Chapter 108
FERTILITY ISSUES IN THE HEMATOLOGIC MALIGNANCIES
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INTRODUCTION

Young adults diagnosed with cancer are living longer than ever due to improved treatment regimens. The 5-year survival rate for certain subtypes of leukemia and Hodgkin’s and non-Hodgkin’s diseases, for example, has dramatically increased to 75%–90%. However, the neoplastic disease per se and/or its treatment commonly impair fertility, leaving many patients unable to bear healthy, biological children.

Hematologic malignancies, in particular, can adversely affect fertility in a number of ways. Since these diseases generally involve the hypothalamus and pituitary, they can directly affect gonadotropin secretion, resulting in secondary hypogonadism and, hence, defective sperm formation and infertility. In addition, chemotherapy and radiation therapy—both being used to treat hematologic malignancies—are toxic to the male and female gonads. Even if fertility does not decline as a result of therapy or returns naturally, patients can still be rendered sterile by cytotoxic therapy, as these drugs can cause genetic mutations in germ cells. Similarly, any cytotoxic therapy administered to pregnant women has the potential for serious teratogenic consequences on the fetus (see Chapter 106).

IMPACT OF MALIGNANCY ON THE REPRODUCTIVE SYSTEM

In the past, infertility associated with malignant disease was considered a side effect of the drugs and radiation used during the course of treatment. However, this view is changing due to strong evidence that decreased fertility sometimes exists before the treatment starts. In a study conducted on 158 male patients with Hodgkin lymphoma, severe damage to fertility was observed in 21% cases before treatment. The decrease in fertility was most prominent in patients with an elevated erythrocyte sedimentation rate (ESR) and in those with advanced disease. In another study, semen analysis showed that 70% of male patients with Hodgkin lymphoma had reduced fertility before therapy.

The effect of Hodgkin lymphoma on testicular function does not involve local tumor or metastasis. Instead, the disease may lead to structural abnormalities in the testicular parenchyma, such as tubular hyalinization. These changes may be caused by an immune-mediated disorder that alters the balance between distinct subpopulations of lymphocytes, which normally inhibit or stimulate the production of spermatozoa. However, this hypothesis requires further testing. In addition, cytokines (e.g., interleukins and tumor necrosis factor) that are secreted by tumor tissue may be partly responsible for the impaired testicular function seen in patients with Hodgkin’s disease.

In general, malignancy is associated with an increased catabolic state and malnutrition. Therefore, most patients experience weight loss and decreased reproductive capacity. In addition, hypothalamic dysfunction can occur and pituitary gonadotropin levels can fall, thus affecting the fertility. Stress hormones may further reduce fertility by leading to a rise in prolactin and endogenous opiate secretion, which in turn suppress gonadotropins.

GONADAL TOXICITY FOLLOWING MALIGNANCY TREATMENT

In addition to Hodgkin and non-Hodgkin’s lymphoma, acute lymphocytic leukemia and acute myeloid leukemia are among the most common neoplastic disorders during the reproductive years. As the mortality rate decreases and the survival rate increases, the consequences of cancer treatment vis-a-vis impaired fertility are more frequently encountered.

SUSCEPTIBILITY OF THE MALE GONAD TO CANCER TREATMENT

Testicular architecture

Chemotherapeutic agents enter testis via blood vessel plexus in the interstitial region. Although the Sertoli
cells usually maintain a protective barrier between the blood and the testicular germ cells, many chemotherapeutic drugs can severely interrupt the integrity of this barrier.

