

## Review

# Implications of systemic malignancies on human fertility



Dr Ashok Agarwal is the Director of Research at the Centre for Advanced Research in Human Reproduction, Infertility, and Sexual Function, and the Director of the Clinical Andrology Laboratory and Reproductive Tissue Bank. He holds these positions at The Cleveland Clinic Foundation, where he is a Professor of Surgery and, since 1993, full staff in the Glickman Urological Institute, Departments of Obstetrics–Gynecology, Anatomic Pathology, and Immunology. Dr Agarwal has published extensively with over 200 original peer reviewed articles, 20 book chapters, and over 450 presentations at scientific meetings. His research is focused on studies of the role of oxidative stress, DNA integrity, and apoptosis in the pathophysiology of male and female reproduction.

*Dr Ashok Agarwal*

Ashok Agarwal<sup>1</sup>, Tamer M Said

Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics-Gynecology, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA

<sup>1</sup>Correspondence: Tel: +1-216-4449485; Fax: +1-216-4456049; e-mail: agarwaa@ccf.org

## Abstract

Cancer patients have now longer life expectancy due to improved treatment modalities. As the mortality rate decreased and the survival rate increased, the consequences of cancer treatment in terms of impaired fertility became more frequently encountered. The objective of this review is to highlight fertility issues associated with systemic malignancies. Systemic malignancies lead to deterioration of human fertility directly or indirectly as a result of cytotoxic treatment regimens. A variety of measures may be used to decrease the incidence of fertility decline that occurs. Gamete cryopreservation represents a widely accepted method for fertility preservation in cancer patients. In addition, other procedures such as germ cell transplantation and ovarian cryopreservation, which are currently being developed, are expected to make significant contribution in these cases. However, there are some ethical issues that should be considered before offering patients any of these options.

**Keywords:** cancer, ethical issues, gamete cryopreservation, germ cell transplantation, ovarian cryopreservation

## Introduction

Improved treatment regimens for malignant diseases have forced a radical change in the way cancer survivors perceive their disease. In the past, patients tended to be most concerned about survival rates and disease recurrence. Nowadays, patients are also concerned about quality of life issues such as fecundity (Meirow and Nugent, 2001). This is evidently clear in diseases such as breast cancer, leukemia and Hodgkin's lymphoma, as they tend to affect young patients at reproductive age.

Unfortunately, neoplastic diseases and their treatment commonly impair fertility, either temporarily or permanently leaving many patients unable to bear children. Systemic malignancies impact fertility in many ways. Some of these diseases tend to metastasize in the hypothalamus and pituitary, thus affecting gonadotrophin secretion, resulting in hypogonadism and infertility. In addition, chemotherapy and radiation, both of which are used to treat systemic malignancies, are toxic to the male and female gonads

(Agarwal *et al.*, 2004). The main objective of this article is to provide an overview of the close relationship between malignant diseases and human fertility. Specifically, it describes the changes that occur in male and female fertility potential as a result of malignancy and/or its treatment regimens. In addition, it also provides a summary of various options for fertility preservation in cancer patients. Such information would be of importance in counseling patients affected with malignancy while still in the reproductive age group.

## Fertility decline associated with systemic malignancies

Infertility associated with malignant disease was considered to be mainly a side effect of the drugs and irradiation used during the course of treatment. However, this view is rapidly changing due to strong evidence that decreased fertility sometimes exists before treatment. In general, malignancy is associated with an increased catabolic state and malnutrition. Therefore, most patients experience weight loss and decreased

reproductive capacity. In addition, hypothalamic dysfunction can occur and pituitary gonadotrophin levels can fall, which in turn impacts fertility (Vigersky *et al.*, 1977). Stress hormones may further reduce fertility by leading to a rise in prolactin and endogenous opiate secretion, which in turn suppress gonadotropins (Schenker *et al.*, 1992).

