

Effects of cancer treatment on ovarian function

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Causes of primary ovarian failure are reviewed, focusing specifically on cancer treatment-related modalities. Strategies and future directions for protection of the ovaries during cancer therapy, including ovarian transposition, and conformal radiation techniques are presented. (*Fertil Steril*® 2009;92:417–27. ©2009 by American Society for Reproductive Medicine.)

Key Words: Ovarian failure, cancer treatment, radiation toxicity

Although cancer incidence rates in women less than 50 years old continue to increase during recent years, mortality rates are dramatically decreasing due to modern advances in treatment (1, 2). In 1990 the prevalence of cancer survivors was 1 in 1,000 for young adults (15–45 years of age). By the year 2010, as many as 1 in 250 patients in this age group will have survived cancer (3). However, increasing numbers of survivors are now confronted with the long-term consequences of exposure to these treatments. Cancer therapy, which includes surgery, radiotherapy, and chemotherapy, can have a profound impact on ovarian function, leading to premature menopause and loss of fertility.

Acute ovarian failure can occur during or shortly after completion of irradiation or chemotherapy and may be transient or permanent. In contrast, premature ovarian failure (POF) or premature menopause typically manifests after a post-treatment return of regular menses with subsequent loss of ovarian function before the age of 40 years. As expected, surgical ablation of the ovaries leads to immediate and permanent loss of function.

Given that the pool of primordial follicles in the ovary is fixed and declines in a predictable manner, generalized models have been established to describe the natural decay of the ovary. Any injury to the ovary can potentially reduce this ovarian reserve, effectively advancing the patient's reproductive age, thus closing her window of reproductive opportunity. The radiosensitivity of the human oocyte has been

studied and a model for predicting the age of ovarian failure after a known dose of radiation has been proposed (4–7). Those women not receiving a sterilizing dose of radiation or chemotherapy may be at increased risk for complications during pregnancy, including spontaneous abortions and delivery of low birth weight babies (8).

Particularly challenging are similar consequences of cancer treatment in the pediatric population. At a time when cancer *survival* is the first priority, questions regarding future reproductive ability and childbearing are difficult issues for physicians, patients, and parents. Nevertheless, having foreknowledge of potential treatment-related ovarian failure will allow the physician to better counsel the patient and her family regarding the importance and timing of fertility preservation given an estimated window of fertility. In prepubertal girls, in whom clinical, biochemical, or radiological detection of ovarian failure is not reliably possible, estimating the risk and age of ovarian failure can potentially facilitate the initiation of hormone replacement therapy (HT) to induce secondary sex characteristics.

Modern techniques for prevention of treatment-induced ovarian ablation include better shielding of the ovaries from the damaging effects of radiation. This can be accomplished by implementing transposition procedures to move the ovaries outside of the area at risk. In addition, knowledge of the precise three-dimensional location of the ovaries allows the physician to limit the dose in these regions with novel beam arrangements, three-dimensional conformal radiation therapy or intensity-modulated radiation therapy. Prevention of the cytotoxic effects of chemotherapy by administering a concurrent GnRH agonist (GnRH-a) appears to be promising in early studies (9, 10).

In the modern era of improved antineoplastic agents and highly conformal, three-dimensionally planned radiation

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therapy, the possibility of limiting the ovaries from excess treatment-related toxicity in certain situations is becoming more of a reality. Herein we describe the effects of multimodal cancer treatment on ovarian function as published in the literature from a historic basis down through more recent efforts to characterize toxicity during this transition into a more technologically advanced age of cancer therapy.

PATHOPHYSIOLOGY

Primary ovarian failure is the loss of ovarian function caused by a process directly affecting the ovaries. In addition to multimodal cancer treatment, other processes including autoimmunity, chromosomal abnormalities, and natural aging can result in secondary ovarian failure, which is easily detected by an increase in serum gonadotropin levels (FSH and LH). Other markers like inhibin A or inhibin B and $17\text{-}\beta\text{-E}_2$ have been shown in preliminary studies to be prognostic factors in predicting the return of ovarian function after cancer treatment (11, 12). Ovarian failure before menarche will preclude development of secondary sex characteristics as the patient ages. Loss of ovarian function at any age thereafter leads to menopausal symptoms including vasomotor instability (hot flashes), fatigue, irritability, anxiety, vaginal dryness, decreased libido, and atrophy of the breasts. Adverse health outcomes of POF include osteoporosis, cardiovascular disease, impaired fecundity, and psychosexual dysfunction.

Any injury to the dividing granulosa cells (GC) that line and support the developing follicles will affect the viability of the maturing oocyte. Inability of the follicle to develop into a mature oocyte available for ovulation indicates loss of ovarian function. The GCs appear to be the initial target for radiation injury. Within a few hours of irradiation, before any changes in the oocyte are detectable, pyknosis—indicating cell death—can be seen in GCs. With sufficient loss of GCs, the oocyte loses viability and the follicle atrophies (13).

Acute ovarian failure (AOF) occurs in those women receiving a dose of irradiation or chemotherapy sufficient to cause permanent sterility during or shortly after treatment. The ovaries of these women reveal complete or nearly complete disappearance of primordial follicles, occasionally with remnants of degenerating follicles. The ovarian cortical stromal cells are mostly replaced with collagen and the ovary shrinks in size. With normal aging, ovarian vessels develop spontaneous sclerosis and myointimal proliferation to the point of occlusion of the vessel lumen. The media may also show hyaline degeneration. Radiation injury accelerates this process of small vessel damage as demonstrated by the signature late effects of organizing thrombi or masses of fibrin around foamy histiocytes within the intima of small vessels (13, 14).

A retrospective cohort multicenter study by the Childhood Cancer Survivor Study assessed AOF in female childhood cancer survivors (15). Of the 3,390 survivors studied, 215

(6.3%) developed AOF defined as the loss of ovarian function (self-reported amenorrhea) within 5 years of cancer diagnosis. Factors associated with the development of AOF were increased age at the time of treatment, diagnosis of Hodgkin's disease, increased radiation doses (particularly >10 Gy), and exposure to alkylating agents (specifically procarbazine and cyclophosphamide). Women who developed AOF despite receiving doses of less than 10 Gy typically had additional risk factors, such as exposure to alkylating agents and older age.

In other women, fertility may remain transiently after treatment if some follicles are relatively radioresistant. This typically occurs in the late stages of maturation when the GCs are no longer rapidly proliferating. Temporary sterility may result because of loss of follicles in the intermediate stages of development when GC proliferation is most intense. Women receiving a dose of irradiation insufficient to result in immediate and permanent sterility may experience POF or premature menopause. Lower radiation doses can lead to a reduction in the total number of remaining follicles, which effectively shortens the reproductive period. The POF typically manifests after a post-treatment return of regular menses with subsequent loss of ovarian function before the age of 40 years.

The incidence and risk factors of POF were recently analyzed using a separate cohort of participants in the multicenter Childhood Cancer Survivor Study (16). Of the 2,819 female cancer survivors that met study entry criteria, 8% had experienced nonsurgical premature menopause compared with 0.8% in a cohort of control siblings. In women who received an alkylating agent and abdominopelvic irradiation, the cumulative incidence of nonsurgical premature menopause approached 30%.

