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## Male Gonadal Toxicity

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### Abstract

Cancer treatment with chemotherapy or radiotherapy causes gonadal toxicity in male patients. The endpoint of most concern for future reproductive options is the induction of prolonged azoospermia, which may or may not be reversible. The immediate effects of therapy and its reversibility are most readily observed in post-pubertal patients, but the same antineoplastic regimens given to prepubertal males can induce permanent azoospermia. The probability of permanent azoospermia is related to the specific agents used and their doses. The most damaging are alkylating agents (particularly chlorambucil, procarbazine, cyclophosphamide, melphalan, and busulfan), cisplatin and radiation to the region of the testicles.

### INTRODUCTION

For male children and young men who have cancer, the success of treatment with regimens that are toxic to testicular function has made infertility an important problem. After the cancer is controlled, the quality of life, which often includes the ability to have a normal child, becomes a major issue.

Chemotherapy and radiotherapy used in the treatment of cancer can cause long-term or permanent gonadal toxicity in male patients. Whereas endocrine dysfunction (e.g., testosterone reduction) only occurs in limited instances [1], the manifestation of the toxicity that is of most concern is the prolonged reduction in sperm count to the point of azoospermia. Damage to other aspects of sperm function, such as loss of motility or morphological abnormalities are less pronounced, as when and if spermatozoa are produced after therapy, their motility and the percentage that exhibit normal morphology are restored to pretreatment levels [2,3]. When sperm count recovers following cytotoxic therapy, fertility is generally restored. However, when the duration of azoospermia is long, sperm count may sometimes plateau in the severe oligospermic range, and the sperm may have morphological abnormalities [4] that are not compatible with fertility.

With current methods of assisted reproductive technologies, including in vitro fertilization, intracytoplasmic sperm injection, and testicular sperm extraction, the limitations on the numbers of sperm and their physical abilities to enter the oocyte can be bypassed. However, there may be an increased risk of passing genetic damage in the spermatozoa on to the children.

## BASIC PRINCIPLES

After a patient starts treatment with chemotherapy or radiotherapy, there may still be sperm produced for the first 2 months because of the relative resistance of the later stage germ cells (Figure 1). Even mild forms of chemotherapy and low gonadal doses of radiotherapy can cause transient reductions in sperm count lasting 2–3 months from the end of treatment because of killing of the very sensitive differentiating spermatogonia. However prolonged reductions in sperm count or azoospermia can occur after stronger chemotherapy regimens or after higher doses of radiation therapy. The eventual recovery of sperm production depends on the survival of the spermatogonial stem cells and their ability to differentiate. In mice the time interval before recovery of fertility is directly related to the degree of stem cell killing [5]; in rats the sterility is due to damage to the somatic environment that prevents surviving stem spermatogonia from differentiating [6]. In human males, both stem cell killing and a block in their differentiation appear to contribute to the duration of the azoospermic period after cytotoxic therapy.

Many post-pubertal males become azoospermic at the completion of their cytotoxic therapy. If low doses of agents that kill stem spermatogonia or affect differentiation are used, recovery to normospermic levels can occur within 1 to 3 years, but at higher doses, azoospermia can be more prolonged or even permanent. Although the probability that spermatogenesis will recover decreases with the duration of azoospermia, in rare cases spermatogenesis has recovered in men after as long as 20 years of azoospermia [7].

Although in most individuals with iatrogenic azoospermia, the seminiferous tubules in testicular biopsies contain only Sertoli cells and no germ cells [8], occasionally a few tubules may contain isolated spermatogonia [9]. This would indicate that there is some potential for recovery but there is a block to spermatogonial development at that time.

## SPECIFIC AGENTS

The duration and permanence of the induced azoospermia depends on the nature of the cytotoxic agent and dose [10]. It is primarily radiation, many of the alkylating chemotherapeutic agents (procarbazine, busulfan, cyclophosphamide, chlorambucil, and melphalan), and cisplatin, which like the alkylating agents produces adducts and cross-links in DNA, that can produce long-term azoospermia. The doses required to produce prolonged azoospermia with many of these agents in adults are known (Table I). At lower doses, recovery to normospermic levels can occur within 1 to 3 years, but at higher doses, azoospermia can be more prolonged or even permanent [11]. Some agents, including the anthracyclines (e.g., doxorubicin), 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 [12]. In addition there are many chemotherapeutic agents that do not produce prolonged azoospermia; these include antimetabolites (e.g., 6-mercaptopurine, methotrexate), topoisomerase inhibitors (e.g., etoposide, mitoxantrone), biologicals (e.g., interferon), corticosteroids (e.g., prednisone), and other agents such as bleomycin (Table II). If treatment is limited to the cytotoxic agents that do not kill stem spermatogonia or block their differentiation, normospermia can be restored at 3 months after the cytotoxic therapy [13]. Although individuals' responses to these agents and combinations may vary, it is possible to predict the probability of prolonged azoospermia from the doses of each agent used in a combination, as the agents appear to have additive effects.