Testicular dysfunction and semen abnormalities were reported in males with Hodgkin's lymphoma prior to the initiation of therapy (Marmor *et al.*, 1986; Fitoussi *et al.*, 2000). In a study conducted on 158 male patients with Hodgkin's lymphoma (Rueffer *et al.*, 2001), severe damage to fertility was observed in 21% of patients before treatment. The decrease in fertility was most prominent in the patients with an elevated erythrocyte sedimentation rate (ESR) and in those with advanced disease. In another study, semen analysis showed that 70% of patients with Hodgkin's lymphoma had reduced fertility before therapy regardless of the disease stage or systemic symptoms (Viviani *et al.*, 1991).

An immune-mediated disorder that alters the balance between subpopulations of lymphocytes may be behind the testicular dysfunction associated with Hodgkin's lymphoma (Barr *et al.*, 1993). Other structural abnormalities in the testicular parenchyma such as tubular hyalinization were also detected in these cases (Chapman *et al.*, 1981). In addition, cytokines (e.g. interleukins and tumour necrosis factor) that are secreted by tumour tissue may be partly responsible for the decline in fertility prior to the initiation of therapy (Marmor *et al.*, 1986).

## Effects of malignancy treatment on human gonads

The effects of cancer therapy on testicular architecture vary with the patient's age and pubertal status. It was initially thought that the testicles of pre- and peri-pubertal males were less vulnerable to toxic effects induced by treatment. However, it is now clear that these patients experience as much testicular structure damage following chemo/radiotherapy as adults (Puscheck *et al.*, 2004).

Although the Sertoli cells usually maintain a protective barrier between the blood and the testicular germ cells, many chemotherapeutic drugs can severely interrupt the integrity of this barrier. Germ cells that do actively differentiate are more susceptible to cytotoxic injury resulting in necrosis. As a result, cytotoxic therapy can deplete germ cells to the point where the seminiferous tubules will only contain Sertoli cells. Some stem cells may survive after cytotoxic insult, but will fail to differentiate into mature spermatozoa for several years (Meistrich *et al.*, 1992).

Unlike male germ cells, female germ cells proliferate only during prenatal life; after birth, they progressively decrease in number due to apoptosis, and ovulation. Germ cells inside the female gonad do not proliferate whereas the somatic cells do. Radiation and chemotherapy induce oocytes to undergo apoptosis, which reduces the number of germ cells (Tilly and Kolesnick, 2002) and results in oestrogen insufficiency. Therefore, when follicles are destroyed by cytotoxic therapy, the frequency of menses decreases and amenorrhoea commonly occurs. Irreversible ovarian failure and menopause occur if the number of follicles falls below that which is required for menstrual cyclicity.

The exact incidence of premature ovarian failure (POF) after chemotherapy is difficult to establish because there are many contributing factors. Depending on the type of chemotherapy regimen used, the incidence of amenorrhoea ranges from 0 to 100% (Bines *et al.*, 1996). Temporary amenorrhoea occurs when cytotoxic drugs destroy maturing follicles whereas permanent amenorrhoea or POF occurs when all primordial follicles are destroyed (Warne *et al.*, 1973; Gradishar and Schilsky, 1989).

## Effects of malignancy treatment on fertility

The seminiferous epithelium inside the testes is most sensitive to the detrimental effects of chemotherapy (Brougham *et al.*, 2003). Therefore, after treatment with gonadotoxic agents, patients may be rendered oligozoospermic or azoospermic. Because testosterone production by the Leydig cells is usually unaffected, patients still develop normal secondary sexual characteristics (Thomson *et al.*, 2002). However, treatment with high, cumulative doses of gonadotoxic chemotherapy can also lead to Leydig cell dysfunction.

Indeed, Leydig cell dysfunction is not observed until doses of 20 Gy are administered to prepubertal boys and up to 30 Gy in sexually mature males (Shalet *et al.*, 1989). On the other hand, doses as low as 0.1–1.2 Gy can have detectable effects on spermatogenesis in adult men, with doses over 4 Gy causing more permanent effects (Centola *et al.*, 1994).

