Fertility Preservation and Management of Gonadal Failure Associated with Lymphoma Therapy

*Simon J. Howell, MD and Stephen M. Shalet, MD, FRCP**

Address
*Christie Hospital NHS Trust, Wilmslow Road, Withington, Manchester M20 4BX, UK.
E-mail: Mmassey@picr.man.ac.uk

Current Oncology Reports 2002, **4**:443–452
Current Science Inc. ISSN 1523-3790

Treatment with cytotoxic chemotherapy and radiotherapy is associated with significant gonadal damage in men and women. The likelihood of gonadal failure following cytotoxic chemotherapy is dependent on the drug and dose, and in women there is also an effect of age at treatment. Irradiation of the testes or ovaries, either directly or indirectly, is also a significant cause of gonadal dysfunction, and the potential to recover from damage is clearly related to the radiation dose received. Several methods of preserving gonadal function during potentially sterilizing treatment have been considered. At present, sperm banking remains the only proven method in men, although hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular germ cells are possibilities for the future. Transposition of the ovaries to allow better shielding during radiotherapy is of use in some women, and the prospect of cryopreservation and reimplanation of ovarian tissue appears to be promising.

Introduction

Cytotoxic chemotherapy and radiotherapy have improved the survival rates in hematologic malignancies. Treatment is, however, associated with significant morbidity in many patients, and alterations in gonadal function are among the most common long-term side effects. Because of the significant reproductive morbidity associated with chemotherapy and radiotherapy, much attention is now directed toward the appropriate management of gonadal dysfunction, whereas research continues to seek ways to prevent damage to, or enhance the recovery of, gonadal function.

Men

Chemotherapy

Many drugs, particularly alkylating agents, have been shown to be gonadotoxic, and the agents most commonly implicated are listed in Table 1. Testicular damage is drug-specific and dose-related [1–4]. The chance of recovery of spermatogenesis following cytotoxic insult, and also the extent and speed of recovery, are related to the agent used and the dose received. It has also been suggested, but not proven, that the germinal epithelium of the adult testis is more susceptible to damage than that of the prepubertal testis [5], implying that patient age or maturation of the testis at the time of cytotoxic insult may influence the degree of damage. The germinal epithelium is far more sensitive to the effects of cytotoxic drugs than are the Leydig cells, and, although complete azoospermia is not uncommon following therapy, evidence of Leydig cell dysfunction is usually limited to raised leuteinizing hormone (LH) levels, with normal or low normal testosterone levels.

The impact on testicular function of chemotherapy used in the treatment of lymphomas, especially Hodgkin's disease, has been widely reported. Several studies have reported azoospermia, with raised follicle-stimulating hormone (FSH) levels in over 90% of men following cyclical chemotherapy with MVPP (mustine, vinblastine, procarbazine, and prednisolone) [6,7]. In an attempt to reduce the gonadotoxic effect of MVPP by halving the alkylating drug and reducing the procarbazine dose, a hybrid combination of chlorambucil, vinblastine, prednisolone, procarbazine, doxorubicin, vincristine, and etoposide (ChlVPP/EVA) has been used. However, in a direct comparison with MVPP, hybrid chemotherapy was found to have the same effect on gonadal function [8].

An alternative regime, however, of Adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) has been shown to be less gonadotoxic. Viviani *et al*. [9•] studied 24 men who received ABVD for Hodgkin's disease. The sperm count was normal a median of 6 months after therapy in 11 men, with oligospermia in a further five. In addition, full recovery of spermatogenesis occurred within 18 months of the first evaluation in all 13 men in whom the sperm count was repeated.

The effect on testicular function has also been assessed in children treated for Hodgkin's disease with ChlVPP (chlorambucil, vinblastine, procarbazine, and prednisolone). Testicular dysfunction, indicated by raised gonadotrophin levels, was found in a significant proportion of a cohort of 46 male patients treated with ChlVPP reported by

Table 1. Gonadotropic drugs

Group	Definite gonadotoxicity	Study
Alkylating agents	Cyclophosphamide	Rivkees and Crawford [5]
	Chlorambucil	
	Mustine	
	Melphalan	
	Busulfan	Sanders *et al.* [17]
	Carmustine	Clayton *et al.* [89]
	Lomustine	Clayton *et al.* [89]
Antimetabolites	Cytarabine	
Vinca alkaloids	Vinblastine	Vilar [90]
Others	Procarbazine	
	Cisplatin	Wallace *et al.* [91]

Mackie *et al*. [10], with 89% and 24% having raised FSH and LH levels, respectively. The COPP regimen (cyclophosphamide, vincristine, procarbazine, and prednisolone), which includes the gonadotoxic agent cyclophosphamide in addition to procarbazine, is associated with even more marked gonadal dysfunction. Charak *et al*. [11] found azoospermia in all 92 patients following treatment with six or more cycles of COPP, along with significant rises in gonadotrophin levels compared with pretreatment values. The median follow-up in this study was 6 years, with 17% of patients having been treated more than 10 years previously, suggesting that germinal epithelial failure is likely to be permanent.

In addition to effects on the germinal epithelium, there is also some evidence of Leydig cell dysfunction following chemotherapy for lymphomas. Howell *et al*. [12] measured testosterone and LH levels in 135 men treated with either MVPP or ChlVPP/EVA hybrid. They demonstrated a significantly higher LH level in patients compared with a cohort of age-matched control subjects (mean LH, 7.8 vs 4.1 IU/l). They suggested that this raised LH level indicated a reduction in hypothalamopituitary negative feedback consequent upon a small reduction in testosterone production. This may still result in testosterone levels that fall within the cross-sectional normal range; mild Leydig cell dysfunction, defined as a raised LH in the presence of a testosterone level in the lower half of the normal range or frankly subnormal, was found in 44 men (31%) following chemotherapy, with a further 10 (7%) having a raised LH level alone. These findings confirm that a significant proportion of men treated with cytotoxic chemotherapy have biochemical abnormalities suggesting mild testosterone deficiency.

Chemotherapy regimens used for the treatment of non-Hodgkin's lymphoma are generally less gonadotoxic than those used for Hodgkin's disease. Pryzant *et al*. [1] reported on 71 patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-based chemotherapy. All men were rendered azoospermic during treatment, but by 5 years 67% had recovered to normospermic levels, with a further 5% oligospermic. The reduced incidence of permanent infertility in men treated for non-Hodgkin's lymphoma compared with Hodgkin's disease is probably related to the absence of procarbazine in the standard regimens used for non-Hodgkin's lymphoma [13], although the reduction in the dose of alkylating agents may also be important. The absence of procarbazine and alkylating drugs is also the likely explanation for the reduced toxicity of ABVD reported by Viviani *et al*. [9•]. Other regimens not containing procarbazine that have been used for non-Hodgkin's lymphoma have also been shown to be less gonadotoxic. VAPEC-B (vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin) [14], VACOP-B (vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide, and bleomycin) [15], MACOP-B (mustine in place of vinblastine) [15], and VEEP (vincristine, etoposide, epirubicin, and prednisolone) [16] have all been associated with normal fertility following treatment in the vast majority of men.