TREATMENT FACTORS

Ultimate fertility depends not only on the reproductive age of the patient and the corresponding ovarian reserve or size of the remaining pool of primordial follicles, but also on treatment-related factors such as the dose of radiotherapy, the dose and class of chemotherapy, and the use of a combination of treatment modalities. In a retrospective analysis of 100 female childhood cancer survivors and 21 age-matched controls from Denmark, the effects of cancer treatment on ovarian function were evaluated (17). Detailed menstrual histories, hormonal measurements, and timed transvaginal ultrasound measurements (menstrual cycle days 2–5) were performed. The median age was 5.4 years at diagnosis and 25.7 years at study entry. All patients received chemotherapy, including 44 who received alkylating agents. Fifty-six patients received radiotherapy with 20 undergoing infradiaphragmatic irradiation (12–40.5 Gy). Cranial (16 patients), supradiaphragmatic (12 patients), and total body irradiation (10 patients) were among the other sites treated. Premature ovarian failure was recorded in 17 patients who were noted to have follicle-depleted or undetectable ovaries, elevated FSH and LH, and reduced inhibin B levels. Thirteen patients used oral contraception (OC) and 70 women had spontaneous menstruation. Compared with the controls, the women

having spontaneous menstruation had smaller ovarian volume, a lower number of antral follicles, lower inhibin B levels, and lower E₂ levels. Independent factors associated with a reduced number of antral follicles were presence of ovaries in the irradiated field, exposure to an alkylating agent, increased age at diagnosis, and increased number of years since treatment. The investigators concluded that sexually mature female cancer survivors with spontaneous cycles should be informed that cessation of fertility might occur much earlier than anticipated.

Radiation Dose Tolerance

Abdominopelvic radiotherapy as part of the management of cancer clearly has deleterious effects on ovarian function. However, the modern age of computer-driven technology has permitted customization of radiation doses to conform to the tumor and spare normal tissues. Image-guided and adaptive radiotherapy allow more precise localization of these regions of interest. The importance of recognizing the tolerance doses of normal tissues in this era of three-dimensional planning cannot be overemphasized.

The dose tolerance of the ovary is dependent on several factors including the volume irradiated, the total radiation dose, the fractionation schedule, and the patient's age at the time of treatment. A British study assessed the effect of cranial versus craniospinal irradiation with and without chemotherapy on ovarian function for 42 girls who had been treated for brain tumors not affecting the hypothalamopituitary axis (18). None of the 14 patients receiving cranial irradiation alone without spinal irradiation or chemotherapy experienced ovarian dysfunction. In contrast, ovarian failure occurred in 7 of 11 girls (64%) treated with craniospinal irradiation and in 9 of 14 (64%) girls treated with craniospinal irradiation and chemotherapy. The association with spinal irradiation was statistically significant. The Children's Cancer Study Group assessed 97 long-term female survivors of childhood acute lymphoblastic leukemia treated between 1972 and 1975 (19). Treatment was administered on protocols involving either 18 or 24 Gy to one of the following fields: cranial, craniospinal, or craniospinal plus 12-Gy abdominal (including the ovaries) divided into 1.2- to 2.0-Gy fractions. After a median follow-up since completion of all therapy of 4.6 years, 35 patients (36%) were found to have elevated serum FSH or LH levels. Of these patients, 93% received craniospinal and abdominal irradiation, 49% received craniospinal irradiation, and 9% received cranial irradiation ($P < .001$). A dose-response relationship between 18 and 24 Gy was observed for the patients receiving craniospinal irradiation. Three of 16 patients treated to 18 Gy had elevated FSH, whereas 14 of 21 patients receiving 24 Gy had elevated FSH.

Doses to the ovaries exceeding 24 Gy conventionally fractionated will invariably produce permanent ovarian ablation (20). In an adult cancer patient, if the ovaries are included within the irradiation fields, the ovarian dose will usually exceed this level. Historic data concerning the effects of low-dose irradiation of the ovary discussed hereafter are ob-

tained from [1] reports of intentional ovarian ablation (castration) schedules for women with metastatic breast cancer, endometriosis, menometrorrhagia, or other benign pelvic inflammatory conditions, and [2] dose schedules historically used to enhance fertility by stimulating ovulation. Because this literature antedates the use of megavoltage, three-dimensionally planned radiotherapy with sophisticated dose computation, the dose limits for ovarian tolerance, which in some reports are expressed as exposure in roentgens (R), should be accepted with caution.

Peck et al. (21) reviewed the records of 334 patients who received a variety of radiotherapeutic techniques to ablate the ovaries. Two hundred eighty-nine patients were more than 40 years old. Successful castration was achieved in 34 of 36 (94%) of those who received 500–624 R in divided doses. All of the 72 women who received more than 625 R were effectively castrated. In patients younger than 40 years, however, results were less predictable. Nathanson et al. (22) reported on 10 premenopausal breast cancer patients who underwent radiation castration with 1,200–1,600 R divided during 4 days. One of six women near the age of 40 years had a menstrual cycle after treatment, whereas five of six women near the age of 30 years had at least one menstruation. Three patients had return of regular menses after a period of amenorrhea. Permanent amenorrhea resulted after a second course of ablative radiotherapy in two relapsing patients (i.e., unsuccessful initial course of therapy). Diczfalusy et al. (23) analyzed 17 premenopausal women with metastatic breast cancer who underwent ovarian ablation with three fractions of 550–650 R per day. Ninety-six-hour urinary estrogen (E) excretion was measured. After oophorectomy approximately 5 months after irradiation, urinary E excretion remained unchanged in all 17 patients indicating successful ablative radiotherapy.

Leung et al. (24) assessed 60 patients with breast cancer undergoing ovarian ablation with one of two dose schedules: 12 Gy in four fractions or 14 Gy in four fractions. All of the women more than 40 years old were effectively castrated with the higher dose schedule, whereas the lower dose schedule had a 14% ovarian ablation failure rate. The overall failure rate for women younger than 40 years old was 35%. Of the women who retained normal menstruation, half had persistent menstruation after irradiation and half had amenorrhea followed by a return of normal menses 5–10 months after irradiation. Meakin (25) reported the results of ovarian ablation with 20 Gy delivered in five fractions. The ovarian ablation failure rate was only 1% in women older than 45 years and 7% in younger women.

