

The determination of reproductive safety in men during and after cancer treatment

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Fertility-related concerns are frequently encountered in the course of providing care to oncologic patients. Male cancer survivors who desire paternity after cancer treatment face the question of whether their posttherapy sperm can be safely used in either natural or assisted conception attempts. Although the reproductive risks of using sperm genetically compromised by chemotherapy or radiotherapy include impaired embryonal development, pregnancy loss, and congenital anomalies in offspring, there is a general lack of consensus in the literature concerning the persistence of sustained genotoxic effects, making it difficult to assuredly quantify the level of risk involved. Transmission of chemotherapeutic agents via seminal plasma is another potential risk that has not yet been well evaluated. Sperm chromosomal aneuploidy rates and DNA fragmentation indices provide means of assessing genomic damage that could prove useful in genetic counseling efforts. Ultimately, additional research is needed to clarify investigational discrepancies and establish a stronger body of evidence that would allow for the development of clinical guidelines to assist cancer patients considering posttreatment conception in their decision-making processes. (*Fertil Steril*® 2013;100:1187–91. ©2013 by American Society for Reproductive Medicine.)

Key Words: Cancer, fertility, genotoxicity, oncofertility, reproductive safety

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Advancements in oncologic treatment have led to improved survival outcomes for cancer patients over the last several decades. Accordingly, the increased longevity afforded to cancer survivors has been accompanied by a shift in provider focus toward ensuring future quality of life. For male patients of reproductive age or younger, one of the key concerns encountered in the consideration of life after cancer therapy is the question of whether they will be able to father children. Studies have demonstrated that after treatment more than half of these young male survivors go on to desire paternity, including 75% of men who were childless at the time of diagnosis (1).

Therapeutic modalities such as chemotherapy and radiation therapy are highly effective at treating cancer, but they also have gonadotoxic properties that can severely impair fertility in an agent- and dose-dependent manner (2). The importance of consistently addressing the issue of fertility preservation in the course of cancer diagnosis and treatment has been increasingly recognized, as entities such as the American Society for Reproductive Medicine (ASRM) and the American Society of Clinical Oncology (ASCO) have issued formal recommendations urging clinicians to discuss with patients the potential impact of cancer treatments on fertility and to present options for

fertility preservation, including sperm cryopreservation, when appropriate (3, 4).

Although the banking of sperm before the onset of cancer treatment is advocated, circumstances may arise that preclude the successful cryopreservation of sperm in individual cases. The resumption of spermatogenesis after chemotherapy or radiotherapy is known to be unpredictable, and studies of spermatogenesis in long-term cancer survivors have demonstrated evidence of persistent azoospermia or severe oligospermia in up to 24% of patients (5). Nevertheless, the eventual return of sperm production in many posttreatment cancer patients brings with it the question of whether the posttherapy sperm is safe to use in attempts at conception either naturally or via assisted reproductive technologies. We examine this issue of reproductive safety and the risks of using sperm from cancer patients during and after cancer treatment.

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REPRODUCTIVE RISKS

In this context, concerns over reproductive safety are principally rooted in the potential presence of DNA damage incurred by the germ line as a result of chemotherapy or radiotherapy. Chemotherapeutic agents, particularly the alkylating agents that are frequently used, may induce genetic damage by cross-linking DNA and introducing single-strand DNA breaks; the similarly mutagenic effects of radiation arise from DNA fragmentation incited within cells (2, 6). The determination of genetic safety hinges largely on the spermatogenic stage at which a mutation is acquired. Owing to down-regulation of DNA repair mechanisms that occurs during late spermatogenesis, spermatogenic cells further along the differentiation pathway typically cannot repair incurred DNA damage, nor are they usually capable of undergoing complete apoptosis (7). As a result, the ejaculated spermatozoa may harbor extensive genomic damage that could theoretically be transmitted to a resultant embryo upon fertilization.