Germ cells that do actively differentiate are more susceptible to cytotoxic injury, resulting in necrosis, whereas testicular somatic cells are affected only in function. As a result, cytotoxic therapy can deplete germ cells to the point where the seminiferous tubules contain only Sertoli cells. The depletion occurs in a time-dependent manner because late-stage germ cells (spermatocytes onward) are relatively more resistant. However, studies in rodents revealed that these late-stage cells are susceptible to mutagenesis, and any mutations in their DNA can be passed on to the next generation.11 Surviving stem cells can remain in the testis, but will fail to differentiate into mature spermatозoa for several years after cytotoxic abuse. The eventual recovery of sperm production depends on the survival of the spermatogonial stem cells, as well as on their ability to differentiate.12

The effects of cancer therapy on testicular architecture vary with the patient's age and pubertal status. It was initially thought that the testes of pre- and peri-pubertal males were less vulnerable to toxic effects induced by treatment. However, it is now clear that these patients experience as much testicular structure damage following chemo/radiotherapy as adults.13

Hormonal imbalances
The loss of germ cells exerts secondary effects on the hypothalamic-pituitary-gonadal axis. Germinal aplasia reduces the size of the testes. Consequently, testicular blood flow decreases, thus reducing the testosterone levels in the circulation.14 Because testosterone is a negative regulator of luteinizing hormone (LH), which is secreted by the pituitary, and LH is the primary stimulator of testosterone synthesis by the Leydig cells, LH increases to maintain constant serum testosterone levels. In addition, inhibition in secretion by the Sertoli cells declines and, as inhibin limits follicle-stimulating hormone (FSH) secretion by the pituitary, serum FSH levels tend to rise.

SUSCEPTIBILITY OF THE FEMALE GONAD TO CANCER TREATMENT
Ovarian architecture
Histological sections of ovaries exposed to cytotoxic drugs show a spectrum of changes, ranging from reduced number of follicles to no follicles and fibrosis.15 The exact incidence of premature ovarian failure (POF) after chemotherapy is difficult to establish because there are many contributing factors. Depending on the type of chemotherapy regimen used, the incidence of amenorrhea ranges from 0% to 100%.16 Cytotoxic drugs may impair follicular maturation and/or deplete primordial follicles.15,17 Temporary amenorrhea occurs when cytotoxic drugs destroy maturing follicles, whereas permanent amenorrhea or POF occurs when all primordial follicles are destroyed. The close structural and functional relationship between the oocyte and the hormone secreting-granulosa cells makes it difficult to identify an exact target for cytotoxic drugs. The destruction of one leads to the demise of the other.

Hormonal imbalances
Unlike male germ cells, female germ cells proliferate only during prenatal life; after birth, these progressively decrease in number due to apoptosis, and ovulation. Germ cells inside the female gonad do not proliferate, whereas the somatic cells do. Radiation and chemotherapy induce oocytes to undergo apoptosis, which reduces the number of germ cells,18 resulting in estrogen insufficiency. Therefore, when follicles are destroyed by cytotoxic therapy, the frequency of menstrual decreases and amenorrhea commonly occurs. Irreversible ovarian failure and menopause occur if the number of follicles falls below that is required for menstrual cyclicity.

EFFECT OF MALIGNANCY TREATMENT ON FERTILITY
Post-treatment of Hodgkin's lymphoma in men with chemotherapy results in testicular germ cell aplasia and decreased libido.5 The seminiferous epithelium inside the testes is most sensitive to the detrimental effects of chemotherapy. Therefore, after treatment with gonadotoxic agents, patients may be rendered oligozoospermic or azoospermic. Because testosterone production by the Leydig cells remains unaffected, patients still develop normal secondary sexual characteristics.19 However, treatment with high, cumulative doses of gonadotoxic chemotherapy can lead to Leydig cell dysfunction.

Doses as low as 0.1 Gy to 1.2 Gy can have detectable effects on spermatogenesis in adult males, with doses over 4 Gy causing more permanent effects.20 Somatic cells are more resistant to chemotherapy and radiation-induced damage than are germ cells. Indeed, Leydig cell dysfunction is not observed until doses of 20 Gy are administered to the prepubertal boy and up to 30 Gy in sexually mature males.21 Testosterone production is therefore relatively preserved below these doses. Thus, many patients develop normal secondary sexual characteristics despite a severe impairment of spermatogenesis.

Young patients with a hematological malignancy are often treated with bone marrow transplantation (BMT).22 During BMT, patients may be given alkylating agents and receive total body irradiation for conditioning, both of which result in POF, hormonal disturbances, and eventually, infertility.23 Long-term female survivors treated with total body irradiation and BMT are at risk for ovarian follicular depletion and impaired uterine growth and blood flow, in addition to early pregnancy loss and premature labor if pregnancy is
achieved. Because women aged above 30 years face a higher incidence of POF following chemotherapy, their treatment regimens should contain fewer alkylating agents.