## COMPARISON BETWEEN POST- AND PRE-PUBERTAL MALES

In the prepubertal testis, the seminiferous tubules contain only immature Sertoli cells and spermatogonia, both of which show very low proliferative rates. There are no recognizable Leydig cells in the interstitium but there are mesenchymal precursors that will proliferate and form the adult Leydig cells, which produce testosterone.

It had been incorrectly assumed that the prepubertal testis would be more resistant to cytotoxic agents because of the low proliferation rates and this assumption was apparently supported by erroneously comparing sterilizing doses of chemotherapy to boys and adult men on a dose per kg basis [14]. However by appropriately expressing chemotherapy doses to boys on a per meter squared basis and calculating radiation doses to the gonad, the doses of a variety of chemotherapy [15] and radiotherapy [16] regimens to produce permanent azoospermia in survivors of childhood and adolescent cancer after they reach puberty appear to be the same as those for adults.

As in adults, radiation, alkylating agents, such as procarbazine, cyclophosphamide, chlorambucil, BCNU, and CCNU, and cisplatin are the most sterilizing and produce prolonged and sometimes permanent azoospermia in boys [17,18]. In addition, high doses of cytosine arabinoside have an additive effect with the above agents in producing germ cell damage [19]. Regimens lacking alkylating agents, such as those used for treatment of acute lymphocytic leukemia do not affect subsequent sperm counts or fertility [20,21].

The only aspect in which prepubertal males and adults display different gonadal sensitivity is with respect to damage to eventual Leydig cell formation and testosterone production, with boys showing greater sensitivity to high doses of radiation [1]. Chemotherapy regimens do not have any marked effect on Leydig cell function, either in pre- or postpubertal males [22].

## GENETIC CONSIDERATIONS

Since anticancer agents damage DNA and interfere with chromosome segregation there is concern that these agents may induce both single gene and chromosomal mutations in germ cells, both of which can cause genetic disease in offspring [23]. It is important to consider the induction of mutations in differentiating germ cells separately from induction of mutations in stem spermatogonia. Animal studies indicate that the differentiating germ cells are more sensitive to induction and transmission of mutations than are stem spermatogonia [24]. Whereas mutations induced in later stages of spermatogenesis will only result in production of mutation-carrying sperm for about 3 months, those induced in stem spermatogonia will continue to produce mutation-carrying sperm for the lifetime of the male (Figure 1). Attempts to assess the risks of paternal transmission of treatment-induced genetic disease to the offspring of male cancer patients have been done using actual data on pregnancy outcomes, measurement of mutations in spermatozoa, which will be transmitted to any resulting fertilizations, and measurement of DNA damage in spermatozoa, which may or may not pose a risk to offspring conceived.

Clinical reports of outcomes of pregnancies in which conception occurred while the father was undergoing cytotoxic therapy, and hence the germ cells were exposed while differentiating, are too limited to evaluate the risks [24]. There are some large studies of genetic disease in offspring, nearly all of which were conceived long after the end of therapy, which is especially true in the studies of long-term survivors of childhood and adolescent cancer. Thus the spermatozoa must be derived from cells that were exposed to the potentially genotoxic agents as stem spermatogonia. The large studies all indicated no significant increase, above the background in the general population of about 4%, in birth

defects or genetic disease in offspring conceived naturally after cytotoxic treatment [25–28]. In addition, the atomic-bomb studies in Japan also showed no significant increase in genetic damage in offspring born to radiation-exposed parents [29]. These observations should reassure those who wish to have children following treatment for cancer. However, the power of these studies can only rule out  $\geq 2$ -fold increases in abnormalities over background; the possibility of a small genetic risk remains. Also these studies do not include many patients receiving the newer chemotherapeutic agents or pregnancies from spermatozoa obtained after treatment and achieved using assisted reproductive technologies.