Testicular function following high-dose chemotherapy used as preparation for bone marrow transplantation has also been studied. Sanders *et al*. [17] reported on a total of 155 men treated with cyclophosphamide (200 mg/kg) or busulfan and cyclophosphamide (busulfan, 16 mg/kg; cyclophosphamide, 200 mg/kg). After an average of 2 to 3 years following transplant, 67 of 109 men who received cyclophosphamide (61%), but only eight of 46 (17%) treated with busulfan and cyclophosphamide, had recovery of testicular function defined by normal LH, FSH, and testosterone levels with evidence of sperm production. The only prospective study to examine testicular function following high-dose treatment reported on 13 men who received either BEAM (BCNU, etoposide, ara-C, and melphalan) (n=11) or melphalan and single-fraction total-body irradiation (TBI) (n=2) [18]. All had previously received multiagent chemotherapy, and four had abnormal semen parameters before transplantation. All patients were azoospermic 2 to 3 months after transplantation, associated with raised FSH levels. LH levels increased and testosterone levels decreased after transplantation, indicating that Leydig cell damage was apparent in addition to germ cell failure.

These findings were also confirmed by Howell *et al*. [12], who studied 68 patients treated with high-dose chemotherapy (either cyclophosphamide, BCNU, etoposide, busulfan, and cyclophosphamide; or BCNU, etoposide, doxorubicin, and melphalan) as conditioning for bone marrow transplant. They demonstrated a raised FSH level in 60 patients (88%) and a raised LH level in 47 (69%), 22 of whom (32%) also had a testosterone level in the lower half of the normal range or frankly subnormal.

In addition to impairment of steroidogenesis and sperm production, concern has been expressed that cytotoxic chemotherapy may result in transmissible genetic damage. Studies have demonstrated untoward effects in offspring of animals treated with cytotoxic agents, but no

clear evidence for this has been reported in humans. Increased aneuploid frequency has been observed in human sperm following chemotherapy for Hodgkin's disease [19,20], and an increase in chromosomal abnormalities has been demonstrated several years after treatment for testicular cancer [21]. However, data concerning the outcome of pregnancies have not shown any increase in genetically mediated birth defects, altered sex ratios, or birth weight effects in offspring of cancer survivors [22], possibly as a result of selection bias against genetically abnormal sperm. On the evidence thus far, it is therefore reasonable to conclude that patients treated with cytotoxic chemotherapy who remain fertile are not at increased risk of fathering children with genetic abnormalities.

Radiotherapy

The testis is one of the most radiosensitive tissues. Very low doses of radiation significantly impair its function. Damage may be caused during direct irradiation of the testis or, more commonly, from scattered radiation during treatment directed at adjacent tissues.

The effects of relatively low-dose, single-fraction irradiation on spermatogenesis in healthy fertile men have been well documented [23], and are illustrated in Figure 1. The more immature cells are more radiosensitive, with doses as low as 0.1 Gy causing morphologic and quantitative changes to spermatogonia. Doses of 2 to 3 Gy result in overt damage to spermatocytes, leading to a reduction in spermatid numbers. At doses of 4 to 6 Gy, numbers of spermatozoa are significantly decreased, implying damage to spermatids. The decline in sperm count following damage to more immature cells, with doses of up to 3 Gy, takes 60 to 70 days, with doses above 0.8 Gy resulting in azoospermia and doses below 0.8 Gy giving rise to oligospermia. A much faster fall in sperm concentration occurs following doses of 4 Gy and above due to damage to spermatids.

Recovery of spermatogenesis takes place from surviving stem cells (type A spermatogonia) and is dependent on the dose of radiation. Complete recovery, indicated by a return to pre-irradiation sperm concentrations and germinal cell numbers, takes place within 9 to 18 months following a dose of 1 Gy or less, 30 months for 2 to 3 Gy, and 5 years or more for doses of 4 Gy and above.

Animal data suggest that fractionation of radiotherapy increases its gonadal toxicity, and the evidence suggests that this is also the case in humans. Speiser *et al.* [24] studied 10 patients who received a testicular dose of radiation of 1.2 to 3.0 Gy, in 14 to 26 fractions, during inverted Y-inguinal field irradiation for Hodgkin's disease. All patients were azoospermic following treatment, and recovery was not seen in a single patient despite follow-up of over 15 months in four patients and up to 40 months in one. An update of these data published in 1994 [25] revealed no recovery of spermatogenesis in patients receiving doses of 1.4 to 2.6 Gy after 17 to 43 months of follow-up, but a return of fertility in the two patients with testicular radiation doses of 1.2 Gy, suggesting that this dose may represent a threshold for permanent testicular damage.

Lower doses of radiation to the testes are, however, associated with better recovery rates for spermatogenesis. Kinsella *et al.* [26] published data concerning 17 patients who had received low-dose scattered radiation during treatment of Hodgkin's disease. Testicular doses of less than 0.2 Gy had no significant effect on FSH levels or sperm counts, whereas doses between 0.2 and 0.7 Gy caused a transient dose-dependent increase in FSH and reduction in sperm concentration, with a return to normal values within 12 to 24 months.

Leydig cells are more resistant to damage from radiotherapy than the germinal epithelium. Significant rises in LH have been demonstrated with single-dose radiation above 0.75 Gy [27•] and fractionated doses above 2 Gy [28]. However, no change in testosterone level was seen at these doses, and LH values showed a gradual return to normal levels over 30 months.

The clinical impact of Leydig cell dysfunction

A significant proportion of men have evidence of impaired Leydig cell function following high-dose chemotherapy, procarbazine-containing chemotherapy, or radiation involving the testis. The biochemical abnormalities are usually mild and consist of a raised LH level associated with a low/normal testosterone level. A deleterious impact of overt testosterone deficiency, and a clear benefit of androgen replacement in such patients on bone density, body composition, and quality of life, have been demonstrated. However, few data are available concerning the impact of replacement therapy on milder forms of testosterone deficiency.

In a cohort of men treated with MVPP, ChlVPP/EVA hybrid, or high-dose chemotherapy for a variety of malignancies, Howell *et al.* [29] identified a cohort of 35 patients with biochemical evidence of mild Leydig cell insufficiency. Insufficiency was defined as a raised LH level and a testosterone level in the lower half of the normal range or frankly subnormal. They demonstrated significantly reduced bone mineral density (BMD) at the hip in these men, compared with a similarly treated cohort with normal hormone levels, and also found evidence of altered body composition, reduced sexual activity, and alterations in mood [30,31]. The men were then enrolled in a 12-month randomized, single-blind, placebo-controlled trial of testosterone replacement [29]. During the 12-month study period, however, there were no significant improvements in BMD, body composition, sexual function, energy levels, or mood in the testosterone-treated group compared with the control subjects.

