Impact of cancers and treatment on male fertility. Radiation effects on spermatogenesis

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2 authors:

F. F. Pasqualotto
Universidade de Caxias do Sul (UCS)
223 PUBLICATIONS 3,221 CITATIONS

Ashok Agarwal
Cleveland Clinic
1,844 PUBLICATIONS 31,897 CITATIONS

Some of the authors of this publication are also working on these related projects:

- Sperm Diagnostics and Selecting Healthier Spermatozoa View project
- Antioxidant therapy in idiopathic oligoasthenoteratozoospermia View project
Introduction

Human fertility is dependent on the maturation of germ cells through meiosis and their association with supporting cells. These processes are sensitive to radiotherapy. Infertility is functionally defined as the inability to conceive after one year of intercourse without contraception [1]. Rates of permanent infertility and compromised fertility after cancer treatment vary and depend on many factors [2]. The effects of radiation therapy depend on the size/location of the radiation field, dose, dose intensity, disease, and the age and pre-treatment fertility status of the patient [2]. Male infertility may result from the disease itself (best documented in patients with testicular cancer and Hodgkin’s disease), anatomic problems (e.g., retrograde ejaculation or anejaculation), primary or secondary hormonal insufficiency, or, more frequently, from damage or depletion of the germinal stem cells [3]. The measurable effects of radiation therapy include a decrease in the number of spermatozoa, decreased motility, abnormal morphology, and reduced DNA integrity. Radiation therapy increases the DNA fragmentation index, whereas this value decreases after chemotherapy [4–6]. The biologic implications of such changes in sperm DNA after cancer therapy have, to date, not been fully elucidated.

Cancer and radiation therapy

Spermatogenesis is a long, complex, and finely tuned process; during this process, the developing germ cells are sensitive to endogenous and exogenous stress. Cancer therapies such as radiation and chemotherapy can cause temporary or permanent impairment of fertility in male cancer patients who are of reproductive age. A total of 1 596 670 new cancer cases and 571 950 deaths from cancer are projected to occur in the United States in 2011. Overall cancer incidence rates were stable in men in the most recent time period after decreasing by 1.9% per year from 2001 to 2005; in women, incidence rates have been declining by 0.6% annually since 1998 [5]. Testicular germ cell carcinoma is the most common malignant disease among young men, and the incidence is increasing. In view of the excellent prognosis, with a cure rate surpassing 95%, the clinical challenge of today lies in minimizing the long-term effects of the treatment. The prognosis of patients with testicular cancer has considerably improved in recent years: whereas in 1970 the mean survival of such patients was only 10%, since 1990 it has risen to 90%. This can be attributed to notable diagnostic and surgical advances and new radiotherapy and chemotherapy protocols, to which testicular tumors are especially sensitive.

From an infertility point of view, patients with testicular germ cell carcinoma represent a particular challenge. Not only is the reproductive function affected by the treatment given (i.e., chemotherapy and/or radiation therapy), but the tumor is also known to be associated with male sub/infertility and undescended testicles, all of which are considered to be part of the testicular dysgenesis syndrome [4].

The cytotoxic effect of radiation therapy on the spermatogenetic cells has led to great interest in studying post-therapy sperm parameter alterations in testicular cancer subjects. Numerous articles report studies of antineoplastic therapy on sperm quality, but these can be limited by the low number of patients examined and methodological errors which reduce their validity. Radiotherapy is used to treat malignancies such as Hodgkin’s lymphoma, lymphosarcoma,
and testicular cancers, many of which strike patients of young or childbearing age. Radiation doses range from 3000 to 7000 cGy – doses that are thousands of times higher than those used in diagnostic radiology. The effects of such high doses can be mutagenic, embryotoxic, embryolethal, and teratogenic. When possible, the gonads are shielded to reduce exposure to the radiation.

Spermatogenesis is initiated from the most primitive type of spermatogonium, the type A-single (As) or stem cell spermatogonium, which has two possible fates: self-renewal or committed differentiation. The As spermatogonia give rise to A-pair (Apr) and then A-aligned (Aal) spermatogonia, which are then able to differentiate into A1, A2, A3, A4, intermediate (In), and B spermatogonia. When a type B spermatogonium enters the last mitotic division, it generates two primary spermatocytes, which initiate meiosis by replicating the DNA before they pass through a number of stages that end with the two nuclear divisions distinguished as meiosis I and II. After the meiotic divisions each primary spermatocyte results in the formation of four haploid round spermatids. The spermatids proceed through a long differentiation process (designated spermiogenesis) resulting in the release of spermatozoa [2–9].

