

# Chapter 1

## Gonadotoxicity of Cancer Therapies in Pediatric and Reproductive-Age Females

Jennifer Levine

### Introduction

In the United States, almost 120,000 females under the age of 50 years are diagnosed with cancer annually [1]. Retaining reproductive potential is extremely important to those diagnosed with cancer, and reproductive compromise can cause distress well into survivorship [2, 3]. Despite this, patients often do not recall discussions at diagnosis regarding fertility and options for fertility preservation [4, 5]. Barriers to these discussions are myriad [6] but include a lack of knowledge of the risk for reproductive compromise [7] in individual premenopausal female patients diagnosed with cancer. It is of critical importance to attempt to understand the factors, both treatment- and host-related, that impact the ability of cancer survivors to have biological children posttreatment. This chapter will summarize normal female reproductive physiology; address the gonadotoxic impact of chemotherapeutic agents, radiation therapy, and surgical intervention on reproductive function; and briefly discuss the current methodologies available for assessing reproductive potential after cancer treatment.

### Female Reproductive Physiology

Although there has been some preliminary work suggesting that female germ cells possess the capacity to proliferate postnatally [8], it is generally accepted that females are born with a fixed number of ovarian primordial follicles, estimated to

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J. Levine, M.D., M.S.W. (✉)  
Division of Oncology, Columbia University Medical Center,  
161 Fort Washington Ave, IP-7, New York, NY 10032, USA  
e-mail: jl175@columbia.edu

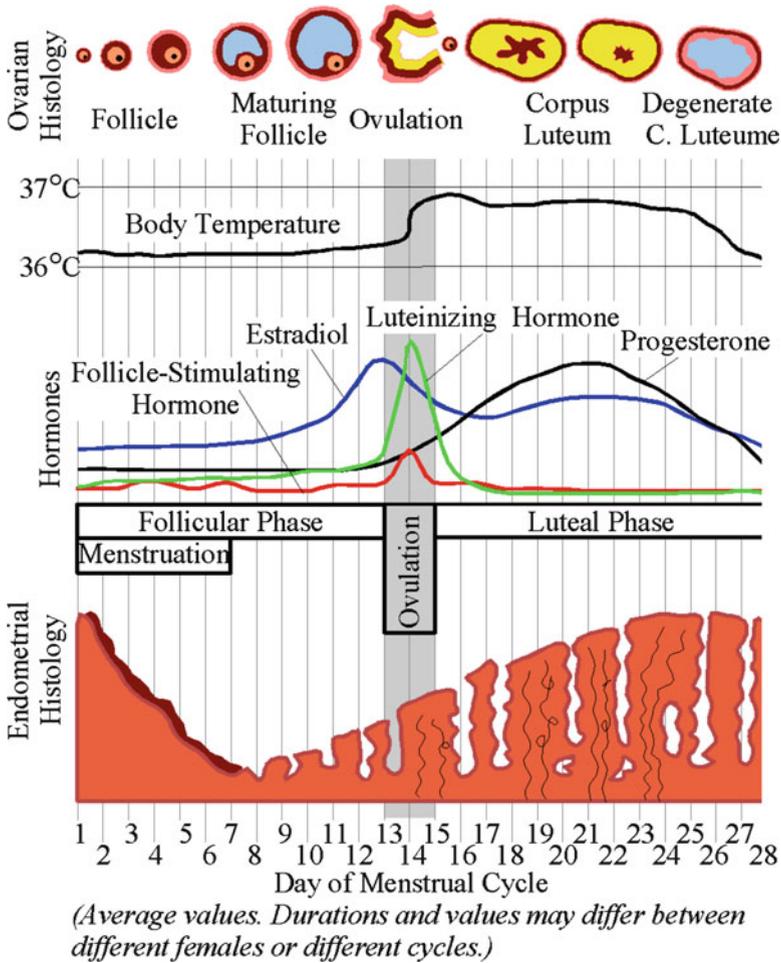
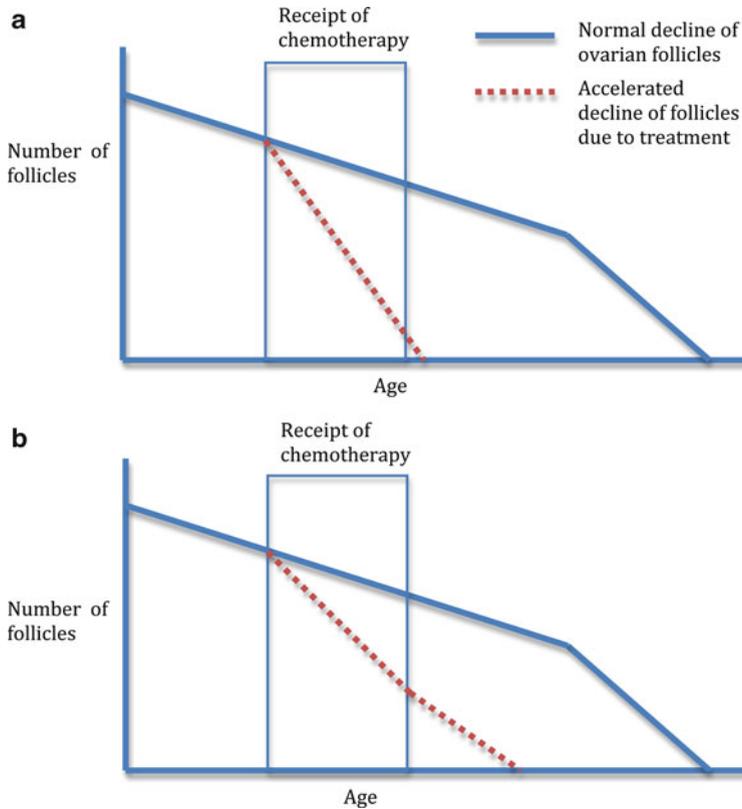