Fortunately, mutations induced exclusively in the later stages of spermatogenesis will yield a risk period for the production of genetically compromised sperm that is limited to about 3 months, or the time it takes for an entire spermatogenic cycle to complete (8). In contrast, mutations incurred by the spermatogonial stem cells can potentially be repaired by inherent DNA repair mechanisms or removed completely by the cell's apoptotic machinery. However, as these spermatogonial cells represent the progenitors from which all future germ lines are derived, any sustained mutations in these cells that escape repair or elimination will continue to be transmitted, resulting in the possible production of mutation-carrying sperm for the duration of a man's lifetime (8).

The risks associated with the propagation of mutated spermatozoal DNA are multifold. Although successful fertilization tends to be inversely correlated with the extent of sperm DNA damage (9), previous research has confirmed that spermatozoa exhibiting significant DNA damage can still retain the capacity for fertilization (10). This risk becomes even more pronounced with the use of intracytoplasmic sperm injection (ICSI), which bypasses several natural barriers, including the requirement for capacitation, acrosomal reaction, and zona pellucida penetration, that might otherwise have prevented functionally incompetent spermatozoa from achieving fertilization. In the event of successful fertilization, the incorporation of the damaged paternal genome confers the risk of serious embryonal developmental disruption and impaired uterine implantation (7, 11). Should pregnancy be achieved, the risk of pregnancy loss is next introduced, as up to 70% of spontaneous abortions can be attributed to aneuploidies (12), and studies have associated sperm chromosomal aneuploidies with unexplained recurrent pregnancy loss (13). Finally, the consequences of a viable pregnancy achieved with genetically compromised sperm are seen in the risk of mutational propagation to potential offspring, which may have a deleterious impact on the development and health of a child. Animal studies have clearly established that spermatozoa damaged by paternal exposure to chemotherapeutic agents can give rise to

offspring with genetic translocations, mutations, and congenital malformations including hydrocephaly and micrognathia (14, 15).

There is a paucity of peer-reviewed literature regarding reproductive outcomes in the acute, posttreatment time interval. Such studies are obviously limited due to ethical and practical considerations. Existing studies involving humans are generally more removed from the acute, posttreatment time frame, and they typically conclude that the frequency of congenital anomalies in the children of men previously exposed to chemotherapeutic agents is no greater than that found among the general population (16). Nevertheless, case studies of men who have managed to conceive in the aftermath of cancer therapy continue to support the possibility of an association between cancer therapy and congenital abnormalities in humans, as instances of resultant syndactyly, tetralogy of Fallot, and anencephaly have been reported (17).

METHODS OF ASSESSMENT

There are numerous methods by which the genetic safety of sperm can be assessed in the laboratory. First, the detection of sperm chromosomal aneuploidy presents an accessible approach to the identification of genetic mutation. Though protocols in the past have used karyotyping after the fusion of human sperm with hamster oocytes (18), more recent techniques routinely employ fluorescent in situ hybridization (FISH) to detect structural chromosomal aberrations, as FISH offers a rapid, less expensive, and technically simpler means of quantifying aneuploidy frequencies (19).

An examination of several key studies from the FISH-based sperm aneuploidy literature enables some broad generalizations regarding time course and sperm safety to be drawn. Frias et al. (20) detected significant inductions of XY, nullisomy sex, and disomy 18, 21, X, and Y aneuploidies in sperm after exposure to Novantrone, Oncovin, Velban, and prednisone (NOVP) combination chemotherapy; comparison of samples 35 to 50 days after treatment with samples 1 to 2 years after treatment revealed that the induced effects did not persist in the latter group. A subsequent study by Tempest et al. (19) found significantly increased frequencies of XY disomy and nullisomy 13 and 21 in sperm at the 6-month mark after either bleomycin, etoposide, and cisplatin (BEP), or doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) chemotherapeutic regimens. They observed a general decline in aneuploidy frequencies at 18 months following treatment, though elevated frequencies persisted in some chromosomes for up to 24 months. Burello et al. (21) confirmed this significant elevation in sperm aneuploidy rate at 6 months after therapy, while noting cases of persistent frequency elevations lasting up to 1 year in their study of patients exposed to chemotherapy and/or radiotherapy. There is a lack of definitive clinical guidelines, but some investigators have suggested that an 18- to 24-month posttreatment time interval may be required to allow for an eventual decline of aneuploidy rates to baseline frequencies.