Long-term female survivors treated with total body irradiation and bone marrow transplantation (BMT) are at risk for ovarian follicular depletion, impaired uterine growth and blood flow in addition to early pregnancy loss and premature labour if pregnancy is achieved (Critchley *et al.*, 2002). During BMT, patients may be given alkylating agents and receive total body irradiation for conditioning, both of which result in POF, hormonal disturbances and eventually, the inability to parent children (Hinterberger-Fischer *et al.*, 1991). Because women who are older than 30 years face a higher incidence of POF following chemotherapy, their treatment regimens should contain fewer alkylating agents (Franchi-Rezgui *et al.*, 2003).

## Impact of cancer treatment on the genetic material

Patients undergoing cancer treatment are at risk of transmitting impaired genetic material to their offspring (Meistrich, 1993). In females, most alkylating agents and a variety of other chemotherapeutic drugs induce chromosome aberrations or other mutations in developing oocytes that result in embryonic death (Witt and Bishop, 1996). On the other hand, radiation and several alkylating agents can produce single-gene mutations and chromosomal translocations in spermatogonia (Witt and Bishop, 1996).

The persistence of a mutation depends mainly on its location. Mutations that occur early in stem spermatogonia will produce mutation-carrying sperm for the lifetime of the male whereas those occurring in later stages of

spermatogenesis will only lead to a mutation-carrying sperm for a few months. Meiotic and post-meiotic germ cells are more susceptible to mutations than are stem spermatogonia. Therefore, the mutational risks are highest when a pregnancy occurs within one spermatogenic cycle after the male is exposed to the damaging agent (Meistrich, 1993).

Although sperm DNA damage can be assessed with various techniques (Agarwal and Said, 2003), none can definitively determine whether the mutations will be passed onto any offspring. Sperm DNA integrity can vary greatly among cancer patients; however, patients with Hodgkin's and non-Hodgkin's diseases generally have a significantly higher prevalence of DNA damage than healthy men (Kobayashi *et al.*, 2001). However, in most instances no major congenital abnormalities can be expected in the offspring of males who received radiation therapy (Hyer *et al.*, 2002).

## Fertility following malignancy treatment

Sperm quality may naturally improve after cancer treatment (Fossa *et al.*, 1993; Marmor and Duyck, 1995; Meistrich *et al.*, 1997; Costabile and Spevak, 1998). However, some defects may persist. The incidence of infertility in men who have recovered sperm production following cytotoxic therapy is generally not higher than that of the general population. Cancer patients with sperm counts below normal (oligozoospermic) are still capable of having children (Marmor and Duyck, 1995). Similarly, infertile women who have menstrual dysfunction following cytotoxic therapy may be treated for menstrual dysfunction and infertility in a manner similar to that of the general population. However, the risk of an adverse pregnancy outcome is higher in these women, and they may require closer observation (Critchley, 1999).

The management of a pregnancy in a woman with a malignant disease may be difficult. Pregnancy itself does not adversely affect the natural course and prognosis of the disease (Griesshammer *et al.*, 1998). However, such women are more likely to experience thrombotic or bleeding complications. Diseases such as chronic myeloid leukaemia (CML) may result in placental insufficiency and increased fetal prematurity and mortality (Miller, 1976). To avoid chemotherapeutic agents during pregnancy, repeated leukapheresis has been recommended as the therapy of choice to control the white blood cell count (Fitzgerald *et al.*, 1986).

Cancer treatment does not seem to affect the outcome results for assisted reproductive techniques. Alkylating agents, which are used extensively in the treatment of breast cancer, lymphomas, and leukaemias, and severe autoimmune disease do not seem to affect the fertilizability of the oocytes (Chen *et al.*, 1998). Although these findings were confirmed in another study (Ginsburg *et al.*, 2001), the number of oocytes obtained in women treated with chemotherapy was somewhat lower due to diminished response to ovulation induction (Ginsburg *et al.*, 2001).

## Fertility preservation following malignancy

### Semen cryopreservation

Semen cryobanking is a widely available and inexpensive option that yields good results and provides a reasonable chance of establishing pregnancy after cancer therapy (Sanger *et al.*, 1992). Cryopreserving semen after the start of therapy would adversely affect their chromosomal structure, causing de-novo mutations. Therefore, it is crucial to cryopreserve spermatozoa before chemotherapy or radiotherapy and also to advocate the use of contraception during therapy and for 6 months after (Meistrich, 1993; ARSAC, 1998).