Fig. 1.1 The normal menstrual cycle [9]

be on the order of one million. By the onset of puberty, approximately 400,000 follicles remain, which mature under the influence of hormones secreted within the hypothalamic-pituitary-ovarian axis. The secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary (Fig. 1.1) [9]. With each cycle, multiple follicles are selected to enter the growing pool and begin to mature; an elevation in FSH results in one of these follicles becoming the dominant follicle that is selected for ovulation while the remaining follicles undergo atresia. The dominant follicle produces estradiol, which triggers an LH surge mid-cycle and results in the mature oocyte being released into the fallopian tube where fertilization can occur. Through this process—and additional atresia and apoptosis of unselected follicles—a progressive decline in the number of ovarian primordial follicles, the



**Fig. 1.2** Advanced ovarian failure and premature menopause after chemotherapy

“ovarian reserve,” occurs during a healthy woman’s reproductive lifespan. When a healthy woman reaches her mid- to late 30s, a threshold number of follicles are reached at which reproductive potential drops significantly, follicular depletion accelerates, and the remaining oocytes are of overall poorer quality [10]. With continued decline in the ovarian reserve, a second threshold occurs when a woman is in her late 40s or early 50s—the start of the menopause—when it is no longer possible to have biological children [11].

The natural depletion of the finite number of follicles present in an individual female’s ovaries can be accelerated by cancer treatment. If the degree of depletion that occurs from cancer treatment is near complete (represented by the dotted line in Fig. 1.2a), then the result is acute ovarian failure, i.e., early menopause with consequent infertility that occurs during or shortly after treatment. In these patients, fertility preservation must be undertaken prior to the start of cancer therapy. If the degree of depletion caused by treatment is more moderate (represented by the dotted line in Fig. 1.2b), then the individual is at risk of premature menopause, i.e., ovarian failure that occurs before the age of 40 years. In the case of premature

menopause, females remain fertile following cancer therapy but have an overall shortened reproductive lifespan. In these patients, fertility preservation may be appropriate prior to the start of cancer therapy, but there is also a window of time during which natural or assisted reproduction can be attempted following the completion of therapy.