Radiation induces germinal depletion in a dose-dependent manner, with immature cells being the most radiosensitive. It has been hypothesized that spermatogonia – which are the most radiosensitive cells because of their intense mitotic activity – and spermatids are affected by ionizing radiation. Spermatids are unprotected because they lose their DNA damage repair mechanisms during post-meiotic differentiation and chromatin condensation. Any radiation damage that occurs may not be apparent initially, as many spermatocytes generally survive the first round of radioactive impact and go on to mature and produce spermatozoa [2]. Ultimately, radiotherapy damages the DNA, impeding the cell from replicating itself and causing its death. This therapy is especially effective in cancer cells, which replicate more quickly, but also affects normal cells, especially those with a high replication rate such as spermatogonia. Radiation induces material ionization both directly, through excitation of the atoms making up the DNA molecule, and indirectly, through its interaction with non-DNA molecules, which induce the ionization of the genetic material by emitting secondary electrons [2,6].

The germinal epithelium is damaged by doses as low as 1 Gy, while Leydig cells are damaged by doses of 20–30 Gy [1]. Doses as low as 0.1–1.2 Gy may damage dividing spermatogonia and may result in oligospermia. In prepubertal males, doses to the testes in excess of 20 Gy may result in delayed puberty. These doses result from irradiation to the pelvis for conditions including bladder, rectal, and anal tumors as well as for germ cell tumors of the testes. Total-body irradiation also causes permanent impairment of spermatogenesis but has variable effects on Leydig cell function.

The observed activation of reserve stem cells after a gonadotoxic insult demonstrates that a single insult is less damaging to the seminiferous epithelium than multiple insults of lower intensity. In men following single measured doses of 6 Gy and above, and low-dose fractionated irradiation of 1.5 Gy and above, irreversible damage of spermatogenesis can be expected [2–10]. Testicular irradiation with doses of 20 Gy is associated with Leydig cell dysfunction in prepubertal boys [7]. Apart from the dose and fractionation, other factors such as source, field of treatment, type of radiation, age, and individual susceptibility influence the gonadotoxicity of irradiation.

Virtually the entire population of spermatogonia will die if exposed to sufficiently high x-ray doses, and especially a fractionated irradiation. Considering the mean dose (2600 rad) as the discriminating value, at 12 months, the reduction in total sperm count is statistically significant in subjects having undergone a total dose > 2600 cGy in comparison with those subjected to a dose ≤ 2600 cGy. This difference is not statistically significant at 24 months after therapy. This confirms that the total dose of radiation administered is a discriminating and predictive factor of the time necessary to recover spermatogenesis [2].

Recovery may however be increased at very high doses with a fractionated irradiation. After exposure to irradiation, spermatocytes and spermatids continue normal development and ultimately leave the testis as spermatozoa. If stem cells (As spermatogonia) survive the irradiation, they may in some cases quickly initiate the recovery of spermatogenesis and repopulate the seminiferous epithelium. The remaining As spermatogonia will either first replenish their own numbers before they enter spermatogenic differentiation, so that in time spermatogenesis spreads along the length of the tubule, or they can remain “arrested” in the testis as isolated spermatogonia in atrophic tubules. In some cases a delay before spermatogenesis reinitiates
Table 12.1. Effects of radiation therapy on spermatogenesis

<table>
<thead>
<tr>
<th>Testicular dose (cGy)</th>
<th>Effect on spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>No effect</td>
</tr>
<tr>
<td>10–30</td>
<td>Temporary oligospermia</td>
</tr>
<tr>
<td>30–50</td>
<td>Temporary azoospermia at 4–12 months after radiation; 100% recovery by 48 months</td>
</tr>
<tr>
<td>50–100</td>
<td>100% temporary azoospermia for 3–17 months after radiotherapy; recovery begins at 8–26 months</td>
</tr>
<tr>
<td>100–200</td>
<td>100% azoospermia from 2 months to at least 9 months; recovery begins at 11–20 months</td>
</tr>
<tr>
<td>200–300</td>
<td>100% azoospermia beginning at 1–2 months; may lead to permanent azoospermia; if recovery takes place, it may take years</td>
</tr>
<tr>
<td>1200</td>
<td>Permanent azoospermia</td>
</tr>
<tr>
<td>2400</td>
<td>Permanent azoospermia</td>
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</table>

has been observed. Currently there is little evidence for damage to the somatic elements of the testis after moderate doses of radiation or chemotherapy. However, as the germ cells are dependent on Sertoli cells for survival, it is difficult to assess whether it is germ cells or somatic cells that are damaged by radiation.

