiving interferon alpha for a stage III melanoma. *Acta Derm Venereol*. 2002; 82:389–90. [PubMed: 12430747]
51. Hattori N, Gopal AK, Shields AT, Fisher DR, Gooley T, Pagel JM, et al. ^{131}I -tositumomab myeloablative radioimmunotherapy for non-Hodgkin's lymphoma: radiation dose to the testes. *Nucl Med Comm*. 2012; 33:1225–31.
52. Petersen PM, Giwercman A, Daugaard G, Rorth M, Petersen JH, Skakkeak NE, et al. Effect of graded testicular doses of radiotherapy in patients treated for carcinoma-in-situ in the testis. *J Clin Oncol*. 2002; 20:1537–43. [PubMed: 11896102]
53. Cremonesi M, Ferrari M, Grana CM, Vanazzi A, Stabin M, Bartolomei M, et al. High-dose radioimmunotherapy with ^{90}Y -ibritumomab tiuxetan: comparative dosimetric study for tailored treatment. *J Nucl Med*. 2007; 48:1871–9. [PubMed: 17978355]
54. Chiesa C, Botta F, Coliva A, Maccauro M, Devizzi L, Guidetti A, et al. Absorbed dose and biologically effective dose in patients with high-risk non-Hodgkin's lymphoma treated with high-activity myeloablative ^{90}Y -ibritumomab tiuxetan (Zevalin). *Eur J Nucl Med Mol Imaging*. 2009; 36:1745–57. [PubMed: 19455328]

Figure 1. Sequence, kinetics, and survival after irradiation of spermatogenic cells in the human male. Drawings cells courtesy of Dr. Y Clermont. Arrows indicate time required for cells to mature from one state to another. Numbers in parentheses are based on histological counts of the surviving fraction of cells at 2 weeks after 1 Gy of irradiation, reflecting the direct killing of cells by irradiation and 2 weeks of maturation depletion (3)

Figure 2.

Declines in sperm counts in patients treated with two different chemotherapy regimens. (A) NOVP (9) chemotherapy consisting of Novantrone (mitoxantrone), Oncovin (vincristine), vinblastine, and prednisone for Hodgkin's disease. Small open circles are individual sperm counts. Large filled circles are averages of counts grouped into time ranges. (Reprinted with permission from Meistrich et al., *Journal of Clinical Oncology* 15: 3488, 1997) The subsequent recovery of sperm counts to normal levels is also shown. (B) CY(V)ADIC (8) chemotherapy consisting of cyclophosphamide, Adriamycin (doxorubicin), DIC (DTIC, dacarbazine), with or without vincristine for Ewing and soft-tissue sarcoma. Pretreatment counts are indicated by solid symbols. Dashed lines connect longitudinal counts for individual patients. (Reprinted with permission from Meistrich et al., *Cancer* 70: 2703, 1992)

Figure 3.

Recovery of sperm counts in individual patients treated with (A) hemi-pelvic radiotherapy for seminoma (22, 23), or (B) pelvic radiotherapy for Hodgkin's disease (24). Gonadal doses are indicated next to each plot.

Figure 4.

(A) Examples of delayed recovery of sperm count occurring after 2 to 5 years of azoospermia in 5 patients treated with chemotherapy agents that are toxic to stem spermatogonia. (○) CVPP-ABDIC consisting of cyclophosphamide, vincristine, procarbazine, prednisone, Adriamycin, bleomycin, dacarbazine, and lomustine (CCNU) (26) treatment for Hodgkin's disease patients; (△) CHOP-Bleo which consists of cyclophosphamide, Adriamycin (hydroxydaunorubicin), Oncovin, prednisone, and bleomycin (27) treatment for a non-Hodgkin's lymphoma. (B) Kaplan-Meier actuarial estimation of sperm count recovery to 10 million/ml showing the overall rates and extents of recovery in sarcoma patients were treated with the CY(V)ADIC regimen, but receiving different total doses of cyclophosphamide (8) (Reprinted with permission from Meistrich et al., Cancer 70: 2703, 1992).

TABLE 1

Antineoplastic Agents that can Cause or Add to Prolonged Azoospermia in Humans^a

Effects	Agents	Mechanism of Action	Dose to produce effect
Prolonged azoospermia			
	Ionizing Radiation	DNA breaks	2.5 Gy
	Chlorambucil	Alkylating	1.4 g/M ²
	Cyclophosphamide	Alkylating	19 g/M ²
	Procarbazine	Alkylating	4 g/M ²
	Melphalan	Alkylating	140 mg/M ²
	Cisplatin	DNA cross-link	500 mg/M ²
Azoospermia in adulthood after treatment prior to puberty			
	BCNU	Alkylating	1 g/M ²
	CCNU	Alkylating	500 mg/M ²
Likely to cause prolonged azoospermia, but always given with other highly sterilizing agents			
	Busulfan	Alkylating	600 mg/M ²
	Ifosfamide	Alkylating	42 g/M ²
	Nitrogen mustard	Alkylating	
	Actinomycin D	DNA intercalating	
Reported to be additive with above agents in causing prolonged azoospermia, but cause only temporary reductions in sperm count when not combined with above agents			
	Adriamycin	DNA intercalating	770 mg/M ²
	Thiotepa	Alkylating	400 mg/M ²
	Cytosine arabinoside	Nucleoside analog	1 g/M ²
	Vinblastine	Microtubule inhibitor	50 mg/M ²

Note: the table in that reference incorrectly gave the dose of busulfan as 600 mg/kg.

^aModified from (30).