## **Effect of Cancer Treatments in Pediatric and Reproductive-Age Females**

Cancer therapies can impact the reproductive capacity of females through a number of mechanisms. Attaining a successful, unassisted biological pregnancy requires that an individual female has adequate ovarian follicular reserve, a functioning hypothalamic-pituitary-ovarian axis, a uterus that can adequately expand to accommodate a growing fetus, and the ability of other organ systems, including the cardiac system, to respond to the changes associated with pregnancy. Alterations in any of these areas due to cancer treatment could compromise the ability of a female survivor to conceive or carry a biologic pregnancy to term.

### ***Chemotherapy***

Most chemotherapeutic agents work by affecting cell cycle division. Therefore, it can be anticipated that anticancer agents would likely affect oocytes that are entering a phase of maturation as well as the growth and proliferation of the somatic cells that support the oocyte within individual ovarian follicles. This mechanism serves to explain the acute impact that chemotherapy frequently has on postpubertal women, namely, the cessation of menses during and immediately following therapy [12]. This includes an acute disruption in reproductive hormone levels that resolves following the completion of therapy in women who presumably have retained fertility potential [12].

What is less well understood is the impact that chemotherapeutic agents have on the relatively quiescent immature oocytes that have not yet been selected into the growing pool. Alkylating agents have a non-cycle-specific mechanism of action leading to single- and double-strand DNA breaks, thereby potentially affecting both quiescent and dividing cells in the ovary [13]. There is also evidence of cortical fibrosis and blood vessel damage in ovarian tissue that has been exposed to alkylating agents compared with tissue that has not been exposed [14]. Cyclophosphamide and procarbazine are particularly toxic to the ovary and therefore present a relatively high risk to maintenance of fertility after treatment [15]. Anthracyclines, such as doxorubicin and the platinum agents cisplatin and carboplatin, also confer a high risk of gonadotoxicity, although usually less than that of alkylating agents [16, 17]. Recent research in humans suggests that the mechanism of ovarian injury by doxorubicin also involves microvascular and stromal damage [18].

Ultimately, the impact of cancer treatment on reproductive potential depends on the age of the patient at the time of treatment, the particular chemotherapeutic agents used, the duration of treatment, the total cumulative dose administered, and, likely, patient-specific factors, as similar regimens can have very different effects on women of the same age. The effect of age is presumably related to the smaller number of follicles in the ovaries of older patients at the time of treatment such that acute ovarian failure may be more likely to occur in older women and premature menopause in younger women or adolescents [19, 20].

Assessing the contribution of individual chemotherapeutic agents on ovarian damage is difficult, as almost all drugs are given as part of multimodality regimen. One approach to describing gonadotoxicity of chemotherapy has been to identify the toxicity of specific treatment regimens rather than single agents (Table 1.1) [21]. Yet, it should be noted that even this approach has its limitations as treatment regimens

**Table 1.1** Effect of cancer treatment on development of amenorrhea (Reprinted with permission from Livestrong/Fertile Hope [21])The following table represents a compilation of both clinical experience and the published research on the impact of common cancer treatments on menstruation. Generally, studies have not focused on other measures of reproductive capacity, such as hormone levels or follicle counts which may more accurately reflect reproductive capacity

Degree of risk	Treatment protocol	Common usage
<i>High risk</i>	Whole abdominal or pelvic radiation doses	Multiple cancers
>80% of women develop amenorrhea posttreatment	≥6 Gy in adult women	Wilms’ tumor, neuroblastoma, sarcoma, Hodgkin lymphoma
	Whole abdominal or pelvic radiation doses	
	≥15 Gy in prepubertal girls ≥10 Gy in postpubertal girls	
	TBI radiation doses	Bone marrow transplant/stem cell transplant (BMT/SCT)
	CMF, CEF, CAF×6 cycles in women 40+	Breast cancer
	Cyclophosphamide 5 g/m <sup>2</sup> in women 40+	Multiple cancers
	Cyclophosphamide 7.5 g/m <sup>2</sup> in girls <20	Non-Hodgkin lymphoma (NHL), neuroblastoma, acute lymphoblastic leukemia (ALL), sarcoma
	Alkylating chemotherapy (e.g., cyclophosphamide, busulfan, melaphan) conditioning for transplant	BMT/SCT
	Any alkylating agent (e.g., cyclophosphamide, ifosfamide, busulfan, BCNU, CCNU)+ TBI or pelvic radiation	BMT/SCT, ovarian cancer, sarcoma, neuroblastoma, Hodgkin lymphoma
	Protocols containing procarbazine: MOPP, MVPP, COPP, ChIVPP, ChIVPP/EVA, BEACOPP, MOPP/ABVD, COPP/ABVD	Hodgkin lymphoma
Cranial/brain radiation ≥40 Gy	Brain tumor	