Patients diagnosed with cancer used to be considered poor candidates for sperm cryopreservation because they present with disease-induced suboptimal semen quality and cryosensitivity. Men with Hodgkin's lymphoma have pre-freeze and post-thaw sperm quality that is below normal (Reed *et al.*, 1986; Agarwal and Newton, 1991). However, almost 40% of patients who cryopreserve their semen may be able to achieve a healthy live birth using one of the assisted reproductive techniques (Agarwal *et al.*, 2004). Based on our experience in the last two decades, the percentage decline in semen quality (from pre-freeze to post-thaw) in patients with cancer is similar to that of normal donors. This suggests that the effect of cryodamage on spermatozoa from patients with cancer is similar to that of normal donors (Hallak *et al.*, 1998; Agarwal, 2000).

As a general rule, there is no cancer group for which spermatozoa cannot be retrieved and stored (Bahadur *et al.*, 2002). Even the absence of spermatozoa in semen should not prevent physicians from attempting to preserve a patient's fertility. In many cancer patients who suffer from azoospermia before treatment, testicular sperm extraction (unilateral or bilateral) 'Onco-tese' may be successfully attempted, and the retrieved spermatozoa may be cryopreserved for future use (Schrader *et al.*, 2003).

It is of interest to note that only a small percentage of patients (<10%) who bank their spermatozoa before chemotherapy or radiotherapy return for assisted reproduction (Audrins *et al.*, 1999; Schover *et al.*, 1999; Lass *et al.*, 2001). This finding may be explained by several reasons: recovery or waiting for possible resumption of spermatogenesis, short period from original illness, anxiety regarding potential risks for the children, and uncertainty about their long-term health and therefore suitability to be parents (Hallak *et al.*, 1998). However, trends have started to change, and awareness of sperm banking has increased over the past 4–5 years, coinciding with the advent of intracytoplasmic sperm injection (ICSI). Our sperm bank records show a steady increase in the number of patients who bank their spermatozoa and also use it for assisted reproduction after their treatments.

### Testicular tissue harvesting

Testicular tissue can be harvested from a biopsy and stored either as a tissue section or as isolated germ cells, before cancer therapy. Following cure, this tissue can be thawed and used to produce offspring in one of two ways: the stored germ

cells can be re-implanted into the patient's own testes to restore natural fertility, a procedure known as germ cell transplantation, or the stored stem cells can be matured *in vitro* until they are able to achieve fertilization via ICSI (Brougham *et al.*, 2003). Although these two measures have been the subject of intensive research in the last decade, further refinements in the protocols used may still be needed before they can be used routinely in clinical practice.

It is possible to reinitiate spermatogenesis after transplantation of cryopreserved testicular germ cell suspensions. Although cell survival is acceptable, current protocols still require improvement (Frederickx *et al.*, 2004). Establishing a successful method for testicular stem cell transplantation of frozen-thawed testicular cells would be of immense benefit for many patients undergoing sterilizing treatment, specifically in pre-pubertal boys with childhood cancer, since no active spermatogenesis is present and no sperm cryopreservation will be feasible.

Before stem cell transplantation can be considered for preserving the fertility of pre-pubertal boys, two issues must be carefully examined (Aslam *et al.*, 2000). First, the testis biopsy taken from the cancer patient may contain malignant cells. These cells must be removed from the cell suspension because studies in rats have shown that one single malignant cell can reintroduce the disease. Second, the cell suspension consists of all testicular cells, and the proportion of spermatogonial stem cells is very low (estimated at 1/5000) (Jahnukainen *et al.*, 2001).