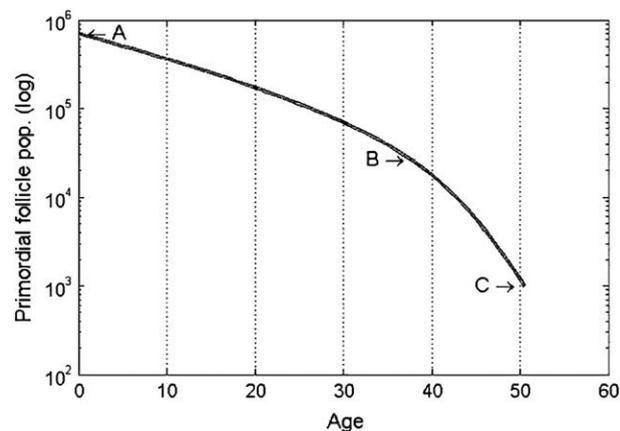
Further data from the historic use of radiotherapy in the treatment of female sterility provide evidence for the tolerance of the ovaries to lower doses of radiation. Payne (26) reported on the treatment of female infertility with x-ray therapy. Sixty-one "infertile" women received low doses of ovarian irradiation to stimulate ovulation. Three fractions of 75 R each (225 R total) were delivered at 2- to 5-day intervals beginning on the third or fourth day of menstrual

bleeding to avoid irradiating an early pregnancy. Some patients also received pituitary irradiation. Successful pregnancy occurred in 27 of 61 (44%), with 44 pregnancies resulting in 28 term births. Four patients who initially failed to become pregnant after the first course of radiotherapy received a second course and subsequently became pregnant, indicating that fertility may be possible after cumulative fractionated doses of 450 R to the ovaries. Kaplan (27) published his experience treating 796 women for “female sterility” between 1924 and 1957. Treatment consisted of ovarian irradiation in three fractions of 60–65 R each using 200 kVp x-rays at 7-day intervals. Three hundred forty-one women subsequently conceived, with the majority bearing living children. Multiple pregnancies occurred in 168 women.

The dose of radiation required to destroy 50% of primordial follicles (LD_{50}) has previously been judged to be less than 4 Gy—an estimate later deemed an oversimplification (5). Using the best available model for follicle decline, Wallace et al. (6) reported their calculation of the radiosensitivity of the human oocyte in terms of LD_{50} . A differential equation was used to describe the decay as an instantaneous rate of temporal change for a specific number of remaining follicles. By solving the equation, the number of remaining follicles could be estimated after a known dose of radiotherapy. The result was an LD_{50} of 1.99 Gy. Using this same model for primordial follicle population decline and published data on age-related ovarian volume as measured by transvaginal sonography, a highly significant correlation was found between ovarian volume and the size of the remaining pool of primordial follicles (4). More recently, Wallace et al. (7) applied knowledge from previous studies regarding the radiosensitivity of the oocyte to the modern era of computed tomography (CT) radiotherapy planning, which allows reliable three-dimensional determination of the dose of radiation received by the ovaries. For a given dose of radiotherapy at a known chronologic age (x_{chron}) (Fig. 1), the surviving fraction of oocytes and thus the age of ovarian failure can be determined with 95% confidence limits given the solution to the Faddy-Gosden equation and the surviving percentage function. An example of the method for predicting ovarian failure after a 12-year-old girl receives a dose of 10 Gy to the ovaries is shown in Figure 2. Using the model, the average population at an age of 12 years (x_{chron}) is 312,000 follicles. After 10-Gy ovarian irradiation, the surviving percentage is $\log_{10} g(10) = 3.01\%$, corresponding to a population of 9,600 follicles. Projecting that number of remaining follicles onto the standard Faddy-Gosden model reveals a reproductive age (x_{reprod}) of 42.9 years. The average 42.9-year-old woman is expected to experience menopause in 7.5 years, at an age of 50.4 years. The patient’s predicted reproductive lifespan is therefore $50.4 - x_{\text{reprod}}$ years. Her predicted age of ovarian failure is $x_{\text{chron}} + (50.4 - x_{\text{reprod}})$ years or $12 + (50.4 - 42.9) = 19.5$ years. Table 1 displays the predicted age of ovarian failure with 95% confidence limits for patients from birth to 30 years old at the time of treatment and for ovarian doses of 3, 6, 9, and 12 Gy.

FIGURE 1

The solution of the Faddy-Gosden differential equation for the primordial follicle population from birth to menopause. (A) At birth, 701,000 follicles. (B) Accelerated decline at age 38 years, 25,000 follicles. (C) At menopause, 1,000 follicles. (From Wallace and Kelsey [4]. Reprinted by permission of the publisher.)

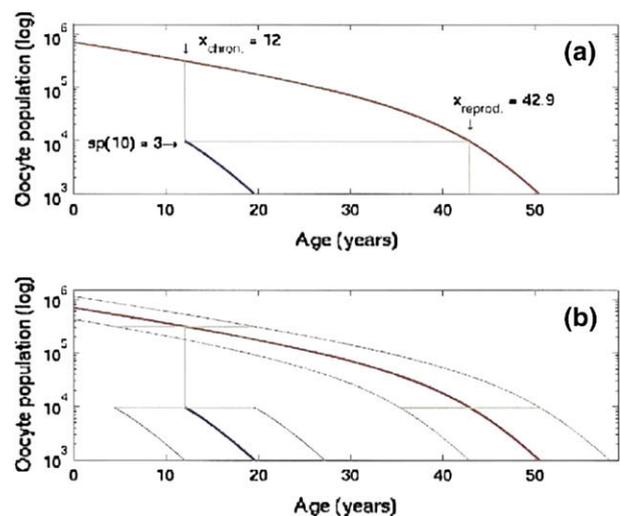


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Application of this model clinically will afford physicians the ability to counsel patients regarding their predicted window of fertility. For instance, when an ovarian dose of 3 Gy or less is delivered to the ovaries, the patient is likely to maintain a significant reproductive lifespan. Increasing

FIGURE 2

(a) Model for predicting ovarian failure after radiotherapy—example case and (b) with confidence intervals included. (From Wallace et al. [7]. Reprinted by permission of the publisher.)



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TABLE 1

Predicted age at ovarian failure with 95% confidence limits for ages at treatment from 0 to 30 years and for doses 3, 6, 9, and 12 Gy.