A study that documented the late effects associated with treatment of early Hodgkin's lymphoma revealed that 43/191 men and 16/149 women had sought medical advice for infertility, while 57/191 men and 54/149 women were able to parent children. In addition, sexual activity was disrupted in 25.8% of cases.

**TRANSMISSION OF GENETIC MATERIAL**
Radiation and several alkylating agents can produce single-gene mutations and chromosomal translocations in spermatogonia. The persistence of a mutation depends mainly on its location. Mutations that occur early in stem spermatogonia will produce mutation-carrying sperm for the lifetime of the male, whereas those occurring in later stages of spermatogenesis will only lead to a mutation-carrying sperm for a few months. Meliotic and post-miotic germ cells are more susceptible to mutations than are stem spermatogonia. Therefore, the mutational risks are highest when a pregnancy occurs within one spermatogenic cycle after the male is exposed to the damaging agent. In females, most alkylating agents and a variety of other chemotherapeutic drugs induce chromosome aberrations or other mutations in developing oocytes that result in embryonic death.

Although sperm DNA integrity can vary greatly among cancer patients, patients with Hodgkin's and non-Hodgkin's diseases generally have a significantly higher prevalence of DNA damage than healthy men. Sperm DNA damage can be assessed with a variety of techniques, but none can definitively determine whether the mutations will be passed onto any offspring.

**POTENTIAL FOR FERTILITY FOLLOWING MALIGNANCY TREATMENT**

**RESTORATION OF FERTILITY**
Sperm quality may naturally improve after cancer treatment. However, some defects may persist. The incidence of infertility in men who have recovered sperm production following cytotoxic therapy is generally not higher than that of the general population. An interesting case report has documented paternity following bone marrow conditioning and transplantation in a patient with acute myeloid leukemia. For the first time, the preservation of fertile sperm was seen despite the use of chemotherapy. Cancer patients with sperm counts below normal (oligozoospermic) are still capable of having children. Similarly, infertile women who have menstrual dysfunction following cytotoxic therapy may be treated for menstrual dysfunction and infertility in a manner similar to that of the general population. However, the risk of an adverse pregnancy outcome is higher in these women, and they may require closer observation.

**ASSISTED REPRODUCTIVE TECHNIQUES**
The reproductive capacity of individuals undergoing malignancy treatment can be preserved by cryopreserving the gametes and using assisted reproductive techniques (ART) when pregnancy is desired. When non-cryopreserved spermatozoa are used in combination with intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI), clinical pregnancy rates of 30-40% per cycle and delivery rates of 30% can be expected at most reproductive clinics. On the other hand, cryopreserved sperm from cancer patients results in complete pregnancies in only 18% of cycles. Similarly, autologous cryopreserved embryos from in vitro-fertilized oocytes can be successfully implanted after cytotoxic therapy if the patient can undergo ovarian hyperstimulation before therapy.

Men who remain azoospermic long after chemotherapy may benefit from testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI). The potential for sperm retrieval is not clearly affected by the chemotherapy regimen or by the disease treated. Therefore, men should not be considered sterile despite prolonged non-obstructive azoospermia after undergoing chemotherapy.

**OPTIONS FOR FERTILITY PRESERVATION**

**SEmen CRYOPRESERVATION**
With the advancement of ART, all men diagnosed with cancer should be offered the option of semen cryobanking, a procedure that provides the only reasonable chance of establishing pregnancy after therapy. Semen cryopreservation is a widely available and inexpensive option (< $1000) that yields good results. Patients diagnosed with cancer used to be considered poor candidates for sperm cryopreservation because they present with disease-induced suboptimal semen quality and cryosensitivity. Men with Hodgkin's lymphoma have pre-freeze and post-thaw sperm quality that is below normal. However, as a general rule, there is no cancer group for which sperm cannot be retrieved and stored. Even the absence of spermatozoa in semen should not prevent physicians from attempting to preserve a patient's fertility. In many cancer patients who suffer from azoospermia before treatment, testicular sperm extraction “Onco-tese” may be successfully attempted (unilateral or bilateral), and the retrieved sperms may be cryopreserved for future use.