Boys with acute leukemia requiring marrow ablative chemoradiotherapy and hematopoietic stem cell transplantation (HSCT) are at extremely high risk for infertility, with 85% of adult patients found to be azoospermic after total-body irradiation and cyclophosphamide administration [8].

Ionizing radiation has adverse effects on gonadal function in men of all ages, with the degree and persistence of the damage being dependent on the dose (Table 12.1) [9]. In many cases, men are azoospermic after treatment, and those who do regain spermatogenesis some time after treatment exhibit low sperm counts, decreased motility, and an increased rate of chromosomal abnormalities [10]. Compared with the testicles, the ovaries are more resistant, mainly because of the biology of the germ cells present in the ovary. These do not divide until completion of the first meiotic division at ovulation, in contrast to the constant cell division that occurs during sperm production in the testes.

Studies in animals

Studies involving animals have shown that radiation has direct mutagenic effects on germ cells in relation to the dose. High doses lead to dominant lethal effects, point mutations, and chromosomal abnormalities [11]. Testicular irradiation in mice, rats, monkeys, and men uniformly decreases the number of differentiating spermatogonia and later depletes the more advanced spermatogenic cells [12].

The mutagenic effects of radiation therapy are well known from animal studies. Total-body irradiation, which is used in myeloablative stem cell transplantation, is highly associated with infertility, while lesser doses or limited radiation fields have less gonadal toxicity [6]. In rhesus monkeys and humans after single doses of 4 and 6 Gy it takes five years or more for spermatogenesis to return to pre-irradiation germinal cell numbers and sperm concentrations [13–18]. Others have used doses of 1–4 Gy for the depletion of the seminiferous epithelium, or for studies on gonadal protection in rhesus monkeys [16–18].

Most of the studies performed in rats and one study in two baboons have shown that blocking gonadotropin secretion can lead to hormonal protection even if the treatment is applied after irradiation [19–24]. In monkeys, after gonadotropin withdrawal, high amounts of testicular androgens remain, which should not severely affect spermatogenic regulation in primates [25–28].

In one study, 20 adult male monkeys were randomized to receive either recombinant human follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH) antagonist, or saline injections for 36 days [29]. Testicular volume and inhibin B decreased significantly in all irradiated groups compared with baseline and with the non-irradiated control group, followed by a gradual recovery of these parameters, which was, especially at the earlier time points, significantly better in the FSH-treated group than in both other irradiated groups. Irradiation caused a drastic decrease of sperm parameters in all groups, followed by a partial recovery of sperm parameters, which was significantly slower in the early phases of recovery in the GnRH antagonist group compared with the vehicle group. Testicular histology showed a significant depletion on study day 261 in all irradiated animals. In contrast to rodent studies, therefore, GnRH antagonist treatment did not provide gonadal protection in this primate model. FSH treatment resulted in slightly better recovery of spermatogenesis, which appears to be of no or only little clinical relevance.

A study in rat testes demonstrated that radiation-induced block in spermatogonial differentiation may in fact be caused by damage to the somatic
environment, i.e., the Sertoli cells, and not to the germ cells [30]. Indeed, transplantation of Sertoli cells into irradiated testes has been shown to stimulate recovery of endogenous host spermatogenesis. Stimulation might, however, be indirect, as the endocrine androgen–estrogen balance seems crucial in stimulating spermatogonial recovery [30,31].

A recent study has shown that irradiation of mice testis created a gap in spermatogenesis, which was initiated by loss of A1 to B spermatogonia [12]. The gap lasted for approximately 10 days and successively extinguished germ cells at different developmental stages. Spermatogonial stem cells were, however, able to repopulate the seminiferous epithelia, which was reconstituted 42 days after irradiation. Also, gene expression can be a useful tool to describe reconstitution of testicular tissue after irradiation or chemotherapy, which otherwise relies on detailed histological descriptions that require carefully trained pathologists.