Age	3Gy			6Gy			9Gy			12Gy		
	Low	Mean	High									
0	31.2	35.1	39.0	18.7	22.6	26.5	9.8	13.7	17.6	4.0	7.9	11.8
1	31.3	35.2	39.1	19.0	22.9	26.8	10.4	14.3	18.2	4.8	8.7	12.6
2	31.5	35.4	39.3	19.3	23.2	27.1	10.9	14.8	18.7	5.5	9.4	13.3
3	31.6	35.5	39.4	19.7	23.6	27.5	11.5	15.4	19.3	6.2	10.1	14.0
4	31.7	35.6	39.5	20.1	24.0	27.9	12.1	16.0	19.9	6.9	10.8	14.7
5	31.9	35.8	39.7	20.5	24.4	28.3	12.7	16.6	20.5	7.7	11.6	15.5
6	32.1	36.0	39.9	20.9	24.8	28.7	13.3	17.2	21.1	8.4	12.3	16.2
7	32.2	36.1	40.0	21.3	25.2	29.1	13.9	17.8	21.7	9.1	13.0	16.9
8	32.4	36.3	40.2	21.7	25.6	29.5	14.6	18.5	22.4	9.9	13.8	17.7
9	32.6	36.5	40.4	22.1	26.0	29.9	15.2	19.1	23.0	10.6	14.5	18.4
10	32.8	36.7	40.6	22.6	26.5	30.4	15.8	19.7	23.6	11.4	15.3	19.2
11	33.0	36.9	40.8	23.0	26.9	30.8	16.5	20.4	24.3	12.1	16.0	19.9
12	33.2	37.1	41.0	23.5	27.4	31.3	17.1	21.0	24.9	12.9	16.8	20.7
13	33.4	37.3	41.2	23.9	27.8	31.7	17.8	21.7	25.6	13.6	17.5	21.4
14	33.6	37.5	41.4	24.4	28.3	32.2	18.5	22.4	26.3	14.4	18.3	22.2
15	33.9	37.8	41.7	24.9	28.8	32.7	19.1	23.0	26.9	15.1	19.0	22.9
16	34.1	38.0	41.9	25.4	29.3	33.2	19.8	23.7	27.6	15.9	19.8	23.7
17	34.3	38.2	42.1	25.9	29.8	33.7	20.5	24.4	28.3	17.0	20.5	24.4
18	34.6	38.5	42.4	26.4	30.3	34.2	21.2	25.1	29.0	18.0	21.3	25.2
19	34.9	38.8	42.7	27.0	30.9	34.8	21.8	25.7	29.6	19.0	22.0	25.9
20	35.1	39.0	42.9	27.5	31.4	35.3	22.5	26.4	30.3	20.0	22.8	26.7
21	35.4	39.3	43.2	28.0	31.9	35.8	23.2	27.1	31.0	21.0	23.5	27.4
22	35.7	39.6	43.5	28.6	32.5	36.4	23.9	27.8	31.7	22.0	24.3	28.2
23	36.0	39.9	43.8	29.1	33.0	36.9	24.6	28.5	32.4	23.0	25.0	28.9
24	36.3	40.2	44.1	29.7	33.6	37.5	25.3	29.2	33.1	24.0	25.7	29.6
25	36.7	40.6	44.5	30.3	34.2	38.1	25.9	29.8	33.7	25.0	26.5	30.4
26	37.0	40.9	44.8	30.8	34.7	38.6	26.6	30.5	34.4	26.0	27.2	31.1
27	37.3	41.2	45.1	31.4	35.3	39.2	27.3	31.2	35.1	27.0	27.9	31.8
28	37.7	41.6	45.5	32.0	35.9	39.8	28.0	31.9	35.8	28.0	28.7	32.6
29	38.0	41.9	45.8	32.5	36.4	40.3	29.0	32.6	36.5	29.0	29.4	33.3
30	38.3	42.2	46.1	33.1	37.0	40.9	30.0	33.2	37.1	30.0	30.1	34.0

Note: (From Wallace et al. [7]. Rwp rinted by permission of the publisher.)

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doses of ovarian irradiation will result in a shortened fertile window. For young girls who will likely be receiving sterilizing doses of radiotherapy before menarche, physicians can assist patients and families in pursuing fertility preservation options. In addition, HT to induce secondary sex characteristics can be anticipated for prepubertal girls.

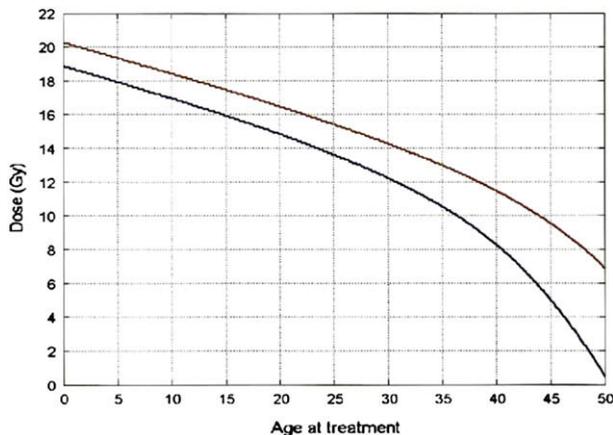
Wallace et al. (7) additionally included their calculations for effective sterilizing dose and mean sterilizing dose from birth to menopause. The effective sterilizing dose is the dose after which the primordial follicle population will decrease below ~1,000. Alternatively, it is the dose that will induce immediate ovarian failure in 97.5% of the female population, effectively taking the patient's reproductive age to 58 years. The mean sterilizing dose is the dose that will in-

duce immediate ovarian failure in 50% of women. Figure 3 shows a graphic representation of the effective sterilizing dose and mean sterilizing dose from birth to 50 years. Of note, the effective sterilizing dose for ovarian irradiation at birth is 20.3 Gy; at 10 years old, 18.4 Gy; at 20 years old, 16.5 Gy; and at 30 years old, 14.3 Gy.

The toxic effects of radiation therapy on ovarian function have been well documented. Ovarian tolerance is a function of the number of remaining primordial follicles, which is related to age. Younger patients have more follicles and, therefore, require higher doses of radiation to ablate ovarian function. The human oocyte is radiosensitive with a low LD₅₀—approximately 2 Gy. The models assist the physician in predicting the age of ovarian failure after treatment with

FIGURE 3

The effective (red, upper) and mean (blue, lower) sterilizing dose of radiation for a given treatment age. (From Wallace et al. [7]. Reprinted by permission of the publisher.)



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TABLE 2

Gonadotoxic chemotherapy agents (38).

- Alkylating agents
 - Cyclophosphamide
 - Ifosfamide
 - Nitrosoureas (BCNU, CCNU)
 - Chlorambucil
 - Melphalan
 - Busulphan
- Vinca alkaloids
 - Vinblastine
- Antimetabolites
 - Cytarabine
- Platinum agents
 - Cisplatin
- Other agents
 - Procarbazine

Note: (From Bokemeyer et al. [45]. Reprinted by permission of the publisher.)

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a known dose of radiotherapy. Early estimation of the age of ovarian failure will help physicians manage young female patients from puberty through premature menopause with appropriate timing of HT and discussion of available fertility preservation options.

Chemotherapy

Chemotherapy administered alone or in combination with irradiation can adversely affect ovarian function. Several investigators have reported on chemotherapy-induced ovarian failure with a wide variety of single agents including busulfan, chlorambucil, vinblastine, and cyclophosphamide (28–38). (See Table 2 for a list of known gonadotoxic chemotherapy agents.) In the treatment of Hodgkin's disease, the use of multiple agents such as the MOPP regimen (mechlorethamine, Oncovin, procarbazine, and prednisone) can result in ovarian failure, as confirmed by decreased E₂ levels and elevation of serum gonadotropins (39–43). More recent evidence from analysis of the Childhood Cancer Survivor Study corroborates older data indicating an increased risk of treatment-induced ovarian failure with exposure to alkylating agents, particularly procarbazine and cyclophosphamide (15, 44, 45). In childhood cancer survivors who received an alkylating agent in addition to abdominopelvic irradiation, the cumulative incidence of nonsurgical POF approached 30% compared with an overall incidence of 8% for all survivors (16). Chemotherapy-induced amenorrhea may be transient, and menstruation may return several months after completion of treatment. As with radiation, the deleterious effects of chemotherapy are strongly age dependent. Women older than 25 years have a substantially higher risk of chemotherapy-induced ovarian failure after treatment for Hodgkin's

disease. Likewise, women more than 30 years old have a 12-fold increased risk of menstrual irregularities (39, 42).

PROPOSED TOXICITY END POINTS

Several tools for grading acute and late effects of cancer treatment on various organ sites are available. However, none of these, including the most recent version of the Common Terminology Criteria for Adverse Events proposed by the National Cancer Institute (CTCAEv3), incorporate the effects of cancer therapy on ovarian function. Grigsby et al. (20) proposed, with specific measurable end points, subjective and objective grading criteria for the effects of cancer therapy on the ovaries (Table 3). Objective end points include measuring serum levels of LH, FSH, and E₂. Other end points include menstruation, ovulation, dysmenorrhea, amenorrhea, infertility, hot flashes, and osteoporosis.