(continued)

**Table 1.1** (continued)

Degree of risk	Treatment protocol	Common usage
<i>Intermediate risk</i>	CMF or CEF or CAF × 6 cycles in women 30–39	Breast cancer
~30–70% of women develop amenorrhea posttreatment	AC in women 40+	Breast cancer
	Whole abdominal or pelvic radiation 10 to <15 Gy in prepubertal girls	Wilm’s tumor
	Whole abdominal or pelvic radiation 5 to <10 Gy in postpubertal girls	Wilm’s tumor, neuroblastoma
	Spinal radiation ≥25 Gy	Spinal tumor, brain tumor, neuroblastoma, relapsed ALL or NHL
<i>Low risk</i>	AC in women 30–39	Breast cancer
<20% of women develop amenorrhea posttreatment	CMF, CEF, or CAF × 6 cycles in women under 30	Breast cancer
	Nonalkylating chemotherapy: ABVD, CHOP, COP	Hodgkin lymphoma, NHL
	AC (anthracycline, cytarabine) Multiagent therapies	Acute myeloid leukemia (AML) ALL
<i>Very low/no risk</i>	MF (methotrexate, 5-FU)	Breast cancer
Negligible effect on menses	Vincristine (used in multiagent therapies)	Leukemia, Hodgkin lymphoma, NHL, neuroblastoma, rhabdomyosarcoma, Wilms’ tumor, Kaposi’s sarcoma
	Radiocactive Iodine	Thyroid cancer
<i>Unknown risk</i>	Paclitaxel, docetaxel (taxanes used in AC protocols)	Breast cancer
	Oxaliplatin	Ovarian cancer
	Irinotecan	Colon cancer
	Bevacizumab (Avastin)	Colon, nonsmall cell lung
	Cetuximab (Erbix)	Colon, head, and neck
	Trastuzumab (Herceptin)	Breast cancer
	Erlotinib (Tarceva)	Nonsmall cell lung, pancreatic
	Imatinib (Gleevec)	Chronic myeloid leukemia (CML), gastrointestinal stromal tumor (GIST)

continue to evolve over time. MOPP therapy (mechlorethamine, vincristine, procarbazine, prednisone) was a common therapy for Hodgkin lymphoma and caused amenorrhea in up to 80% of females and acute ovarian failure in 39–46% of young adults [22, 23]. More contemporary regimens that contain alkylating agents, such as COPP/ABVD (cyclophosphamide, vincristine, procarbazine, prednisone/doxorubicin, bleomycin, vinblastine, dacarbazine) and BEACOPP (bleomycin/etoposide/adriamycin/cyclophosphamide/oncovin/procarbazine/prednisone), are also associated with ovarian failure. Kreuser et al. identified premature ovarian failure in 77% of patients following COPP/ABVD therapy [24]. Regimens that limit the use of alkylating agents, such as ABVD, have demonstrated lower rates of gonadotoxicity than regimens such as dose-escalated BEACOPP [25]. One small

study of 40 women treated with ABVD and radiation therapy demonstrated no acute ovarian failure in women younger than 25 years of age and transient amenorrhea in 33% of women younger than 45 years of age [26].

CMF (cyclophosphamide, methotrexate, 5-fluorouracil), once used in the treatment of breast cancer, was found to be associated with rates of amenorrhea ranging from 21% to 71% in women under 40 years of age and 40–100% of women older than 40 years of age [27, 28]. Newer regimens such as AC (doxorubicin, cyclophosphamide) have been associated with a significantly lower rate of amenorrhea (55%), whereas the addition of taxane is associated with a significantly higher rate of amenorrhea (64%) [29]. In this study, women over the age of 40 years who received a taxane had a higher odds ratio of developing amenorrhea as well as a higher risk of the amenorrhea remaining permanent than women who did not receive a taxane.