Studies in humans

In humans, most men are azoospermic for at least a year following radiation therapy, and studies of semen samples taken three years following treatment have found an increased frequency of sperm chromosomal abnormalities (both numerical and structural) in 20.9% of the patients compared with a control rate of 8.5%. These studies have also found a greater frequency of hypohaploidy relative to hyperhaploidy, suggesting that radiation therapy causes chromosomal loss rather than non-dysfunction [32]. There is also a suggestion that high doses of paternal irradiation preconception may increase induction of lymphomyeloid malignancies in the offspring if they are exposed after birth to a recognized inducer of leukemia [33].

In humans, a number of studies have shown that the frequency of autosomal and sex chromosome aneuploidy in sperm samples increases after radiation therapy, but the data suggest that these changes are transient. Results of studies of patients with testicular germ cell carcinoma indicate that sperm DNA might be damaged post-orchiectomy, even before radiation therapy [4]. The risk of damage to sperm DNA due to cancer or its treatment is a source of potential concern. Children who are fathered by cancer survivors do not seem to be at an increased risk for genetic aberrations, although this conclusion is largely based on data from small case series. In addition, the vast majority of large studies represent a follow-up of survivors of pediatric cancer [2–4]. It can be assumed that the sensitivity of DNA damage of a prepubertal germ cell is less pronounced than in the proliferating postpubertal cells of spermatogenesis. Finally, these surveys were based on conceptions that had occurred naturally.

Paternal exposure to ionizing radiation can produce dramatic effects on reproduction resulting in a decrease in fertility, which may be associated with a drop in sperm production and an increase in dominant lethal mutations in offspring [34]. Evidence from animal studies suggests that a high risk of transmissible defects exists that results from increases in the frequency of germline mutations [35,36], predisposing future generations to risks such as cancer [36].

Even though radiation therapy may damage DNA, the extent, duration, and biologic significance of such an effect on sperm chromatin integrity is not known [33]. A dose-dependent increase in DNA damage in testis cells 14 days after radiation therapy has been reported [37]. The overall results show that DNA damage induced in pre-meiotic germ cells is detectable in primary spermatocytes and is still present in mature spermatozoa. Other animal studies have shown that pregnancies resulting from mating with an irradiated male are associated with impaired fetal development, pregnancy loss, abnormal somatic development, and tumor induction in the fetus [38].

Genetic instability was detected in children whose fathers previously had been exposed to radiation therapy [39], but no increase in malformation rates or in tumor induction was observed [40].

The DNA fragmentation index (DFI) was found to be significantly increased in men who received radiation therapy [33]. The potential risk of using sperm samples with post-irradiation DNA damage for advanced techniques of assisted reproduction is emphasized by the results of studies using the human sperm–hamster oocyte technique. In such models, the fertilization ability remained despite radiation-induced sperm DNA damage. Therefore, the risk of transmitting defective DNA to the offspring is apparent [41,42].

Regarding semen parameters, there is a statistically significant decrease in ejaculate volume, sperm concentration per mL, total sperm count, and forward motility and a statistically significant increase in abnormal forms up to 12 months following cancer therapy. In one study, there were no differences at the beginning and 24 months post-therapy for
any semen parameter except volume, indicating that sperm quality had returned to pre-radiotherapy values [4]. However, even after 24 months, sperm volume remained lower than before treatment. In contrast with the chemotherapy group, alteration in sperm parameters were most relevant six months after the end of radiotherapy.

**Gonadal shielding**

Semen quality has been reported in men who received external-beam irradiation for testicular cancer without gonadal shielding, and it has been found that while 51% of patients had low sperm counts before therapy, all of them had low sperm counts two years after treatment, and 82% of them had persistently low counts at a mean of eight years after therapy [3]. With pelvic irradiation of 5000 cGy, a shield should provide a 99% block. It has also been demonstrated that gonadal shielding before radiation is effective in protecting testicular function following bone marrow transplantation in childhood and adolescence [43].

**Conclusions**

Quality of life is an important issue for childhood and adult cancer patients. Radiation treatment for testicular or other cancers may damage the testis, leaving permanent problems with sperm production. Reduced fertility or sterility after radiation therapy depends on the dose received by the testis. Therefore, physicians should discuss the side effects of radiation therapy on fertility potential with their patients before beginning the treatment.

**References**