Thus, it seems likely that the mild biochemical abnormalities (*ie*, raised LH and low/normal testosterone) observed in many men following cytotoxic chemotherapy are not clinically important in the vast majority of patients

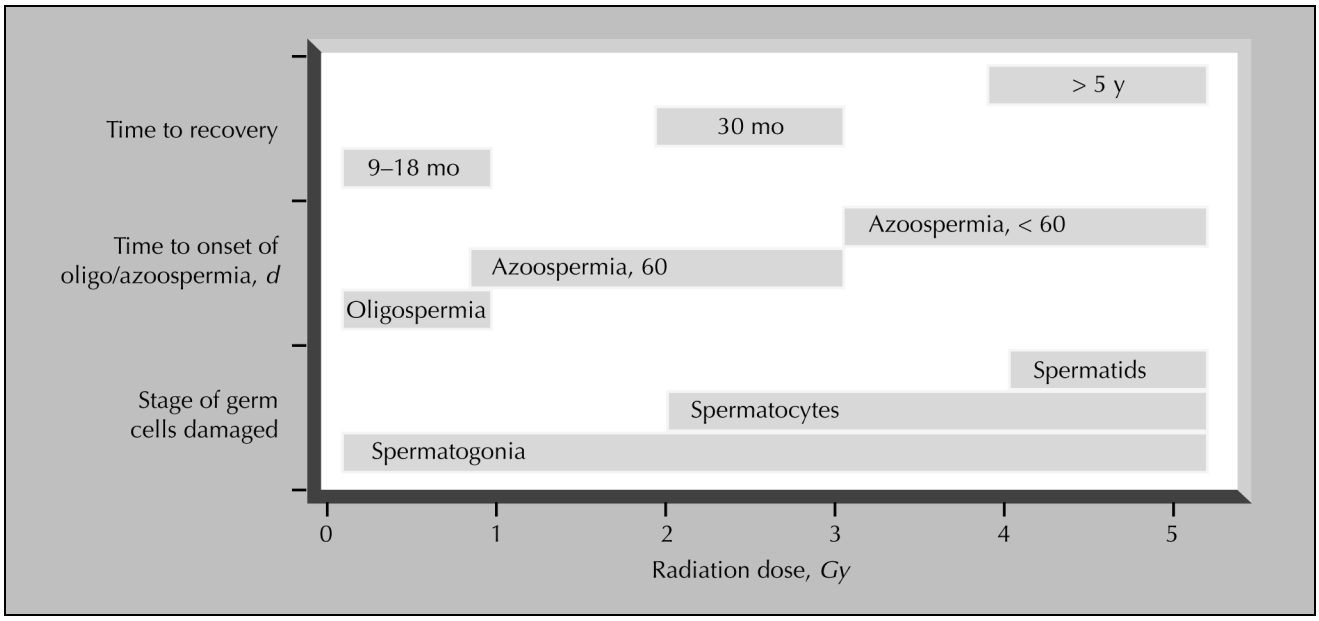

Figure 1. Impairment of spermatogenesis following single-dose radiotherapy is depicted, including the effect of radiation dose on stage of germ cell damage, time to onset, and recovery from germ cell damage.

and that androgen replacement is not routinely indicated. However, it remains possible that a minority of men with more marked biochemical abnormalities may benefit from androgen therapy.

Protection of testicular function during cancer treatment

The deleterious effect of chemotherapy and radiotherapy on germinal epithelial function has initiated a search for strategies to preserve fertility in men undergoing therapy.

Semen cryopreservation and assisted reproduction

Cryostorage of semen has become standard practice, and it should be offered to all men before they undergo potentially sterilizing therapy. Improvements in the techniques used to store semen [32] and advances in the field of assisted reproduction, such as intracytoplasmic sperm injection (ICSI), have increased the chance of successful pregnancies using cryopreserved sperm. However, some limitations exist with this method of preserving fertility. First, it is not a feasible option for prepubertal patients. Furthermore, testicular function in adult males with malignant disease is often impaired before treatment, resulting in poor sperm quality or difficulty providing semen for storage [33]. Oligospermia is found in a third to half of patients with Hodgkin's disease, non-Hodgkin's lymphoma, and testicular cancer prior to treatment, and it also occurs in men with leukemia and soft tissue cancer [34]. Sperm motility is impaired in these patients as well, and the process of freezing and thawing semen further reduces the sperm quality. Whereas successful fertilization may be achieved with only a few viable sperm using ICSI, pregnancy rates using this method are lower with abnormal semen than with normal semen [35]. As a result, methods for protecting or enhancing the recovery of normal spermatogenesis following gonadotoxic therapy have been pursued.

Hormonal manipulation

The belief that prepubertal boys have a lower rate of permanent chemotherapy-induced gonadal damage [5] led many investigators to propose that suppression of testicular function in adult men (*ie*, inducing a "prepubertal state") will provide a degree of protection against cytotoxic therapy. Irrespective of the validity of the hypothesis, data derived from animal models have been encouraging, but there is at present no convincing evidence of similar success in humans. Ward *et al*. [36] demonstrated enhanced recovery of spermatogenesis in procarbazine-treated rats by administration of the gonadotropin-releasing hormone (GnRH) analogue Zoladex (AstraZeneca, Wilmington, DE) for 2 weeks before and during chemotherapy. Increased stem cell survival was evident by 50 days, and at 90 days sperm count was close to normal and significantly higher than it was in rats treated with procarbazine alone. Similar protective effects of hormonal treatment have been described following the use of testosterone [37], testosterone and estradiol [38], GnRH and testosterone [39], and GnRH and the antiandrogen flutamide [40,41], following gonadal insult with procarbazine, cyclophosphamide, or radiotherapy. Pogach *et al*. [39] suggested that testosterone administered after treatment with procarbazine enhanced the recovery of spermatogenesis. More recently, Meistrich and Kangasniemi [42••] confirmed that treatment with either testosterone or Zoladex following a 3.5-Gy radiation dose markedly improves the recovery of spermatogenesis, even if treatment is delayed for 10 weeks after irradiation. The same group had previously shown that spermatogenesis did not occur

after a similar dose of radiation despite the presence of A spermatogonia in the seminiferous tubules [43]. They postulated that the role of hormonal treatment in the "protection" of germinal epithelial function may be to enhance recovery of surviving A spermatogonia and to facilitate their differentiation to more mature cells, rather than to protect them from damage during cytotoxic therapy or radiotherapy. These authors suggested that a reduction of intratesticular testosterone, or one of its metabolites, is involved in the mechanism by which hormone therapy stimulates recovery of spermatogenesis.

In humans, attempts to reproduce the protective effects seen in animals have been unsuccessful. Several groups have used GnRH analogues, with and without testosterone, to suppress testicular function during MOPP [44] or MVPP [45] chemotherapy for lymphoma, cisplatin-based chemotherapy for teratoma [46], and testicular irradiation for seminoma [47]. None of these regimens has demonstrated any significant protective effect in terms of maintaining spermatogenesis or increasing the rate of recovery. However, none of the studies involved the continuation of gonadal-suppressive therapy for a significant period of time after the completion of chemotherapy or radiotherapy. The most recent animal data suggest that hormonal treatment may enhance recovery of spermatogenesis from surviving stem cells rather than protect them from damage during cytotoxic or radiation insult. Thus, suppression of gonadal function with a GnRH agonist or testosterone for a fixed time after radiotherapy or chemotherapy may prove more successful in reducing the impact of these treatments on fertility.