The high dose of cyclophosphamide that is often used as conditioning for hematopoietic stem cell transplant (HSCT)—in conjunction with other chemotherapy agents or total body irradiation (TBI)—carries a high risk for ovarian failure [30]. Little information is available on newer chemotherapy agents and targeted therapies, and it is therefore important that this be a future research focus within the field of oncofertility.

## ***Radiation Therapy***

Irradiation of the ovaries—as a consequence of TBI; direct pelvic, abdominal, or spinal irradiation; or scatter irradiation—in doses as low as 1–2 Gy in girls [31] and 4–6 Gy in adults [32] can have a permanent negative effect on the ovaries by causing the depletion of follicles. In the Childhood Cancer Survivor Study (CCSS), pelvic radiation was found to be one of the significant risk factors for acute ovarian failure and premature menopause [15].

Using the estimate that the lethal dose required to kill half the number of existing oocytes (the LD50) is less than 2 Gy, Wallace et al. developed a model to determine when ovarian failure will occur for a given dose of radiation to the gonads [33]. As with chemotherapy exposure, older age confers an increased risk, with an effective sterilizing radiation dose at birth estimated to be 20.3 Gy that decreases to 14.3 Gy by 30 years of age. Increasing doses of radiation and the schedule of delivery also determine the ultimate degree of ovarian damage [34]. Irradiation on the order of 14–30 Gy may also impact fertility by causing damage to the uterine musculature and vascular structures, thereby limiting the ability of a survivor to carry a pregnancy to term [35], as well as increasing the incidence of intrauterine growth retardation and spontaneous miscarriage [36].

High-dose cranial irradiation, in the dose range of 35–40 Gy or greater, can cause hypogonadism through effects on the hypothalamus and pituitary [37]. Impaired fertility achieved via this mechanism differs from direct irradiation of the ovaries in that it can be treated with hormone replacement therapy if the ovarian reserve has otherwise been preserved.

## ***Surgery***

The surgical resection of reproductive organs, i.e., hysterectomies or oophorectomies, has obvious implications for infertility. In earlier stage cervical cancers, use of conservative surgical techniques can preserve the uterus such that carrying a future pregnancy is possible. Such techniques include cervical conization—the surgical resection of a cone-shaped portion of the cervix that includes the cancerous tissue with an unaffected margin—which is appropriate for the earliest stages, and radical trachelectomy—the surgical removal of part or the entire cervix, surrounding connective tissue, and adjacent lymph nodes—for later stages. Both of these procedures retain the uterus. Conception rates of 53% have been reported post-radical trachelectomy, although premature labor and miscarriage remain as complications [38].

## ***Impact of Cancer Therapy on Cardiac Function***

The impact of cancer treatments on the ability to bear biologic children is not limited to reproductive organs. Late effects from chemotherapy can also impact a female's ability to tolerate the physiologic changes that are associated with carrying a pregnancy to term. For example, the use of anthracyclines can result in cardiomyopathy that limits the ability of the body to acclimate to the vastly increased blood volume associated with pregnancy or the stress of delivery. The survivorship guidelines from the Children's Oncology Group recommend that women who have received 300 mg/m<sup>2</sup> of doxorubicin or equivalent anthracycline dosage, radiation at a dose of 30 Gy or higher to the heart tissue or surrounding tissues, or radiation at any dose in combination with an anthracycline or high doses of cyclophosphamide undergo cardiac evaluation before and periodically during pregnancy [39].