Two types of mutations have been analyzed in spermatozoa of patients treated with cytotoxic agents for cancer. Minisatellite repeat number mutations represent a change in the number of tandem repeats in a type of DNA sequence called a minisatellite. Indeed, such mutations were reported to be increased in offspring of men exposed to radiation from Chernobyl [30]. However, the lack of any increases in these mutations in spermatozoa from nearly all men analyzed after treatment with radiotherapy or chemotherapy indicates that they are not a significant concern [31,32]. Chromosomal abnormalities have been measured by sperm karyotyping after fusion with hamster eggs or by fluorescence in situ hybridization (FISH). Structural chromosomal aberrations were present in sperm more than 5 years after the end of MOPP chemotherapy combined with radiation therapy indicating they are induced in stem cells [33]. Abnormal numbers of chromosomes (aneuploidy) can be present during and within a defined period after chemotherapy (up to 4 or 18 months, depending on the study) and then return to baseline [34–36]. These results demonstrate that there can be significant genetic risks if conception or storage of sperm occurs during or shortly after cytotoxic therapy, but that this risk declines after the end of such therapy (Figure 1).

Recently, there have been a few studies of DNA damage in spermatozoa after cancer therapy. The presence of high levels of DNA damage in spermatozoa has been correlated with reduced fertilizing capacity. In addition DNA damage sites are considered to be potentially premutational lesions. If they are repaired correctly in the oocyte or zygote, they may not have any consequence. However, if they are not repaired or repaired incorrectly, they may lead to a mutation in DNA sequence or chromosome content. One study showed a transient increase in DNA damage just at the completion of chemotherapy [37]. Several other studies have failed to show any persistent increase in DNA damage in spermatozoa obtained after chemotherapy, but one of these studies did show that there may be a persistent increase in DNA damage for up to 2 years after radiation therapy [38–40]. Since these techniques only measure what might be premutational damage and knowledge of repair of such damage within the oocyte is incomplete, no estimates of mutational risk to the offspring are yet possible from these data.

In summary, mutational risks will be highest when a pregnancy occurs during or within several months after the male is exposed to the damaging agent. After this time, the incidence of mutations declines to a lower level, which so far has not been detectable as adverse pregnancy outcomes.

## FUTURE DIRECTIONS

Monitoring and minimizing the gonadal toxicity of cancer treatments is an ongoing need. New treatment regimens are being developed that may have unexpected gonadal toxicity. Although it is less likely that new specifically targeted therapies, as opposed to the DNA-damaging therapies that constitute the bulk of antineoplastic agents, will display prolonged gonadal toxicities, they still must be monitored, especially for possible synergistic effects when used in combination with existing therapies.

A better understanding of the mechanisms causing prolonged azoospermia following anticancer therapies is needed. As stated above, the eventual recovery of sperm production depends on the survival of the spermatogonial stem cells and their ability to differentiate. It is generally thought that the prolonged period of azoospermia is caused by killing many of the stem spermatogonia by antineoplastic agents and, until they gradually recover their number, they will not produce differentiating cells and spermatozoa. However in rats exposed to certain antineoplastic agents, the stem spermatogonia that survive and recover their number still are blocked from differentiating [41] as a result of damage to the somatic environment within the testis, not to the spermatogonia [6,42]. It is important to determine whether such damage to the somatic environment of the testis occurs in human males as well and the relative roles of germ cell and somatic cell damage in the gonadal toxicity from anticancer therapy.

Methods to prevent these adverse effects on sperm production and to restore gonadal function after the toxic treatment are of great importance to young male patients. A variety of biochemical and biological approaches have been tested in experimental animal models to protect the testes against radiation and chemotherapy (reviewed in [43]). However, the greatest research interest and all clinical trials have involved hormonal modulation in attempts to prevent or reverse damage to the germline from radiotherapy and chemotherapy [44]. Gonadotropin and testosterone suppression in rats before or after cytotoxic therapy dramatically enhances the recovery of spermatogenesis and fertility [41]. Although the mechanism of this phenomenon is not fully known, it is known that it is not a consequence of the induction of quiescence as originally speculated but rather involves the reversal, by suppression of testosterone, of the block in differentiation of surviving spermatogonia caused by damage to the somatic environment [6]. However, in men, only one of eight clinical trials using hormonal suppression was successful in protecting or restoring spermatogenesis after cytotoxic therapy [45]. It is therefore important to identify the specific beneficial effects of hormonal suppression in the rat model that may be applicable to human

Since many chemotherapy and radiotherapy regimens may result in complete killing of the stem spermatogonia, cryopreservation of spermatogonia from prepubertal males and autologous transplantation after therapy is considered a potential method for restoring spermatogenesis and possibly rescuing fertility [46]. However, possible damage to the somatic cells that might render the testis unable to support differentiation of transplanted cells may have to be considered [6]. Methods such as gonadotropin and testosterone suppression, which promotes the survival and differentiation of spermatogonia that were transplanted into testes of rodents depleted of endogenous stem cells, might restore a favorable somatic environment [6,47].