This approach relies on enhancing recovery of sperm production, and therefore a prerequisite for its success is the survival of stem cells during the gonadotoxic insult. Few data are available, however, regarding testicular histology after chemotherapy or radiotherapy. Following chemotherapy for Hodgkin's disease with procarbazine-containing regimens and high-dose radiotherapy, recovery to oligo- or normospermia is uncommon. Testicular biopsies taken after standard chemotherapy (MVPP and COPP) for Hodgkin's disease have shown complete germinal aplasia with a Sertoli-cell only pattern [6,11,33,48,49]. Recent reports have been made of the isolation of mature sperm in the testicular parenchyma of some men with biopsy evidence of Sertoli-cell only, suggesting that even in this situation there may be small foci of spermatogenesis [50]. In addition, recovery of spermatogenesis occurs in a minority of these patients, indicating that some germ cells survive in some patients. However, the absence of histologic evidence of any spermatogenesis at biopsy in many men suggests that all spermatogonia may be eradicated during chemotherapy.

Hormonal manipulation after treatment to enhance the recovery of spermatogenesis is therefore likely to be the most beneficial in those patients in whom the testicular insult is less severe, as it is these patients in whom there is significant preservation of A spermatogonia. The success of this approach in those patients who have undergone more gonadotoxic therapy will depend on whether any stem cells remain. Complete ablation of the germinal epithelium may occur in many men following treatment with procarbazine-based chemotherapy for Hodgkin's disease, and this will clearly be irreversible.

Stem cell cryopreservation

Results from recent animal experiments indicate another possible method of preserving testicular function during gonadotoxic therapy. In 1994, Brinster *et al*. [51••] demonstrated that stem cells isolated from a donor mouse could be injected into the seminiferous tubules of a sterile recipient mouse and result in the initiation of spermatogenesis. More recently, the same group demonstrated that spermatogenesis can be achieved in previously sterile mice following cryopreservation and subsequent injection of donor stem cells into the testis. Potentially, therefore, stem cells could be harvested from the human testis before the start of sterilizing therapy, freeze-stored, and reimplanted at a later date, with a subsequent return of spermatogenesis.

A clinical trial testing this hypothesis is currently underway in adults: 16 men had testicular tissue harvested shortly before commencing treatment with sterilizing chemotherapy for Hodgkin's disease or non-Hodgkin's lymphoma. In each case, a 0.5-cm cube of testicular tissue was subjected to enzymatic digestion to produce a single cell suspension which, following equilibration in cryoprotectant, has been stored in liquid nitrogen [52]. Seven men have now successfully completed chemotherapy, and thawed testicular suspension has been reinjected into the donor testis. Semen analysis has demonstrated a return of spermatogenesis in one man at the time of writing. However, this may simply represent spontaneous recovery of spermatogenesis, as is seen in a small proportion of men following treatment, rather than repopulation from cryopreserved stem cells. The lack of greater success may relate to problems reinjecting the testicular suspension. The seminiferous tubules in adult men are too fibrous to allow direct injection, and therefore an indirect approach is necessary. This approach consisted of injecting into the rete testes and relying on retrograde flow to fill the tubules, which may not occur to a sufficient extent to allow repopulation and a return of spermatogenesis. Further studies are currently being undertaken using non-disaggregated testicular tissue, and the results are awaited with interest.

Women

Chemotherapy

The gonadotoxic effects of chemotherapy in women were first reported in 1956 by Louis *et al.* [53] in women treated with busulfan for chronic myeloid leukemia. Ovarian damage has been clearly shown to be dependent on dose and age [54,55], with progressively smaller doses required to induce permanent amenorrhea with increasing age [54].

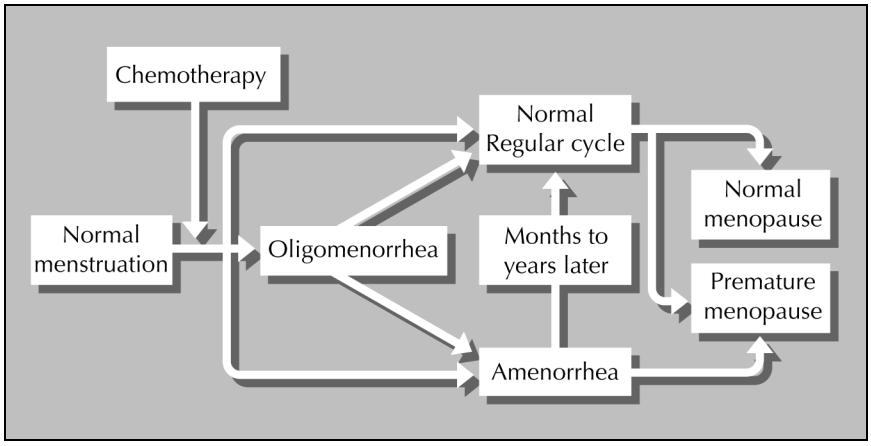

Figure 2. The impact of chemotherapy on the menstrual cycle is shown.

Premature ovarian failure has also been observed following treatment for lymphomas in both children and adults. The incidence of ovarian dysfunction varies according to the chemotherapy regime used and is also strongly age dependent. Treatment of Hodgkin's disease with MVPP [56], COPP [13,57], and ChlVPP [10] results in ovarian failure in between 38% and 57% of patients, and maintenance of normal ovarian function in women aged over 35 years at the time of treatment is unusual. High-dose chemotherapy, employed as conditioning for bone marrow transplant, is also gonadotoxic [17,58–61]. Several studies have suggested high rates of ovarian dysfunction following treatment, although rates vary according to the regimen used. The use of busulfan is almost invariably associated with permanent ovarian failure [17,58–60], whereas regimens not containing busulfan have a better outcome. The use of melphalan-based regimens is associated with retention of fertility in a higher proportion of patients. Jackson *et al*. [61] reported pregnancies in 10 of 23 women aged under 40 years who were treated for lymphoma with melphalan, with or without etoposide. In addition, there is evidence that a younger age at bone marrow transplant is protective. Matsumoto *et al*. [62] studied a group of girls who underwent bone marrow transplant before the age of 12 years. They received cyclophosphamide, ara-C, and etoposide either alone or in combination in addition to TBI. A high proportion (67%) went through puberty spontaneously, suggesting preservation of ovarian function, and those who did not achieve menarche were significantly older at treatment.

Longitudinal studies have demonstrated that the course of ovarian function after chemotherapy can be varied, as illustrated in Figure 2. There is evidence of a reduction in ovarian follicle numbers after chemotherapy [63], and the evolution of ovarian dysfunction with time is consistent with the destruction of a fixed number of oocytes. Older patients have a smaller pool of remaining oocytes before cytotoxic treatment and are therefore more likely to become permanently amenorrheic following therapy. Younger patients often continue with normal ovarian function after cytotoxic insult, but they may undergo premature menopause. Byrne *et al*. [64] took menstrual histories from over 1000 women who were still menstruating at the age of 21 after receiving treatment for malignancy during childhood. They found a much higher rate of premature menopause in these women compared with a control population, with 42% of the treated women having reached menopause by the age of 31, compared with only 5% of the control subjects.