PREVENTION AND MANAGEMENT OF OVARIAN FAILURE

The physician's role in preventing and managing ovarian dysfunction in patients being treated for malignant disease is vital. The initial step is recognizing that ovarian protection and fertility preservation are not only desirable but also potentially attainable. New advances in cancer therapy and reproductive medicine offer hope for biologic parenthood to women who previously would have been rendered infertile by surgery, chemotherapy, or radiation therapy.

Irradiation

Modern improvements in radiation delivery allow the radiation oncologist to tailor highly conformal dose distributions to complex treatment volumes to maximize target coverage and spare normal structures. Radiation treatment planning

TABLE 3**Measurable end points.**

	Grade 1	Grade 2	Grade 3	Grade 4
Subjective				
Hot flashes	Occasional	Intermittent	Persistent	
Dysmenorrhea	Occasional	Intermittent	Persistent	
Menstruation		Oligomenorrhea	Amenorrhea	
Objective				
Ovulation			Anovulation in premenopausal women	
Involuntary infertility			Infertility	
Osteoporosis			Radiographic evidence	Fracture
Management				
Dysmenorrhea, hot flashes		Hormone replacement	Hormone replacement	Hormone replacement
Menstruation		Hormone replacement	Hormone replacement	Hormone replacement
Osteoporosis		Hormone replacement, calcium supplements	Hormone replacement, calcium supplements	Hormone replacement, calcium supplements
Analytic				
FSH/LH /estradiol	Assessment of hormonal production			Y/N Date:
Bone densitometry	Quantify bone density			Y/N Date:
<i>Note:</i> (From Grigsby et al. [20]. Reprinted by permission of the publisher.)				
<i>Stroud. Effects of cancer therapy on ovarian function. Fertil Steril 2009.</i>				

systems now allow for meaningful assessment of the planned dose to the ovaries through dose–volume histograms. Using this information, the treating physician can clinically apply the data regarding the tolerance dose of the ovary as well as the tools for predicting the age of ovarian failure after a known dose of radiotherapy.

Knowledge of the precise location of the ovaries during treatment is essential in accurately determining ovarian dose during irradiation. Counsell et al. (46) reviewed 81 pelvic CT scans of 30 healthy premenopausal women to assess the variation of the position of the ovaries. In 23 of the 30 women (77%), one or both of the ovaries were identified. It was determined that the ovaries of 17% of the women were located in the upper pelvis outside of traditional ovarian ablation fields. In those cases, treatment would have likely been inadequate. Rigsby and Siegel (47) from Mallinckrodt Institute of Radiology evaluated pelvic CT scans of 125 young girls between 1 and 18 years old. Ovaries were identified in only 6.3% of patients less than 8 years old, and 75% of patients more than 8 years old. Thus, in the majority of female patients older than 8 years of age, the ovaries should

be detectable on CT simulation for radiation planning purposes. The position of the ovaries, however, can vary based on filling and emptying of the bladder. Nicholson et al. (48) reported on 12 women of childbearing age who underwent pelvic magnetic resonance imaging (MRI) with full and empty bladders. Significant variation in the position of the ovaries was evident and around the pelvic inlet would have resulted in displacement of the ovaries into a region of high dose irradiation. Instructing the patient on bladder filling and including a safety margin when delineating the ovary as a normal structure during radiation treatment planning may provide for better sparing of the ovaries. Alternatively, the ovaries can be imaged and contoured on a daily basis before treatment using on-board CT imaging—a recent advance in image-guided radiotherapy. Daily three-dimensional imaging provides the framework for adaptive radiotherapy in which treatments can be customized daily to the appropriate anatomy immediately before treatment.

Craniospinal irradiation has been reported to be an inciting factor in ovarian failure (18, 19). Several techniques have been reported for reducing gonadotoxicity when treating

the craniospinal axis. Some investigators have proposed that, instead of the standard posterior-anterior inferior spine field, opposed lateral fields for the inferior spine (similar to cranial fields) can result in reduced doses to the ovaries (49). Other investigators have reported reduced doses with proton therapy for craniospinal irradiation in which a posterior-anterior proton beam stops just after the thecal sac with no unwanted exit dose (50–52).

Ovarian Transposition

Curtailling the ovarian dose as much as possible without compromising target coverage is the ultimate dosimetric goal in preserving ovarian function. Oftentimes, however, the ovaries lie directly in the path of the beam or in close proximity to the targeted high dose region. Transfixing the ovaries out of the radiation fields before therapy, a procedure called oophoropexy or ovarian transposition, can increase the chance of preserving ovarian function by allowing better sparing of the ovaries from the harmful effects of irradiation. Historically, rates of cessation of ovarian function after transposition alone have been reported to be as high as 40% (53–56). However, laparoscopic techniques have been used to minimize operative morbidity (57, 58).

Typically, surgical clips are placed to mark the transposed ovaries, which, in the case of lymphoid irradiation, are frequently moved medially behind the uterus and away from the pelvic lymph node fields. For other primary pelvic malignancies, a lateral ovarian transposition technique is used. Hadar et al. (59) reported on seven patients with cervical cancer that had lateral ovarian transposition and nine patients with Hodgkin's disease that had medial ovarian transposition. After surgery, CT scans were performed to evaluate the position of the transposed ovaries. Eleven of the 13 ovaries laterally placed were outside of a generic anterior-posterior pelvic field. In contrast, of the 13 identifiable ovaries transposed medially, 3 were outside of the generic inverted Y field used to treat abdominopelvic lymph nodes. These data suggest an improved likelihood of shielding the ovaries during pelvic radiotherapy when a lateral ovarian transposition is performed, as opposed to a medial transposition.

LeFloch et al. (60) reviewed Stanford's experience with Hodgkin's disease. Nine women became pregnant and two-thirds of women retained ovarian function after oophoropexy before total nodal irradiation. A central 10-cm thick lead block was used to shield the medially transposed ovaries. The women who became pregnant ranged in age from 16–31 years, and they successfully conceived 2–5 years after completion of pelvic irradiation. Based on film dosimetry in a phantom, pelvic nodal doses were estimated to range from 43.75–44 Gy during 35–46 days, and minimum ovarian dose ranged from 3.52–3.96 Gy. In contrast to these results at Stanford, data by Peck et al. (21) would suggest an ovarian failure rate of 60% in this dose range.

Thibaud et al. (61) reported on 18 girls (median age 9.4 years) who underwent ovarian transposition before radiotherapy for

various malignancies including Hodgkin's disease (5 patients), Ewing's sarcoma (3 patients), medulloblastoma (2 patients), and other gynecologic tumors (8 patients). The ovaries were surgically transfixed just below the iliac crest (15 cases) or posterolateral to the uterus (3 cases). The calculated ovarian dose was up to 9.5 Gy. At follow-up (8.6 ± 0.9 years after ovarian transposition) 16 of the 18 girls were menstruating. Basal plasma gonadotropin values were normal in all patients. Seven girls had documented ovulation and two pregnancies had occurred. Four patients experienced complications including intestinal obstruction, dyspareunia, functional ovarian cysts, and tubal obstruction caused by adhesions.