Almost 40% of patients who cryopreserve their semen may have a healthy live birth using ART.
last two decades, the percentage decline in semen quality (from pre-freeze to post-thaw) in patients with cancer shows a similar trend that of normal donors. This suggests that the effect of cryodamage on spermatozoa from patients with cancer is similar to that of normal donors.\textsuperscript{42,43} Cryopreserving semen after the start of therapy can adversely affect their chromosomal structure, causing de novo mutations, but should still be attempted if the imperativeness of starting therapy outweighs the chance for cryopreservation, as viable sperm may still be recovered. Therefore, it is crucial to cryopreserve sperm before chemotherapy or radiotherapy and also to advocate the use of contraception during therapy and for 6 months after.

Only a small percentage of patients (< 10%) who bank their spermatozoa before chemotherapy or radiotherapy return for assisted reproduction.\textsuperscript{44-46} This finding may be explained by several reasons: recovery or waiting for possible resumption of spermatogenesis, short period from original illness, anxiety regarding potential risks for the children, and uncertainty about long-term health and, therefore, suitability to be parents.\textsuperscript{42} However, trends have started to change, and awareness of sperm banking has increased over the past 4 to 5 years, coinciding with the advent of ICSI.

In males with cancer, the extent of sperm DNA damage plays an important role in determining how semen should be cryopreserved before therapy begins. Specimens with high sperm concentration and motility and low levels of DNA damage can be preserved in relatively large aliquots suitable for IUI. If a single specimen of good quality is available, then it should be preserved in multiple small aliquots suitable for IVF or ICSI.\textsuperscript{28}

**TESTICULAR TISSUE HARVESTING**

Although spermatogenesis does not occur in prepubertal testes, and prepubertal testes do not produce mature spermatozoa, these do contain the diploid stem germ cells from which haploid spermatozoa can be derived. Therefore, testicular tissue can be harvested from a biopsy and stored either as a tissue section or as isolated germ cells, before cancer therapy. Following cure and on entering adulthood, this tissue can be thawed and used to produce offspring in either of the two ways: the stored germ cells can be re-implanted into the patient's own testes to restore natural fertility, a procedure known as germ cell transplantation, or the stored stem cells can be matured in vitro until they are able to achieve fertilization via ICSI.\textsuperscript{47} Although these two measures have been the subject of intensive research in the last decade, further refinements in the protocols may still be needed before they can be used routinely in clinical practice.

**Germ cell transplantation**

Germ cells isolated from the testes of donor male mice can repopulate immunologically compatible testes when injected into the seminiferous tubules of recipient animals; the recipients show normal morphological features characteristic of the donor species.\textsuperscript{48} Similarly, mouse germ cells transplanted into the testes of fertile mice colonize the recipient seminiferous tubules and initiate donor spermatogenesis in more than 70% of recipients.\textsuperscript{49} The most striking result of these experiments was that healthy offspring (by mating) were produced from spermatozoa generated within the recipient testes by donor germ cells.

Establishing a successful method for testicular stem cell transplantation of frozen, thawed testicular cells would be of immense benefit to boys with childhood cancer undergoing sterilizing treatment. It is possible to reinitiate spermatogenesis after cryopreservation of testicular germ cell suspensions. Although cell survival is acceptable, current protocols need further improvement.\textsuperscript{50} Male germ cells obtained before chemotherapy can be frozen and, after thawing, can be transplanted into animals to maintain the entire genetic information of the donor for a limited period.