In addition to chromosomal analysis, the quantification of DNA damage found within spermatozoa provides an alternate method of assessing reproductive safety after

genotoxic insult. Multiple laboratory techniques are available for the evaluation of sperm DNA integrity, including the sperm chromatin structure assay (SCSA), which measures the extent of DNA denaturability in the presence of an acid-detergent resulting from the presence of accumulated strand breaks (22); the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, which quantifies the incorporation of fluorescently labeled dUTP at break points in the DNA strand (23); and the Comet assay, which employs gel electrophoresis to determine the degree of DNA damage (24).

Perhaps as a result of the wide variety of methodologic approaches, study designs, and end points assessed, discrepancies are prevalent among the findings from studies evaluating DNA integrity in the wake of chemotherapeutic or radiotherapeutic exposure. It is generally well established that high levels of DNA damage are seen during and directly after treatment, as demonstrated by Chatterjee et al. (25) using spermatozoa exposed to fludarabine. Ambiguity exists, however, regarding the posttreatment persistence of these genetic effects. A study by Thomson et al. (26) reported no significant differences in sperm DNA integrity between nonazoospermic cancer survivors and age-matched controls. More recently, a large, multicenter, prospective study by Bujan et al. (27) serially assessed men undergoing chemotherapy or radiotherapy for testicular germ cell tumors; in measuring sperm DNA fragmentation via the TUNEL assay and chromatin compaction via the SCSA, they found no higher proportion of patients with increased sperm DNA fragmentation after cancer treatment but did report more men having chromatin defects at 6 months after treatment. Studies by O'Flaherty et al. (28, 29) demonstrated significantly higher levels of sperm DNA damage compared with controls in addition to low DNA compaction, persisting up to 2 years after chemotherapy. Similarly, Spermon et al. (30) found abnormally elevated rates of sperm DNA damage after cisplatin-based chemotherapy as compared with controls. It is interesting that Smit et al. (31) found posttreatment sperm DNA fragmentation index levels to be significantly higher in patients treated with radiotherapy versus those treated with chemotherapy alone. Data from Stahl et al. (23) have also shown patients one or more years out from exclusive radiotherapy to have a greater extent of sperm DNA fragmentation compared with controls. These findings may shed light on a more prominent role played by radiation in inducing genetic damage, though additional studies comparing the effects of chemotherapy and radiotherapy on DNA integrity are needed.

CHEMOTHERAPY IN SEMEN

Aside from the concern for potential damage to the spermatozoal genome, an additional reproductive safety consideration deals with the theoretical possibility of chemotherapeutic compounds being carried via the seminal plasma, thereby exposing the patient's partner and any prospective embryo to cytotoxic agents. Prior work has documented the presence of environmental and pharmaceutical chemicals in human semen, though for the majority of

chemical compounds the seminal concentrations tend to be markedly lower than their serum correlates (32, 33). There is a pronounced paucity of data in the literature, however, with respect to the detection of chemotherapeutic agents in human semen. As part of an investigation into the effects of thalidomide on patients who are seropositive for human immunodeficiency virus (HIV), Teo et al. (34) obtained semen samples from two patients that revealed detectable seminal levels of thalidomide at 4 and 8 weeks after oral administration. In a later study by Chen et al. (35) to measure seminal levels of lenalidomide, a thalidomide analog, and to track the time course of its elimination from the semen, the semen samples collected 2 and 24 hours after oral lenalidomide administration contained measurable levels of the drug. However, samples taken at 72 and 168 hours bore no detectable traces of lenalidomide, which led the investigators to conclude that the agent was eliminated from seminal reservoirs by 72 hours after dosage. Given the small sample sizes evaluated in these protocols and the lack of additional corroborative studies, it is difficult to draw conclusions from these conflicting findings regarding the risk of chemotherapeutic agents being transferred by semen. Although the presence of these particular agents has been detected in human semen, most chemotherapeutic agents have not been similarly investigated.