Belinson et al. (53) calculated the scatter dose to transposed ovarian tissue under various clinical conditions when delivering 45 Gy to the pelvis with conventional radiation. The scatter dose to the transposed ovaries using opposed 15 x 15 cm posterior-anterior pelvic fields was 15.8 Gy and using a four-field box technique (posterior-anterior, right and left laterals) 21.9 Gy.

Ovarian transposition should be offered to all patients who have a high likelihood of receiving radiation to the ovaries. The patient should be informed that the risks of surgical complications including the chance that oophoropexy itself would cause ovarian failure.

Chemotherapy

Several investigators have reported a protective effect of concurrent administration of GnRH-a during combination chemotherapy (9, 10, 62). In a prospective clinical trial from Israel, 18 normally menstruating women with either Hodgkin's disease (13) or non-Hodgkin's lymphoma (3) aged 15–40 years were administered monthly injections of GnRH-a for a maximum of 6 months starting before chemotherapy (9). This group was compared with a matched control group being treated with a similar combination chemotherapy also for Hodgkin's disease (14) and non-Hodgkin's lymphoma (4). Gonadal function was assessed clinically, hormonally (LH, FSH, E₂, and P), and sonographically. Of the group that received GnRH-a, 93.7% resumed menstruations within 3–8 months after completion of treatment, compared with 39% of the control group. The other 61% of women in the control group experienced POF. However, a major criticism of this study was that it was not a blinded trial and that the ovarian failure rate in the control group was very high. Other reports show promise for gonadal protection with concurrent administration of OC agents during polychemotherapy (62) and prospective clinical trials are in progress (63).

Cryopreservation

In addition to minimizing the radiation dose to the ovaries with the mentioned surgical and radiotherapeutic techniques and administering protective agents concurrently with gonadotoxic chemotherapy, the most reliable option for fertility preservation in young sexually mature women with male

partners and enough time before treatment (i.e., chemotherapy or radiotherapy can be delayed 4–6 weeks) is embryo cryopreservation (64). The procedure consists of ovarian stimulation and collection of mature oocytes for IVF with subsequent cryopreservation of the resulting embryos. At the desired time, these embryos can be thawed and implanted into the uterus of either the biologic or the surrogate mother in hopes of a successful pregnancy. Survival rates per thawed embryo range from 35%–90%, whereas implantation rates range from 8%–30%. Cumulative pregnancy rates (PR) have been reported up to and even more than 60% (65–70).

For women who refuse the use of a sperm donor, embryo cryopreservation may not be an option. Instead, these women may choose to attempt cryopreservation of mature or immature oocytes. Historically, rates of survival, fertilization, and subsequent pregnancy after IVF of cryopreserved and thawed oocytes have been dismal (71–73). However, a recent review of 21 studies reported a mean survival rate of 47%, mean fertilization rate of 52.5%, and a mean PR per thawed oocytes of 1.52% (74). Further improvement has been reported with vitrification using the cryoprotectants ethylene glycol and dimethyl sulfoxide (DMSO) with a mean survival rate of 68.4%, fertilization rate of 48.5%, and PR of 1.7% per vitrified-thawed oocytes (74–76). Cobo and associates (77) reported the results of a cohort prospective randomized study of vitrification by the Cryotop method. In their study, the survival rate was 97% with a PR of 65% and an implantation rate of 41%. The Cryotop method demonstrated excellent clinical outcome and a high potential for establishing oocyte banking.

Clearly, the most challenging cases for preserving fertility involve children in whom embryo and oocyte cryopreservation is deemed by most to be unethical. Ovarian tissue cryopreservation is one of the only options for fertility preservation in these young girls (74). It has been suggested that the primordial follicles contained within the ovarian cortex are more resistant to cryodamage because of the low metabolic rate and absence of zona pellucida (ZP). More recent advances in effective cryoprotectants have led to successful births in a number of animal studies with cryopreserved ovarian tissue. However, ovarian tissue cryobanking remains experimental without standard guidelines for freezing–thawing and surgical grafting (78, 79). Fresh ovarian tissue has been successfully transplanted with resulting pregnancy and delivery from a fertile woman into her sterile monozygotic twin (80). Recently, Meirou et al. (81) reported on a young patient who, after being rendered sterile by her treatment for non-Hodgkin's lymphoma, became pregnant and delivered a healthy baby after transplantation of cryopreserved ovarian cortical tissue.

Future directions in fertility preservation are focused on the development of ovarian transplantation techniques. Orthotopic transplants involve grafting the ovarian tissue onto an atrophied ovary or near the infundibulopelvic ligaments. Heterotopic transplants, however, involve grafting the tissue into various subcutaneous sites. Xenografting of human ovarian tissue into immunodeficient mice has provided a model

for studying human autotransplantation and is being studied as a means for banking ovarian tissue, albeit with some difficulty (74). Although most fertility options are still considered experimental, with efficacy and reliability yet to be determined, the future of fertility preservation for female cancer survivors is promising.

CONCLUSIONS

Recent advances in cancer therapy have resulted in increasing numbers of long-term survivors who are then left to deal with the consequences of their treatments. The sensitivity of the ovaries to irradiation and chemotherapy often results in impaired fertility and premature menopause. Models are available for predicting the age of ovarian failure after a known dose of radiotherapy. The treating physician should take an active role in both reducing potential ovarian toxicity in every way possible and in providing the means whereby the patient can make an informed decision regarding her options for fertility preservation. Radiation doses more than 24 Gy will produce permanent ovarian ablation. Lesser doses may ablate the ovaries depending on individual patient variation. Chemotherapy agents either alone or in combination with radiation can ablate the ovaries. The most common gonadotoxic drugs are the alkylating agents and the vinca alkaloids. Ongoing research in fertility preservation shows promise for those female cancer survivors who face POF and infertility. Patient-to-patient variability is problematic and better definitions of outcomes and controlled trials of post-therapy management are warranted.

REFERENCES

1. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence—SEER 9 Regs Public-Use, Nov 2005 Sub (1973–2003), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006, based on the November 2005 submission.
2. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER * Stat Database: Mortality—All COD, Public-Use With State, Total U.S. (1969–2003), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006. Underlying mortality data provided by NCHS (<http://www.cdc.gov/nchs>).
3. Bleyer WA. The impact of childhood cancer on the United States and the world. *CA Cancer J Clin* 1990;40:355–67.
4. Wallace WH, Kelsey TW. Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography. *Hum Reprod* 2004;19:1612–7.
5. Wallace WH, Shalet SM, Hendry JH, Morris-Jones PH, Gattamaneni HR. Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte. *Br J Radiol* 1989;62:995–8.
6. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. *Hum Reprod* 2003;18:117–21.
7. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int J Radiat Oncol Biol Phys* 2005;62:738–44.
8. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood* 1996;87:3045–52.