A proportion of women who are initially amenorrheic after treatment, with biochemical evidence of premature ovarian failure, recover ovarian function with a return of normal menses and fertility [8]. There are no indicators that allow the identification of this subgroup of patients other than the fact that it is more likely to occur in younger women. The duration of amenorrhea in these women varies from a few months to several years, and the mechanisms underlying the recovery of ovarian function are unclear.

In women who remain fertile following chemotherapy, there is no evidence of an increase in birth defects during subsequent pregnancies. There is, however, some evidence for teratogenicity of several cytotoxic agents when they are administered during pregnancy [65,66]. Antimetabolites, such as methotrexate and, to a lesser extent, alkylating agents, are associated with an increase in spontaneous abortions and congenital malformations following exposure during the first trimester. The risk of birth defects when chemotherapy is given during the second and third trimesters is probably no greater than the background rate. Women should therefore be advised to avoid conception during chemotherapy, and adequate contraception should probably be continued until the patient is in stable remission and is unlikely to require further cytotoxic therapy.

Radiotherapy

Information regarding the impact of irradiation on ovarian function has been acquired from women treated with pelvic radiotherapy for dysfunctional uterine bleeding or malignancies such as Hodgkin's disease. Information has also been acquired regarding those treated with TBI prior to bone marrow transplant. The effects of radiation are dose and age dependent. Ovarian doses of less than 4 Gy do not usually result in permanent ovarian dysfunction

[48], which is not surprising [67], because the calculated LD_{50} of the human oocyte does not exceed 4 Gy [68].

Women aged under 40 years are less sensitive to radiation-induced ovarian damage, with an estimated dose of 20 Gy required to produce permanent ovarian failure, compared with 6 Gy in older women [69]. Higher ovarian radiotherapy doses will almost inevitably result in ovarian failure irrespective of age.

In addition to radiotherapy for pelvic and abdominal disease, TBI used as conditioning for bone marrow transplant may also cause ovarian dysfunction [58,70,71]. Treatment with TBI (10 to 15 Gy given as a single dose or fractionated), often used in combination with cyclophosphamide or melphalan, is almost invariably followed by a cessation of menses. Late recovery of ovarian function, however, has been reported following a spell of amenorrhea. Sanders *et al*. [70] studied a group of 187 women who underwent bone marrow transplant for various hematologic conditions; 144 received TBI and cyclophosphamide and were evaluated between 1 and 7 years later. All 144 had evidence of ovarian failure with amenorrhea, raised gonadotrophins, and decreased estradiol for the first 3 years after the transplant. Recovery of ovarian function occurred in nine women between 3 and 7 years after transplant, all of whom were aged below 25 years. Thus, whereas a few younger women may recover ovarian function following TBI, the outlook is universally poor for older women.

Although infertility is often the major concern for women undergoing potentially gonadotoxic therapy, the consequences of ovarian failure are not limited to the cessation of oocyte production but also include the loss of ovarian steroidogenesis. Symptoms of estrogen deficiency, such as hot flashes, irritability, and vaginal dryness, are not uncommon, and often necessitate hormone replacement therapy (HRT). The long-term effects of estrogen deficiency have been well documented, with a possible increase in cardiovascular mortality, alterations in lipid profile, and reduction in BMD. Whereas some of these changes have been confirmed in women with chemotherapy- and radiotherapy-induced ovarian failure [72], evidence suggests that ovarian dysfunction resulting from chemotherapy alone may not have exactly the same deleterious effects. Saarto *et al*. [73] have shown that high-density lipoprotein (HDL) levels rise in women with cyclophosphamide-induced ovarian failure in contrast to the fall in HDL levels seen after natural and surgical menopause. This may in part negate the adverse effect of other changes in the lipid profile with respect to cardiovascular risk. In addition, although significant reductions in BMD have been reported in women with ovarian failure following gonadotoxic therapy [72,74,75], the one cohort in whom treatment was restricted to chemotherapy alone displayed only small mean reductions in BMD, with the majority of patients showing BMD z-scores well within the normal range.

Prevention of ovarian failure

Because of the consequences of ovarian failure in young women, several strategies aimed at reducing the gonadotoxic effect of cancer treatment have been investigated. Transposition of the ovaries before radiotherapy can reduce the dose to approximately 10% of the given dose during pelvic radiotherapy [48,76,77]. This practice has been shown to reduce the incidence of ovarian dysfunction [77–79]. However, routine use of ovarian transposition in Hodgkin's disease patients treated with radiotherapy remains controversial. Hormonal methods of maintaining fertility during treatment with radiotherapy or chemotherapy have also been investigated. Results with animal models suggest that suppression of the pituitary-gonadal axis with GnRH may provide some protection against ovarian follicular depletion in rats [80] and monkeys [81]. There are few published data in humans, and results have been contradictory [45,82,83]. Two recent studies found that GnRH agonists may partially protect against chemotherapy-induced ovarian damage [83,84]. These results need to be confirmed in larger studies before GnRH analogues can be considered for use in routine clinical practice.

Whereas sperm banking may provide a means of preserving the reproductive capacity of some men, a similar approach in women is not feasible for several reasons. First, although treatment can often be deferred for a few days to allow the collection of semen, the longer delay required to allow the harvesting of mature oocytes would often be unacceptable. Furthermore, only a handful of pregnancies have been established with cryopreserved oocytes, and there is some concern that freezing may be associated with chromosomal abnormalities. Cryopreservation of embryos is much more successful, but there is no guarantee of success, time restraints are still a problem, and the technique is of no use to women who have no permanent partner.

One possible method of preserving both the reproductive and steroidogenic capacity of the ovary is the cryopreservation of strips of ovarian tissue, to be reimplanted at a later date. Several recent reports have demonstrated the potential for this method for preservation of ovarian function. Endocrine function and ovulation have been demonstrated in ovarian strips autologously transplanted into the pelvis and forearm [85,86]. There is a theoretic possibility of reintroducing tumor cells during the implantation of ovarian tissue, but this seems unlikely in conditions such as Hodgkin's disease, in which tumor involvement of the ovaries is extremely unusual. In addition, results from initial studies involving implantation of ovarian tissue harvested before chemotherapy for Hodgkin's disease into SCID mice have been encouraging [87]. The first study examining the use of reimplantation of cryopreserved ovarian tissue in women following cytotoxic chemotherapy has demonstrated endocrine function in the transplanted ovarian strip [88••]. This method may thus provide a realistic possibility of maintaining fertility in women who

would otherwise be rendered infertile, although it remains experimental at this stage.