Prospective, quantitative studies are needed to determine the appearance and persistence of chemotherapeutic agents in the semen of treated males. In considering a hypothetical mechanism of drug absorption by the vaginal mucosa, even for an unlikely agent that happens to be concentrated more than 10 times in seminal plasma versus serum, the female's expected blood exposure would be three orders of magnitude lower than the male's total exposure (33). Thus, an exceptionally high serum drug concentration in the male would be necessary to incite a clinically relevant exposure in his partner via semen. Nevertheless, as the threshold exposure levels for the induction of teratogenic effects by chemotherapeutic agents have not been well established, patients should be advised to use barrier contraception in the aftermath of chemotherapeutic exposure, though an exact time frame for this precautionary measure is difficult to propose because of the limited evidence available. This is another area in need of additional study.

CHALLENGES OF SAFETY DETERMINATION

In the absence of evidence-based clinical consensus, reproductive risk in the context of cancer therapy has proven difficult to quantify, making the determination of safety a challenge. The lack of concordance between study findings directly reflects the immense heterogeneity of study criteria, methodologies, cohorts, variables, and end points that populate the existing body of literature. Further complicating the matter is the probability that the extent of any deleterious effects manifested may vary substantially with the particular chemotherapeutic agents used, variations in treatment regimens, and even the type of malignancy being treated. Furthermore, it is necessary to acknowledge the possibility that a genetic insult to sperm may be incurred independent of the chemotherapeutic or radiation exposure, perhaps as a

function of the underlying malignancy itself; this possibility is exemplified by the fact that elevated sperm aneuploidy rates have been detected in cancer patients before the initiation of any therapy (21).

The ambiguities and prognostic uncertainties introduced by these variables hinder broad statements and conclusions regarding reproductive safety after cancer treatment, particularly with respect to offering a defined posttreatment time course for minimizing risk. In fact, the only guidelines currently available, issued by the European Society for Medical Oncology (ESMO), advocate the deferral of childbearing for at least 12 months after cancer therapy, as a Grade C recommendation based on level IV evidence (36). Clearly, more prospective clinical trials with this patient population are warranted to achieve a more robust evidence base from which broader guidelines can be formulated.

In the meantime, pretreatment sperm cryopreservation should continue to be emphasized in oncologic practice. Although the acquisition of viable, genetically healthy sperm cannot be guaranteed, this method nonetheless provides the most optimal approach to circumventing the genotoxic effects of chemotherapy and radiotherapy. For cancer patients who wish to conceive in the acute, posttreatment time interval and do not have cryopreserved sperm, genetic counseling and testing should be strongly considered to help quantify sperm aneuploidy and DNA damage levels. Testing methodologies, including the FISH, SCSA, TUNEL, and Comet assays, can be used to provide some insight into the genetic integrity of a patient's sperm after therapy. Abnormal results in these tests are clearly linked to an increased risk of adverse reproductive outcomes, as previously discussed. These techniques have thus far been used in a limited fashion clinically in the postoncologic treatment setting, and further study may provide more clarity as to when it is truly "safe" to use sperm after cancer therapy.

These initiatives will undoubtedly help clarify existing discrepancies in the literature and contribute to the overall assessment of male reproductive safety in cancer survivors. The insight gained from such endeavors will be instrumental in helping both patients and providers better understand the reproductive risks encountered in life after cancer.

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