The technique of in-vitro maturation of germ cells may be used in adults who received high doses of gonadotoxic drugs/irradiation to the extent that the somatic Sertoli cells become incapable of performing their function of supporting spermatogenesis. In these cases, transplanted germ cells will not have a suitable environment to develop; therefore in-vitro maturation of germ cells may be considered. However, the procedure represents another technical challenge and despite multiple reports and methodologies (Tesarik and Mendoza, 2003), no current protocol can be described as reliable.

## Oocyte cryopreservation

The therapeutic role of oocyte freezing for young cancer patients has been generally well received and welcomed (Lockwood, 2003). Although successful fertilization and embryonic cleavage have been reported after injection of cryopreserved thawed oocytes, the pregnancy rate is not high enough to justify its routine use in clinical practice (Gook *et al.*, 1994). The main reason for poor outcomes after oocyte cryopreservation is related to the oocyte's structural complexity. Nevertheless, recent technical modification such as changes in the freezing protocol greatly improved the clinical efficiency of oocyte cryopreservation (Porcu, 2001). Preliminary reports from 18 patients suffering from various malignancies suggest that oocyte storage may be an alternative pragmatic option for fertility preservation, as the duration of oocyte storage did not interfere with its survival and pregnancies occurred even after several years of gamete cryopreservation (Porcu *et al.*, 2004). However, the presence of other factors such as uterine impairment would be of major concern. In addition, complications during pregnancy and pre-

term deliveries will be expected in these cases (Larsen *et al.*, 2000).

## Ovarian tissue cryopreservation

Ovarian tissue banking in humans is being considered to restore fertility in patients who lose ovarian function because of chemotherapy or radiotherapy (Gosden, 2002). Ovarian tissue cryopreservation and transplantation first emerged in rodent studies and then in sheep and human ovarian xenograft studies (Oktay, 2001). Although promising, there is a theoretical risk that malignant stem cells will be re-implanted along with the thawed cryopreserved ovary (Kim *et al.*, 2001; Blumenfeld *et al.*, 2002). Ovarian tissue cryopreservation is currently under evaluation and is not offered as a routine service. However, a live birth has been recently reported following the autotransplantation of cryopreserved ovarian tissue (Donnez *et al.*, 2004). This report offers promising information that may advocate the use of ovarian tissue cryopreservation from patients before cancer treatment.

## Choice of cytotoxic regimens

Currently, treatment regimens for systemic malignancy include a variety of chemotherapeutic agents, all of which affect reproductive functions differently. For young patients, it is important to select an agent with minimal toxicity but maximal therapeutic effect. For example, NOVP (mitoxantrone, vincristine, vinblastine, prednisone) may be preferred over MOPP (mustine, vincristine, procarbazine and prednisone) for the treatment of diseases such as Hodgkin's lymphoma. Although NOVP markedly affects spermatogenesis, sperm production recovers rapidly after treatment, usually within 3–4 months. This rapid recovery is due to the fact that NOVP chemotherapy damages spermatogenic germ cells rather than inhibiting stem cells (Meistrich *et al.*, 1997).

Similarly, ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) is used to treat Hodgkin's disease instead of MOPP because the former dramatically reduces gonadal toxicity (Viviani *et al.*, 1985). VAPEC-B (doxorubicin, cyclophosphamide, etoposide, vincristine, bleomycin and prednisolone), which is used in the treatment of non-Hodgkin's lymphoma, minimizes the dose of cyclophosphamide and therefore results in less gonadal failure than CHOP-Bleo (cyclophosphamide, vincristine, prednisone, bleomycin) (Radford *et al.*, 1994).

## Gonadal shielding

The gonads must be outside the field of radiation or shielded from the direct radiation beam unless they are being irradiated directly as a result of actual or potential neoplastic involvement. Although gonadal shields can reduce the amount of radiation 2- to 5-fold, some radiation may still reach the gonads. For example, the gonads typically receive 2–3 Gy with an inverted Y-field, which is used for Hodgkin's disease (Bieri *et al.*, 1999). To minimize ovarian exposure, oophoropexy may be performed to relocate the ovaries away from the direct beam (Sy Ortin *et al.*, 1990; Morice *et al.*, 2000). Laparoscopic oophoropexy may be of benefit in cases of Hodgkin's disease if performed before pelvic irradiation (Williams *et al.*, 1999).