Before stem cell transplantation can be considered for preserving the fertility of pre-pubertal boys, two issues must be carefully examined.\textsuperscript{51} First, the testis biopsy taken from the cancer patient may contain malignant cells. These cells must be removed from the cell suspension because studies in rats have shown that one single malignant cell can reintroduce the disease. Second, the cell suspension consists of all testicular cells, and the proportion of spermatogonial stem cells is low (estimated at 1/5000).\textsuperscript{52}

**In vitro maturation**

In vitro germ cell maturation would be particularly useful in patients who have received extremely gonadotoxic therapy and in whom the supporting Sertoli cells would be unable to support spermatogenesis. Mouse spermatogonial stem cells can survive up to 4 months in culture and retain their ability to commence spermatogenesis following transplantation into a recipient.\textsuperscript{53} However, it appears that current methods for in vitro maturation of diploid stem cells into haploid spermatozoa are not well developed. Ongoing research may improve their feasibility.

**OOCYTE CRYOPRESERVATION**

Although successful fertilization and embryonic cleavage have been reported after injection of cryopreserved thawed oocytes, the pregnancy rate is not high enough to justify its routine use in clinical practice.\textsuperscript{54} The main reason for poor outcomes after oocyte cryopreservation is related to the oocyte's structural complexity. Oocyte subcellular organelles are far more complex and perhaps more sensitive to thermal injury than preimplantation embryos. Oocyte donation may be considered in cases characterized by complete ovarian depletion. However, the presence of other factors, such as uterine impairment, would be of
major concern. In addition, complications during pregnancy and pre-term deliveries would be expected in these cases.55

OVARIAN TISSUE CRYOPRESERVATION
Ovarian tissue banking in humans is being considered to restore fertility in patients who lose ovarian function because of chemotherapy or radiotherapy.56 Ovarian tissue cryopreservation and transplantation was first examined in rodent studies and then in sheep and human ovarian xenograft studies.57 However, no pregnancies have been reported in humans from the use of cryopreserved ovarian tissue. Although promising, there is a theoretical risk that malignant stem cells will be reimplanted along with the thawed cryopreserved ovary.58,59 With the publication of promising data from humans, ovarian tissue cryopreservation from selected patients before cancer treatment and in those requiring oophorectomy may be advocated. However, this option is currently under experimental evaluation, and few centers offer this to patients.

EMBRYO CRYOPRESERVATION
Embryo cryopreservation was introduced to maximize the chances of conception during a single menstrual cycle. Cryopreservation of preimplantation embryos is currently an integral part of patient care in clinical practice. This option may not be socially acceptable in prepubertal females and adolescents. However, acceptable, long-term data are available about the outcome of children born from these procedures.60

CHOICE OF CYTOTOXIC REGIMENS
Currently, treatment regimens for hematologic malignancy include a variety of chemotherapeutic agents, all of which affect reproductive functions differently. For young patients, agents with minimal toxicity but maximal therapeutic effect are selected. For example, NOVP (Novantrone (mitoxantrone), Oncovin (vincristine), Vinblastine, Prednisone) may be preferred over MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) for the treatment of Hodgkin’s lymphoma. Although NOVP markedly affects spermatogenesis, sperm production recovers rapidly after treatment, usually within 3 to 4 months. This rapid recovery is due to the fact that NOVP chemotherapy damages spermatogenic germ cells rather than inhibiting stem cells.61

Similarly, ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) is used to treat Hodgkin’s disease instead of MOPP because the former dramatically reduces gonadal toxicity.62 VAPEC-B (adriamycin, cyclophosphamide, etoposide, vincristine, bleomycin, and prednisolone), which is used in the treatment of non-Hodgkin’s lymphoma, minimizes the dose of cyclophosphamide and therefore results in less gonadal failure than CHOP-Bleo (cyclophosphamide, adriamycin, Oncovin (vincristine), prednisone, bleomycin).63

GONADAL SHIELDING
The gonads must be outside the field of radiation or shielded from the direct radiation beam unless they are being irradiated directly as a result of actual or potential neoplastic involvement. Although gonadal shields can reduce the amount of radiation two- to fivefold, some radiation may still reach the gonads. For example, the gonads typically receive 2 to 3 Gy with an inverted Y-field, which is used for Hodgkin’s disease.64 To minimize ovarian exposure, oophoropexy may be performed to relocate the ovaries away from the direct beam.65,66 Laparoscopic oophoropexy may be of benefit in cases of Hodgkin’s disease, if performed before pelvic irradiation.67