Conclusions

Cytotoxic chemotherapy and radiotherapy are associated with significant gonadal damage in men and women. Alkylating agents, such as cyclophosphamide and procarbazine, are the most common agents implicated. The vast majority of men receiving procarbazine-containing regimens for the treatment of lymphomas, and high-dose chemotherapy as conditioning for bone marrow transplant, are rendered permanently infertile. Treatment with ABVD is associated with a much better outcome, with temporary oligo- or azoospermia being the rule. There is also evidence of Leydig cell impairment in a proportion of these men, although this does not appear to be clinically significant in the majority of patients. The germinal epithelium is very sensitive to radiation-induced damage, with changes to spermatogonia following as little as 0.1 Gy, and permanent infertility after fractionated doses of 2 Gy and above.

Cytotoxic-induced premature ovarian failure is age and drug dependent and ensues in approximately half of the women treated with procarbazine-containing chemotherapy for lymphomas. High-dose chemotherapy, TBI, and radiation at an ovarian dose above 6 Gy usually result in permanent ovarian failure. The course of ovarian function after chemotherapy is variable, and late recovery occurs in some patients.

Several methods of preserving gonadal function during potentially sterilizing treatment have been considered. At present, sperm banking remains the only proven method in men, although hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular germ cells are possibilities for the future. Transposition of the ovaries to allow better shielding during radiotherapy is of use in some women, and the prospect of cryopreservation and reimplantation of ovarian tissue appears promising. Suppression of the pituitary ovarian axis with GnRH analogues has been studied, but results are contradictory. Further study is required to ascertain whether this approach can be used to preserve fertility.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Pryzant RM, Meistrich ML, Wilson G, *et al.*: **Long-term reduction in sperm count after chemotherapy with and without radiation therapy for non-Hodgkin's lymphomas.** *J Clin Oncol* 1993, **11:**239–247.
2. Meistrich ML, Chawla SP, Da Cunha MF, *et al.*: **Recovery of sperm production after chemotherapy for osteosarcoma.** *Cancer* 1989, **63:**2115–2123.
3. da Cunha MF, Meistrich ML, Fuller LM, *et al.*: **Recovery of spermatogenesis after treatment for Hodgkin's disease: limiting dose of MOPP chemotherapy.** *J Clin Oncol* 1984, **2:**571–577.
4. Watson AR, Rance CP, Bain J: **Long term effects of cyclophosphamide on testicular function.** *Br Med J (Clin Res Ed)* 1985, **291:**1457–1460.
5. Rivkees SA, Crawford JD: **The relationship of gonadal activity and chemotherapy-induced gonadal damage.** *JAMA* 1988, **259:**2123–2125.
6. Chapman RM, Sutcliffe SB, Rees LH, *et al.*: **Cyclical combination chemotherapy and gonadal function: retrospective study in males.** *Lancet* 1979, **1:**285–289.
7. Whitehead E, Shalet SM, Blackledge G, *et al.*: **The effects of Hodgkin's disease and combination chemotherapy on gonadal function in the adult male.** *Cancer* 1982, **49:**418–422.
8. Clark ST, Radford JA, Crowther D, *et al.*: **Gonadal function following chemotherapy for Hodgkin's disease: a comparative study of MVPP and a seven-drug hybrid regimen.** *J Clin Oncol* 1995, **13:**134–139.
9. • Viviani S, Santoro A, Ragni G, *et al.*: **Gonadal toxicity after combination chemotherapy for Hodgkin's disease: comparative results of MOPP vs ABVD.** *Eur J Cancer Clin Oncol* 1985, **21:**601–605.

Demonstrates the reduced long-term gonadal toxictity of ABVD compared with MOPP.

10. Mackie EJ, Radford M, Shalet SM: **Gonadal function following chemotherapy for childhood Hodgkin's disease.** *Med Pediatr Oncol* 1996, **27:**74–78.
11. Charak BS, Gupta R, Mandrekar P, *et al.*: **Testicular dysfunction after cyclophosphamide-vincristine-procarbazine-prednisolone chemotherapy for advanced Hodgkin's disease: a long-term follow-up study.** *Cancer* 1990, **65:**1903–1906.
12. Howell SJ, Radford JA, Shalet SM: **Testicular function following cytotoxic chemotherapy: evidence of Leydig cell insufficiency.** *J Clin Oncol* 1999, **17:**1493–1498.
13. Bokemeyer C, Schmoll HJ, van Rhee J, *et al.*: **Long-term gonadal toxicity after therapy for Hodgkin's and non-Hodgkin's lymphoma.** *Ann Hematol* 1994, **68:**105–110.
14. Radford JA, Clark S, Crowther D, Shalet SM: **Male fertility after VAPEC-B chemotherapy for Hodgkin's disease and non-Hodgkin's lymphoma.** *Br J Cancer* 1994, **69:**379–381.
15. Muller U, Stahel RA: **Gonadal function after MACOP-B or VACOP-B with or without dose intensification and ABMT in young patients with aggressive non-Hodgkin's lymphoma.** *Ann Oncol* 1993, **4:**399–402.
16. Hill M, Milan S, Cunningham D, *et al.*: **Evaluation of the efficacy of the VEEP regimen in adult Hodgkin's disease with assessment of gonadal and cardiac toxicity.** *J Clin Oncol* 1995, **13:**387–395.
17. Sanders JE, Hawley J, Levy W, *et al.*: **Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation.** *Blood* 1996, **87:**3045–3052.
18. Chatterjee R, Mills W, Katz M, *et al.*: **Germ cell failure and Leydig cell insufficiency in post-pubertal males after autologous bone marrow transplantation with BEAM for lymphoma.** *Bone Marrow Transplant* 1994, **13:**519–522.
19. Monteil M, Rousseaux S, Chevret E, *et al.*: **Increased aneuploid frequency in spermatozoa from a Hodgkin's disease patient after chemotherapy and radiotherapy.** *Cytogenet Cell Genet* 1997, **76:**134–138.
20. Robbins WA, Meistrich ML, Moore D, *et al.*: **Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm.** *Nat Genet* 1997, **16:**74–78.
21. Genesca A, Benet J, Caballin MR, *et al.*: **Significance of structural chromosome aberrations in human sperm: analysis of induced aberrations.** *Hum Genet* 1990, **85:**495–499.
22. Robbins WA: **Cytogenetic damage measured in human sperm following cancer chemotherapy.** *Mutat Res* 1996, **355:**235–252.

23. Shalet SM: **Effect of irradiation treatment on gonadal function in men treated for germ cell cancer.** *Eur Urol* 1993, **23:**148–151.
24. Speiser B, Rubin P, Casarett G: **Aspermia following lower truncal irradiation in Hodgkin's disease.** *Cancer* 1973, **32:**692–698.
25. Centola GM, Keller JW, Henzler M, Rubin P: **Effect of low-dose testicular irradiation on sperm count and fertility in patients with testicular seminoma.** *J Androl* 1994, **15:**608–613.
26. Kinsella TJ, Trivette G, Rowland J, *et al.*: **Long-term follow-up of testicular function following radiation therapy for early-stage Hodgkin's disease.** *J Clin Oncol* 1989, **7:**718–724.
27.• Rowley MJ, Leach DR, Warner GA, Heller CG: **Effect of graded doses of ionizing radiation on the human testis.** *Radiat Res* 1974, **59:**665–678.