## ***Cohort Studies***

The Childhood Cancer Survivor Study (CCSS) was conducted in a cohort of 5,000 female cancer survivors who were diagnosed between 1970 and 1986 [15]. The study identified acute ovarian failure, defined as self-reported amenorrhea, in 6.3% of survivors surveyed, the majority of whom had received abdominal or pelvic radiation. The prevalence of nonsurgical premature menopause was 13 times higher among survivors than in sibling controls. Risk factors included attained age, exposure to increasing doses of alkylating agents, radiation to the ovaries, and a diagnosis of Hodgkin lymphoma [15]. The relative risk for achieving pregnancy was 0.81 compared to sibling controls. Individuals less likely to conceive were those who had hypothalamic-pituitary radiation dose of 30 Gy, ovarian/uterine radiation dose greater than 5 Gy, higher doses of total alkylating agents, or treatment with lomustine

or cyclophosphamide [40]. While uterine radiation doses of more than 5 Gy were associated with a greater number of infants that were small for their gestational age, there was no evidence of an increased risk of congenital malformations compared to that in the general population [15].

Letourneau et al. conducted a survey on the reproductive health history of 1,041 women of reproductive age who were diagnosed with one of the following malignancies: breast cancer, Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), leukemia, or gastrointestinal cancer. The incidence of acute ovarian failure was lowest in patients who had been diagnosed with leukemia (3%) and highest in patients with NHL (10%), Hodgkin lymphoma (8%), and breast cancer (9%). The rate of acute ovarian failure increased significantly with age [41].

## Assessing Individual Female Reproductive Capacity

Traditional assessments of ovarian reserve have relied on amenorrhea and sustained elevation of FSH, measures which become abnormal only in the perimenopausal period when fertility has already been compromised. More recently, ultrasonographic measures, such as ovarian volume and antral follicle counts, and serum measurements such as anti-Müllerian hormone (AMH) have been utilized to assess diminished but not depleted ovarian reserve [42, 43]. AMH is an indicator of follicular function in the ovary and has been demonstrated to be a more reliable and reproducible indicator of ovarian reserve [44] as well as longer term ovarian function compared with FSH [45]. A small study of young adult breast cancer patients revealed menopausal levels of FSH and estradiol during cancer therapy with subsequent normalization 6 months post-therapy, while AMH declined and remained low post-therapy, indicating a diminished ovarian reserve [12]. These hormone patterns suggest that reproductive endocrine function can resume, but it may be potentially falsely reassuring [12]. Other small studies in young adults with cancer have shown that pretreatment AMH level was the only predictor of post-therapy amenorrhea and long-term ovarian function [45, 46]. Cross-sectional studies of pediatric cancer survivors have found lower levels of AMH, smaller ovarian volume, a lower number of antral follicles, and higher levels of day 2/3 FSH compared with healthy controls, even in patients who continue to menstruate, suggesting the presence of a diminished ovarian reserve as a result of cancer therapy [47, 48].

## Conclusions

Based on cumulative experience and the findings of cohort studies, we can predict which patients may be at higher risk of gonadotoxicity from their cancer therapy (Table 1.2). Older women, women receiving radiation to the ovaries or pelvis (including TBI), and those receiving regimens that include high-dose alkylating

**Table 1.2** Highest risk factors for impaired fertility

Older age
Treatment with high-dose cyclophosphamide or procarbazine
Radiation to the pelvis >2 Gy
Treatment with radiation and alkylating agent
Total body irradiation
Surgical resection of uterus or ovaries

agents (including myeloablative stem cell transplants) represent those at the highest risk for infertility. Individuals falling into this highest risk group should routinely be offered the opportunity for fertility preservation prior to the start of cancer therapy, as they are unlikely to have other opportunities to have biological children as cancer survivors. For those individuals who did not undergo fertility preservation procedures prior to cancer therapy, close attention should be paid to assessing post-therapy reproductive capacity and desire for biologic children, as options for fertility preservation still exist for certain patients in that time frame. Nevertheless, it remains difficult to predict which individuals are at risk for the development of either acute ovarian failure or premature menopause, thereby complicating targeted counseling regarding fertility preservation at diagnosis and post-therapy. Ongoing research is needed to expand our understanding of the gonadotoxicity of contemporary chemotherapeutic regimens, ascertain the role that pretreatment fertility and other patient-based factors play when determining outcomes for a given individual, and gauge the length of the “reproductive window” that remains post-therapy.

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