MEDICAL TREATMENT
Hormones
When testosterone suppressors such as gonadal steroids, GnRH analogs, and antiandrogens were used before and during cytotoxic therapy in male rats, they enhanced the recovery of spermatogenesis and fertility.68 These suppressors may work by enhancing the potential of the somatic cells in the testis to support the recovery of spermatogenesis.69 For a while, it was assumed that recovery of stem spermatogonia cells could possibly be stimulated after prolonged periods of iatrogenic azoospermia, but research does not support this theory. Hormone treatment given before and during cytotoxic therapy was found to protect spermatogenesis in only one of eight clinical trials.40

Gonadotropin-releasing hormone (GnRH) agonists may protect ovarian function from the effects of cyclophosphamide71 by decreasing the recruitment of primordial follicles. Strong evidence supports the use of GnRH agonistic analogues to minimize the gonadotoxic effect of chemotherapy because they induce a pre-pubertal milieu.59,72 However, the feasibility of using oral contraceptives or GnRH agonists to protect women against ovarian damage has not been established.73 Another hormone, medroxyprogesterone, helps protect primordial follicles from the acute toxic effects of chemotherapy. Nevertheless, the quality of the follicles will be impaired, and many will undergo atresia, resulting in a shortened fertility period.74

Hormone replacement therapy (HRT) should be considered in young pre-menopausal women who have developed ovarian failure due to malignancy or cancer treatment.75 Even with the use of HRT, though, uterine size can decrease by 40%.24 Importantly, any residual ovarian function remaining after chemotherapy is considered a good prognostic sign because the ovaries may be stimulated with steroid hormones and/or gonadotropins.76

Anti-apoptotic drugs
Oocytes exposed to chemotherapeutic agents in vitro undergo various changes leading to apoptosis.77 Because a series of specific signaling events are
activated in the cell that is bound for apoptosis, inhibiting these signaling events could potentially stop the apoptotic process and protect the patient from POF. Sphingosine-1-phosphate is an example of an apoptotic inhibitor. The oocytes of mice that had been treated with sphingosine-1-phosphate therapy resisted apoptosis that was induced by doxorubicin. This concept offers a promising experimental alternative to guard against apoptosis. With the eventual identification of the molecular and genetic framework of chemotherapy-induced germ cell death, apoptotic inhibitors may some day play a role in preventing oocyte loss.

**ETHICAL AND LEGAL ISSUES**

Options for future fertility following cancer treatment must be considered in the patient's best interests. Thus, the advantages of any intervention or of an active decision not to intervene must outweigh any disadvantages, both in the short and long term. Any intervention intended to preserve fertility must have a sound evidence base as well as moral provenance. It should neither raise unrealistic expectations, nor have long term adverse effects on the patient or his or her offspring.

Informed consent should be given voluntarily by a competent person. However, in view of the complexity of the issues surrounding fertility preservation, the anxieties of both patients and their families at the time of diagnosis, and the limited time for discussion due to the urgency of commencing treatment, the validity of such consent may be impaired. The first stage of consent is for the collection and storage of the germinal tissue or gametes. The second stage is for use of the collected material for fertilization. In addition, it is important to consider what will happen to stored cells in the event of divorce or the patient's death. While some would advocate destruction of the tissue in the latter situation, others have suggested allowing the parents to donate the tissue for research purposes.

**SUMMARY**

Patients with hematologic malignancies have impaired fertility indirectly as a result of necessary cytotoxic treatment regimens. The deterioration in fertility potential may be temporary or permanent. However, the decreasing mortality rate and the increasing survival rate as a result of effective treatment have made fertility issues more frequently encountered.

A variety of measures may be used to minimize the deleterious effects of malignancy and its treatment on the human fertility potential. Moreover, assisted reproductive techniques in combination with our rapidly evolving understanding of cryobiology offer encouraging measures to preserve productiveness following malignancy treatment. These measures should be considered in young adults, and patients should be counseled regarding the pros and cons of each of the available options for fertility preservation.

**REFERENCES**


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