9. Blumenfeld Z, Avivi I, Linn S, Epelbaum R, Ben-Shahar M, Haim N. Prevention of irreversible chemotherapy-induced ovarian damage in young women with lymphoma by a gonadotrophin-releasing hormone agonist in parallel to chemotherapy. *Hum Reprod* 1996;11:1620–6.
10. Marchesoni D, Driul L, Fruscalzo A, Santuz M, Calcagno A, Ianni A, et al. Premature ovarian failure in patients affected by oncohematological disease. *Minerva Ginecol* 2005;57:545–50.
11. Blumenfeld Z. Preservation of fertility and ovarian function and minimalization of chemotherapy associated gonadotoxicity and premature ovarian failure: the role of inhibin-A and -B as markers. *Mol Cell Endocrinol* 2002;187:93–105.
12. Blumenfeld Z, Ritter M, Shen-Orr Z, Shariki K, Ben-Shahar M, Haim N. Inhibin A concentrations in the sera of young women during and after chemotherapy for lymphoma: correlation with ovarian toxicity. *Am J Reprod Immunol* 1998;39:33–40.
13. Rubin P, Casarett GW. The female genital tract. In: Rubin P, Casarett GW, eds. *Clinical radiation pathology*. Philadelphia: W.B. Saunders, 1986:396–422.
14. Fajardo LF. Female reproductive organs. In: Fajardo LF, ed. *Pathology of radiation injury*. New York: Mason Publishing USA, 1982:108–28.
15. Chemaitilly W, Mertens AC, Mitby P, Whitton J, Stovall M, Yasui Y, et al. Acute ovarian failure in the childhood cancer survivor study. *J Clin Endocrinol Metab* 2006;91:1723–8.
16. Sklar CA, Mertens AC, Mitby P, Whitton J, Stovall M, Kasper C, et al. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. *J Natl Cancer Inst* 2006;98:890–6.
17. Larsen EC, Muller J, Schmiegelow K, Rehnitz C, Andersen AN. Reduced ovarian function in long-term survivors of radiation- and chemotherapy-treated childhood cancer. *J Clin Endocrinol Metab* 2003;88:5307–14.
18. Livesey EA, Brook CG. Gonadal dysfunction after treatment of intracranial tumours. *Arch Dis Childhood* 1988;63:495–500.
19. Hamre MR, Robison LL, Nesbit ME, Sather HN, Meadows AT, Ortega JA, et al. Effects of radiation on ovarian function in long-term survivors of childhood acute lymphoblastic leukemia: a report from the Children's Cancer Study Group. *J Clin Oncol* 1987;5:1759–65.
20. Grigsby P, Russell A, Bruner D, Eifel P, Koh W, Spanos W, et al. Late injury of cancer therapy on the female reproductive tract. *Intern J Rad Oncol Biol Phys* 1995;31:1281–99.
21. Peck WS, McGreer JT, Kretzschmar NR, Brown WE. Castration of the female by irradiation: the results in 334 patients. *Radiology* 1940;34:176–86.
22. Nathanson IT, Rice C, Meigs JV. Hormonal studies in artificial menopause produced by roentgen rays. *Am J Obstet Gynecol* 1940;40:936–5.
23. Diczfalusy E, Notter G, Edsmyr F, Westman A. Estrogen excretion in breast cancer patients before and after ovarian irradiation and oophorectomy. *J Clin Oncol* 1959;19:1230–44.
24. Leung SF, Tsao SY, Teo PML, Choi PHK, Shiu WCT. Ovarian ablation failures by radiation. *Br J Radiol* 1991;64:537–8.
25. Meakin JW. Review of Canadian trials of adjuvant endocrine therapy for breast cancer. *NCI Monogr* 1986:111–3.
26. Payne S. The treatment of female infertility by x-ray therapy. *W J Surg Obstet Gyn* 1954;62:173–6.
27. Kaplan I. The treatment of female sterility with x-ray therapy directed to the pituitary and ovaries. *Am J Obstet Gynecol* 1958;76:447–53.
28. Belohorsky B, Siracky J, Sandor L, Klauber E. Comments on the development of amenorrhea caused by myleran in cases of chronic myelosis. *Neoplasma* 1960;7:397–403.
29. Chapman RM. Cytotoxic therapy on sexuality and gonadal function. *Semin Oncol* 1982;9:84–94.
30. Fisher B, Sherman B, Rockett H, Redmond C, Margolese R, Fisher E. L-phenylalanine mustard (L-PAM) in the management of premenopausal patients with primary breast cancer: lack of association of disease-free survival with depression of ovarian function. National Surgical Adjuvant Project for Breast and Bowel Cancers. *Cancer* 1979;44:847–57.
31. Fosdick WM, Parsons JL, Hill DF. Preliminary report: long-term cyclophosphamide therapy in rheumatoid arthritis. *Arthritis Rheum* 1968;11:151–61.
32. Koyama H, Wada T, Nishizawa J, Iwanaga T, Aoki Y. Cyclophosphamide-induced ovarian failure and its therapeutic significance in patients with breast cancer. *Cancer* 1977;39:1403–9.
33. Miller JJ, Williams GF, Leissring JC. Multiple late complications of therapy with cyclophosphamide, including ovarian destruction. *Am J Med* 1971;50:530–5.
34. Rose DP, Davis TE. Ovarian function in patients receiving adjuvant chemotherapy for breast cancer. *Lancet* 1977;1:1174–6.
35. Shalet SM. Disorders of the endocrine system due to radiation and cytotoxic chemotherapy. *Clin Endocrinol* 1983;18:637–59.
36. Shalet SM. Effects of cancer chemotherapy on gonadal function of patients. *Cancer Treat Res* 1980;7:141–52.
37. Warne GL, Fairley KF, Hobbs JB, Martin FL. Cyclophosphamide-induced ovarian failure. *N Engl J Med* 1973;289:1159–62.
38. Thomson AB, Critchley HO, Wallace WH. Fertility and progeny. *Eur J Cancer* 2002;38:1634–44 [discussion 45–6].
39. Andrieu JM, Ochoa-Molina ME. Menstrual cycle, pregnancies and offspring before and after MOPP therapy for Hodgkin's disease. *Cancer* 1983;52:435–8.
40. Chapman RM, Sutcliffe SB, Malpas JS. Cytotoxic-induced ovarian failure in women with Hodgkin's disease. I. Hormone function. *JAMA* 1979;242:1877–81.
41. Morganfeld MC, Goldberg V, Parisier H, Bugnard SC, Bur GE. Ovarian lesions due to cytostatic agents during the treatment of Hodgkin's disease. *Surg Gynecol Obstet* 1972;1134:826–8.
42. Schilsky RL, Sherins RJ, Hubbard SM, Wesley MN, Young RC, DeVita VT. Long-term follow-up of ovarian function in women treated with MOPP chemotherapy for Hodgkin's disease. *Am J Med* 1981;71:552–6.
43. Sobrinho LG, Levine RA, DeConti RC. Amenorrhea in patients with Hodgkin's disease treated with antineoplastic agents. *Am J Obstet Gynecol* 1971;109:135–9.
44. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. *Endocrinol Metab Clin North Am* 1998;27:927–43.
45. Bokemeyer C, Schmoll HJ, van Rhee J, Kuczyk M, Schuppert F, Poliwooda H. Long-term gonadal toxicity after therapy for Hodgkin's and non-Hodgkin's lymphoma. *Ann Hematol* 1994;68:105–10.
46. Counsell R, Bain G, Williams MV, Dixon AK. Artificial radiation menopause: where are the ovaries? *Clin Oncol (R Coll Radiol)* 1996;8:250–3.
47. Rigsby CK, Siegel MJ. CT appearance of pediatric ovaries and uterus. *J Comput Assist Tomogr* 1994;18:72–6.
48. Nicholson R, Coucher J, Thornton A, Connor F. Effect of a full and empty bladder on radiation dose to the uterus, ovaries and bladder from lumbar spine CT and X-ray examinations. *Br J Radiol* 2000;73:1290–6.
49. Harden SV, Twyman N, Lomas DJ, Williams D, Burnet NG, Williams MV. A method for reducing ovarian doses in whole neuro-axis irradiation for medulloblastoma. *Radiother Oncol* 2003;69:183–8.
50. Yuh GE, Loreda LN, Yonemoto LT, Bush DA, Shahnaazi K, Preston W, et al. Reducing toxicity from craniospinal irradiation: using proton beams to treat medulloblastoma in young children. *Cancer J* 2004;10:386–90.
51. Miralbell R, Lomax A, Russo M. Potential role of proton therapy in the treatment of pediatric medulloblastoma/primitive neuro-ectodermal tumors: spinal theca irradiation. *Int J Radiat Oncol Biol Phys* 1997;38:805–11.
52. Lee CT, Bilton SD, Famiglietti RM, Riley BA, Mahajan A, Chang EL, et al. Treatment planning with protons for pediatric retinoblastoma, medulloblastoma, and pelvic sarcoma: how do protons compare with other conformal techniques? *Int J Radiat Oncol Biol Phys* 2005;63:362–72.
53. Belinson JL, Doherty M, McDay JB. A new technique for ovarian transposition. *Surg Gynecol Obstet* 1984;159:157–60.
54. Hodel K, Rich WM, Austin P, DiSaia PJ. The role of ovarian transposition in conservation of ovarian function in radical hysterectomy followed by pelvic radiation. *Gynecol Oncol* 1982;13:195–202.
55. Husseinzadeh N, Nahhas WA, Velkly DE, Whitney CW, Mortel R. The preservation of ovarian function in young women undergoing pelvic radiation therapy. *Gynecol Oncol* 1984;18:373–9.