Illustrates the effects of single-dose radiation on germinal epithelial function.

28. Shapiro E, Kinsella TJ, Makuch RW, *et al.*: **Effects of fractionated irradiation of endocrine aspects of testicular function.** *J Clin Oncol* 1985, **3:**1232–1239.
29. Howell S, Radford J, Adams J, *et al.*: **Randomised placebo controlled trial of testosterone replacement in men with mild Leydig cell insufficiency following cytotoxic chemotherapy.** *Clin Endocrinol* 2001, **55:**315–324.
30. Howell SJ, Radford JA, Adams JE, Shalet SM: **The impact of mild Leydig cell dysfunction following cytotoxic chemotherapy on bone mineral density (BMD) and body composition.** *Clin Enocrinol (Oxf)* 2000, **52:**609–616.
31. Howell SJ, Radford JA, Smets EMA, Shalet SM: **Fatigue, sexual function and mood following treatment for haematological malignancy: the impact of mild Leydig cell dysfunction.** *Br J Cancer* 2000, **82:**789–793.
32. Royere D, Barthelemy C, Hamamah S, Lansac J: **Cryopreservation of spermatozoa: a 1996 review.** *Hum Reprod Update* 1996, **2:**553–559.
33. Chapman RM, Sutcliffe SB, Malpas JS: **Male gonadal dysfunction in Hodgkin's disease: a prospective study.** *JAMA* 1981, **245:**1323–1328.
34. Padron OF, Sharma RK, Thomas AJ Jr, Agarwal A: **Effects of cancer on spermatozoa quality after cryopreservation: a 12-year experience.** *Fertil Steril* 1997, **67:**326–331.
35. Aboulghar MA, Mansour RT, Serour GI, *et al.*: **Fertilization and pregnancy rates after intracytoplasmic sperm injection using ejaculate semen and surgically retrieved sperm.** *Fertil Steril* 1997, **68:**108–111.
36. Ward JA, Robinson J, Furr BJ, *et al.*: **Protection of spermatogenesis in rats from the cytotoxic procarbazine by the depot formulation of Zoladex, a gonadotropin-releasing hormone agonist.** *Cancer Res* 1990, **50:**568–574.
37. Delic JI, Bush C, Peckham MJ: **Protection from procarbazine-induced damage of spermatogenesis in the rat by androgen.** *Cancer Res* 1986, **46:**1909–1914.
38. Kurdoglu B, Wilson G, Parchuri N, *et al.*: **Protection from radiation-induced damage to spermatogenesis by hormone treatment.** *Radiat Res* 1994, **139:**97–102.
39. Pogach LM, Lee Y, Gould S, *et al.*: **Partial prevention of procarbazine induced germinal cell aplasia in rats by sequential GnRH antagonist and testosterone administration.** *Cancer Res* 1988, **48:**4354–4360.
40. Meistrich ML, Parchuri N, Wilson G, *et al.*: **Hormonal protection from cyclophosphamide-induced inactivation of rat stem spermatogonia.** *J Androl* 1995, **16:**334–341.
41. Kangasniemi M, Wilson G, Huhtaniemi I, Meistrich ML: **Protection against procarbazine-induced testicular damage by GnRH-agonist and antiandrogen treatment in the rat.** *Endocrinology* 1995, **136:**3677–3678.
42.•• Meistrich ML, Kangasniemi M: **Hormone treatment after irradiation stimulates recovery of rat spermatogenesis from surviving spermatogonia.** *J Androl* 1997, **18:**80–87.

Demonstrates that hormone manipulation after potentially sterilizing treatment enhances recovery of spermatogenesis in rats.

43. Kangasniemi M, Huhtaniemi I, Meistrich ML: **Failure of spermatogenesis to recover despite the presence of a spermatogonia in the irradiated LBNF1 rat.** *Biol Reprod* 1996, **54:**1200–1208.
44. Johnson DH, Linde R, Hainsworth JD, *et al.*: **Effect of a luteinizing hormone releasing hormone agonist given during combination chemotherapy on posttherapy fertility in male patients with lymphoma: preliminary observations.** *Blood* 1985, **65:**832–836.
45. Waxman JH, Ahmed R, Smith D, *et al.*: **Failure to preserve fertility in patients with Hodgkin's disease.** *Cancer Chemother Pharmacol* 1987, **19:**159–162.
46. Kreuser ED, Hetzel WD, Hautmann R, Pfeiffer EF: **Reproductive toxicity with and without LHRHA administration during adjuvant chemotherapy in patients with germ cell tumors.** *Horm Metab Res* 1990, **22:**494–498.
47. Brennemann W, Brensing KA, Leipner N, *et al.*: **Attempted protection of spermatogenesis from irradiation in patients with seminoma by D-Tryptophan-6 luteinizing hormone releasing hormone.** *Clin Investig* 1994, **72:**838–842.
48. Ortin TT, Shostak CA, Donaldson SS: **Gonadal status and reproductive function following treatment for Hodgkin's disease in childhood: the Stanford experience.** *Int J Radiat Oncol Biol Phys* 1990, **19:**873–880.
49. Das PK, Das BK, Sahu DC, *et al.*: **Male gonadal function in Hodgkin's disease before and after treatment.** *J Assoc Physicians India* 1994, **42:**604–605.
50. Mulhall JP, Burgess CM, Cunningham D, *et al.*: **Presence of mature sperm in testicular parenchyma of men with nonobstructive azoospermia: prevalence and predictive factors.** *Urology* 1997, **49:**91–95.
51.•• Brinster RL, Zimmermann JW: **Spermatogenesis following male germ-cell transplantation.** *Proc Natl Acad Sci U S A* 1994, **91:**11298–11302.

Demonstrates spermatogenesis following implantation of donor germ cells into sterile mice.

52. Brook P, Radford J, Shalet S, *et al.*: **Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation.** *Fertil Steril* 2001, **75:**269–274.
53. Louis J, Limarzi LR, Best WR: **Treatment of chronic granulocytic leukaemia with Myleran.** *Arch Intern Med* 1956, **97:**299–308.
54. Koyama H, Wada T, Nishizawa Y, *et al.*: **Cyclophosphamide-induced ovarian failure and its therapeutic significance in patients with breast cancer.** *Cancer* 1977, **39:**1403–1409.
55. Bines J, Oleske DM, Cobleigh MA: **Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer.** *J Clin Oncol* 1996, **14:**1718–1729.
56. Whitehead E, Shalet SM, Blackledge G, *et al.*: **The effect of combination chemotherapy on ovarian function in women treated for Hodgkin's disease.** *Cancer* 1983, **52:**988–993.
57. Kreuser ED, Xiros N, Hetzel WD, Heimpel H: **Reproductive and endocrine gonadal capacity in patients treated with COPP chemotherapy for Hodgkin's disease.** *J Cancer Res Clin Oncol* 1987, **113:**260–266.
58. Chatterjee R, Mills W, Katz M, *et al.*: **Prospective study of pituitary-gonadal function to evaluate short-term effects of ablative chemotherapy or total body irradiation with autologous or allogenic marrow transplantation in post-menarcheal female patients.** *Bone Marrow Transplant* 1994, **13:**511–517.
59. Grigg A, McLachlan R, Zaja J, Szer J: **Reproductive status in long-term bone marrow transplant survivors receiving busulfan-cyclophosphamide (120 mg/kg).** *Bone Marrow Transplant* 2000, **26:**1089–1095.
60. Teinturier C, Hartmann O, Valteau Couanet D, *et al.*: **Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulfan is a major cause of ovarian failure.** *Bone Marrow Transplant* 1998, **22:**989–994.
61. Jackson GH, Wood A, Taylor PR, *et al.*: **Early high dose chemotherapy intensification with autologous bone marrow transplantation in lymphoma associated with retention of fertility and normal pregnancies in females. Scotland and Newcastle Lymphoma Group, UK.** *Leuk Lymphoma* 1997, **28:**127–132.