Finally and most importantly the information available must be regularly and proactively used by health care professionals to inform the cancer patients about the probability of sterility and the genetic risk from their disease and its treatment. Options for preservation of fertility, including choice of less gonadotoxic chemotherapy regimens, gonadal shielding during radiation therapy, semen cryopreservation, and the investigational approach of testicular tissue cryopreservation for later use, should be discussed with the patient.

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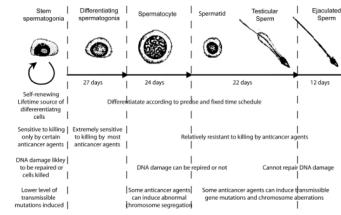
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**Figure 1.** Sequence of spermatogenic cells showing the cell morphology, kinetics, relative sensitivity to killing by anticancer agents, ability to accumulate and repair DNA damage, and sensitivity to induction of transmissible mutations.

TABLE I

## Agents with Significant Long-Term Effects on Sperm Production in Men

Agents (cumulative dose for effect)	Effect
Radiation (2.5 Gy to testis) [48]	Prolonged azoospermia
Busulfan (600 mg/m <sup>2</sup> ) [49]	
Chlorambucil (1.4 g/m <sup>2</sup> ) [50]	
Cyclophosphamide (19 g/m <sup>2</sup> ) [51]	
Procarbazine (4 g/m <sup>2</sup> ) [52]	
Melphalan (140 mg/m <sup>2</sup> ) [53]	
Cisplatin (500 mg/m <sup>2</sup> ) [54]	
BCNU (1 g/m <sup>2</sup> ) [17]	Azoospermia in adulthood after treatment prior to puberty
CCNU (500 mg/m <sup>2</sup> ) [17]	
Ifosfamide (42 g/m <sup>2</sup> ) [55]	Likely, but always given with other highly sterilizing agents
BCNU (300 mg/m <sup>2</sup> ) [53]	
Nitrogen mustard	
Actinomycin D	

This table was modified from Ref. [10]. Note that the dose of busulfan that contributes significantly to sterility was given incorrectly in that reference as 600 mg/kg; the correct value is 600 mg/m<sup>2</sup>. BCNU: Carmustine; CCNU: Lomustine

TABLE II

## Agents with Limited, No, or Unknown Long-Term Effects on Sperm Production in Men

Agents (cumulative dose for effect)	Effect
Carboplatin (2 g/m <sup>2</sup> ) [56]	Contributes less to prolonged azoospermia than does cisplatin.
Adriamycin (770 mg/m <sup>2</sup> ) [2] Thiotepa (400 mg/m <sup>2</sup> ) [4] Cytosine arabinoside (1 g/m <sup>2</sup> ) [19] Vinca alkaloids [56]*	Can be additive with highly gonadotoxic agents (see Table I) in causing prolonged azoospermia, but cause only temporary reductions in sperm count when not combined with those agents
Amsacrine, bleomycin, dacarbazine, daunorubicin, epirubicin, etoposide, fludarabine, 5-fluorouracil, 6-mercaptopurine, methotrexate, mitoxantrone, thioguanine [13,57–59]	Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible
Prednisone [11] Interferon- $\alpha$ [11]	No effects on sperm production
Taxanes Tyrosine kinase inhibitors Monoclonal antibodies Nanoparticles	No published data available

This table was modified from Ref. [10]. Note that the doses of vinblastine and vincristine that appear to have additive effects were given incorrectly in that reference as 50 g/m<sup>2</sup> and 8 g/m<sup>2</sup>, respectively; the correct values are 50 mg/m<sup>2</sup> and 300 mg/m<sup>2</sup>.

\* Patients treated with vinblastine and vincristine were grouped in the analysis. Average vinblastine doses were 50 mg/m<sup>2</sup> and vincristine doses were 300 mg/m<sup>2</sup>.