56. Husseinzadeh N, van Aken ML, Aron B. Ovarian transposition in young patients with invasive cervical cancer receiving radiation therapy. *Int J Gynecol Cancer* 1994;4:61–5.
57. Scott SM, Schlaff W. Laparoscopic medial oophoropexy prior to radiation therapy in an adolescent with Hodgkin's disease. *J Pediatr Adol Gynecol* 2005;18:355–7.
58. Williams RS, Littell RD, Mendenhall NP. Laparoscopic oophoropexy and ovarian function in the treatment of Hodgkin disease. *Cancer* 1999;86:2138–42.
59. Hadar H, Loven D, Herskovitz P, Bairey O, Yagoda A, Levavi H. An evaluation of lateral and medial transposition of the ovaries out of radiation fields. *Cancer* 1994;74:774–9.
60. LeFloch O, Donaldson SS, Kaplan HS. Pregnancy following oophoropexy and total nodal irradiation in women with Hodgkin's disease. *Cancer* 1976;38:2263–8.
61. Thibaud E, Ramirez M, Brauner R, Flamant F, Zucker JM, Fekete C, et al. Preservation of ovarian function by ovarian transposition performed before pelvic irradiation during childhood. *J Pediatr* 1992;121:880–4.
62. Franke HR, Smit WM, Vermes I. Gonadal protection by a gonadotropin-releasing hormone agonist depot in young women with Hodgkin's disease undergoing chemotherapy. *Gynecol Endocrinol* 2005;20:274–8.
63. Monnier A. Clinical management of adverse events in adjuvant therapy for hormone-responsive early breast cancer. *Ann Oncol* 2007;18(Suppl):viii36–44.
64. Gook DA, Edgar DH. Human oocyte cryopreservation. *Human Reprod Update* 2007;13:591–605.
65. al-Shawaf T, Dave R, Harper J, Linehan D, Riley P, Craft I. Transfer of embryos into the uterus: how much do technical factors affect pregnancy rates? *J Assist Reprod Genet* 1993;10:31–6.
66. Frederick JL, Ord T, Kettel LM, Stone SC, Balmaceda JP, Asch RH. Successful pregnancy outcome after cryopreservation of all fresh embryos with subsequent transfer into an unstimulated cycle. *Fertil Steril* 1995;64:987–90.
67. Selick CE, Hofmann GE, Albano C, Horowitz GM, Copperman AB, Garrisi GJ, et al. Embryo quality and pregnancy potential of fresh compared with frozen embryos—is freezing detrimental to high quality embryos? *Hum Reprod* 1995;10:392–5.
68. Senn A, Vozzi C, Chanson A, De Grandi P, Germond M. Prospective randomized study of two cryopreservation policies avoiding embryo selection: the pronucleate stage leads to a higher cumulative delivery rate than the early cleavage stage. *Fertil Steril* 2000;74:946–52.
69. Wang JX, Yap YY, Matthews CD. Frozen-thawed embryo transfer: influence of clinical factors on implantation rate and risk of multiple conception. *Hum Reprod* 2001;16:2316–9.
70. Son WY, Yoon SH, Yoon HJ, Lee SM, Lim JH. Pregnancy outcome following transfer of human blastocysts vitrified on electron microscopy grids after induced collapse of the blastocoele. *Hum Reprod* 2003;18:137–9.
71. Mandelbaum J, Junca AM, Plachot M, Alnot MO, Salat-Baroux J, Alvarez S, et al. Cryopreservation of human embryos and oocytes. *Hum Reprod* 1988;3:117–9.
72. Imoedemhe DG, Sigue AB. Survival of human oocytes cryopreserved with or without the cumulus in 1,2-propanediol. *J Assist Reprod Genet* 1992;9:323–7.
73. Oktay K, Kan MT, Rosenwaks Z. Recent progress in oocyte and ovarian tissue cryopreservation and transplantation. *Curr Opin Obstet Gynecol* 2001;13:263–8.
74. Sonmezer M, Oktay K. Fertility preservation in female patients. *Hum Reprod Update* 2004;10:251–66.
75. Katayama KP, Stehlik J, Kuwayama M, Kato O, Stehlik E. High survival rate of vitrified human oocytes results in clinical pregnancy. *Fertil Steril* 2003;80:223–4.
76. Yoon TK, Kim TJ, Park SE, Hong SW, Ko JJ, Chung HM, et al. Live births after vitrification of oocytes in a stimulated in vitro fertilization–embryo transfer program. *Fertil Steril* 2003;79:1323–6.
77. Cobo A, Kuwayama M, Pérez S, Ruiz A, Pellicer A, Remohí J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril* 2008;89:1657–64.
78. Wallace WH, Pritchard J. Livebirth after cryopreserved ovarian tissue autotransplantation. *Lancet* 2004;364:2093–4.
79. Kim SS. Fertility preservation in female cancer patients: current developments and future directions. *Fertil Steril* 2006;85:1–11.
80. Silber SJ, Lenahan KM, Levine DJ, Pineda JA, Gorman KS, Friez MJ, et al. Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. *N Engl J Med* 2005;353:58–63.
81. Meirov D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med* 2005;353:318–21.