62. Matsumoto M, Shinohara O, Ishiguro H, *et al.*: **Ovarian transplant after bone marrow transplantation performed before menopause.** *Arch Dis Child* 1999, **80:**452–454.
63. Nicosia SV, Matus Ridley M, Meadows AT: **Gonadal effects of cancer therapy in girls.** *Cancer* 1985, **55:**2364–2372.
64. Byrne J, Fears TR, Gail MH, *et al.*: **Early menopause in long-term survivors of cancer during adolescence.** *Am J Obstet Gynecol* 1992, **166:**788–793.
65. Sorosky JI, Sood AK, Buekers TE: **The use of chemotherapeutic agents during pregnancy.** *Obstet Gynecol Clin North Am,* 1997, **24:**591–599.
66. Barnicle MM: **Chemotherapy and pregnancy.** *Semin Oncol Nurs,* 1992, **8:**124–132.
67. Faddy MJ, Gosden RG, Gougeon A, *et al.*: **Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause.** *Hum Reprod* 1992, **7:**1342–1346.
68. Wallace WH, Shalet SM, Hendry JH, *et al.*: **Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte.** *Br J Radiol* 1989, **62:**995–998.
69. Lushbaugh CC, Casarett GW: **The effects of gonadal irradiation in clinical radiation therapy: a review.** *Cancer* 1976, **37(suppl 2):**1111–1125.
70. Sanders JE, Buckner CD, Amos D, *et al.*: **Ovarian function following marrow transplantation for aplastic anemia or leukemia.** *J Clin Oncol* 1988, **6:**813–818.
71. Spinelli S, Chiodi S, Bacigalupo A, *et al.*: **Ovarian recovery after total body irradiation and allogeneic bone marrow transplantation: long-term follow up of 79 females.** *Bone Marrow Transplant* 1994, **14:**373–380.
72. Bruning PF, Pit MJ, de Jong Bakker M, *et al.*: **Bone mineral density after adjuvant chemotherapy for premenopausal breast cancer.** *Br J Cancer* 1990, **61:**308–310.
73. Saarto T, Blomqvist C, Ehnholm C, *et al.*: **Effects of chemotherapy-induced castration on serum lipids and apoproteins in premenopausal women with node-positive breast cancer.** *J Clin Endocrinol Metab* 1996, **81:**4453–4457.
74. Ratcliffe MA, Lanham SA, Reid DM, Dawson AA: **Bone mineral density (BMD) in patients with lymphoma: the effects of chemotherapy, intermittent corticosteroids and premature menopause.** *Hematol Oncol* 1992, **10:**181–187.
75. Redman JR, Bajorunas DR, Wong G, *et al.*: **Bone mineralization in women following successful treatment of Hodgkin's disease.** *Am J Med* 1988, **85:**65–72.
76. Haie Meder C, Mlika Cabanne N, Michel G, *et al.*: **Radiotherapy after ovarian transposition: ovarian function and fertility preservation.** *Int J Radiat Oncol Biol Phys* 1993, **25:**419–424.
77. Ray GR, Trueblood HW, Enright LP, *et al.*: **Oophoropexy: a means of preserving ovarian function following pelvic megavoltage radiotherapy for Hodgkin's disease.** *Radiology* 1970, **96:**175–180.
78. Williams R, Littell R, Mendenhall N: **Laparoscopic oophoroplexy and ovarian function in the treatment of Hodgkin's disease.** *Cancer* 1999, **86:**2138–2142.
79. Yarali H, Demirol A, Bukulmez O: **Laparoscopic high lateral transposition of both ovaries before pelvic irradiation.** *J Am Assoc Gynecol Laparosc* 2000, **7:**237–239.
80. Bokser L, Szende B, Schally AV: **Protective effects of D-Trp6-luteinising hormone-releasing hormone microcapsules against cyclophosphamide-induced gonadotoxicity in female rats.** *Br J Cancer* 1990, **61:**861–865.
81. Ataya K, Rao LV, Lawrence E, and Kimmel R: **Luteinizing hormone-releasing hormone agonist inhibits cyclophosphamide-induced ovarian follicular depletion in rhesus monkeys.** *Biol Reprod* 1995, **52:**365–372.
82. Blumenfeld Z, Avivi I, Linn S, *et al.*: **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:**1620–1626.
83. Pereyra Pacheco B, Mendez Ribaz J, Milone G, *et al.*: **Use of GnRH analogues for functional protection of the ovary and preservation of fertility during cancer treatment in adolescents: a preliminary report.** *Gynecol Oncol* 2001, **81:**391–397.
84. Blumenfeld Z: **Ovarian rescue/protection from chemotherapeutic agents.** *J Soc Gynecol Investig* 2001, **8(suppl 1):**S60–S64.
85. Oktay K, Economos K, Khan M, *et al.*: **Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm.** *JAMA* 2001, **286:**1490–1493.
86. Oktay K, Karlikaya G: **Ovarian function after transplantation of frozen, banked autologous ovarian tissue.** *N Engl J Med* 2000, **342:**1919.
87. Kim SS, Radford JA, Harris M, *et al.*: **Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation.** *Hum Reprod* 2001, **16:**2056–2060.
88.•• Radford JA, Leiberman BA, Brison BR, *et al.*: **Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma.** *Lancet* 2001, **357:**1172–1175.

Demonstrates the potential for reimplantation of cryopreserved ovarian tissue after treatment for Hodgkin's disease.

89. Clayton PE, Shalet SM, Price DA, Campbell RH: **Testicular damage after chemotherapy for childhood brain tumors.** *J Pediatr* 1988, **112:**922–926.
90. Vilar O: **Effect of cytostatic drugs on human testicular function.** In *Male Fertility and Sterility.* Edited by Mancini RE, Martini L. London: Academic Press; 1974:423–440.
91. Wallace WH, Shalet SM, Crowne EC, *et al.*: **Gonadal dysfunction due to cis-platinum.** *Med Pediatr Oncol* 1989, **17:**409–413.