## Medical treatment

Testosterone suppressors such as gonadal steroids, gonadotropin-releasing hormone (GnRH) analogues and anti-androgens when used before and during cytotoxic therapy in male rats can enhance the recovery of spermatogenesis and fertility (Meistrich and Shetty, 2003). It was assumed that recovery of stem spermatogonia cells could possibly be stimulated after prolonged periods of iatrogenic azoospermia, but research does not necessarily support that theory. Hormone treatment given before and during cytotoxic therapy was found to protect spermatogenesis in only one of eight clinical trials (Masala *et al.*, 1997).

GnRH agonists may protect ovarian function from the effects of cyclophosphamide (Ataya *et al.*, 1995) by decreasing the recruitment of primordial follicles. Strong evidence supports the use GnRH agonistic analogue to minimize the gonadotoxic effect of chemotherapy because it induces pre-pubertal milieu (Blumenfeld 2002; Blumenfeld *et al.*, 2002). However, the feasibility of using oral contraceptives or GnRH agonists to protect women against ovarian damage has not been established (Waxman *et al.*, 1987).

Hormone replacement therapy (HRT) should be considered in young pre-menopausal women who have developed ovarian failure due to malignancy or cancer treatment (Mulder, 1999). Even with the use of HRT, though, uterine size can still decrease by 40% (Critchley *et al.*, 2002). Finally, it is important to mention that any residual ovarian function remaining after chemotherapy is considered a good prognostic sign because it may be stimulated with steroid hormones and/or gonadotropins (Chatterjee and Goldstone, 1996).

Oocytes exposed to chemotherapeutic agents *in vitro* undergo various changes leading to apoptosis (Tilly, 1998). Sphingosine-1-phosphate may be an example of an apoptotic inhibitor. The oocytes of mice that had been treated with sphingosine-1-phosphate therapy resisted apoptosis that was induced by doxorubicin (Morita *et al.*, 2000). The concept offers a promising experimental alternative to guard against apoptosis. With the eventual identification of the molecular and genetic framework of chemotherapy-induced germ cell death, apoptotic inhibitors may some day play a role in preventing oocyte loss.

## Ethics of fertility preservation

Options for future fertility following cancer treatment must be considered in the patient's best interests. Thus, the advantages of any intervention or of an active decision not to intervene must outweigh any disadvantages, both in the short and long term. Any intervention intended to preserve fertility must have a sound evidence base as well as moral provenance. It should neither raise unrealistic expectations nor have long-term adverse effects on the patient or their offspring (Grundy *et al.*, 2001).

A competent person must give informed consent voluntarily. However, in view of the complexity of the issues surrounding fertility preservation, the anxieties of both patients and their families at the time of diagnosis and the limited time for discussion due to the urgency of commencing treatment, the

validity of such consent may be impaired. The first stage of consent is for the collection and storage of the germinal tissue or gametes. The second stage is for use of the collected material for fertilization.

In addition, it is important to consider what will happen to stored cells in the event of divorce or the patient's death. While some would advocate destruction of the tissue in the latter situation, others have suggested allowing the parents to allow the tissue to be used for research purposes (Wallace and Walker, 2001). Procedures such as embryo cryopreservation have always generated legal conflicts. The disputes regarding ownership and rights of access to frozen embryos created in relationships that have ended, provide further evidence for the desirability of separating the preservation of fertility potential from the creation of embryos (Lockwood, 2003). Proper regulations and accurate definitions are still needed to govern the new opportunities for preservation of fertility potential for cancer patients receiving damaging treatment regimens (Bahadur *et al.*, 2001).

## Conclusions

Today between 50 and 60% of all cancer patients survive for more than 5 years (Fossa, 2004). Many systemic malignancies such as Hodgkin's lymphoma and breast cancer affect predominantly young patients in the reproductive age group. The decreasing mortality rate and the increasing survival rate as result of effective treatment have made fertility issues more frequently encountered. Patients with systemic malignancies have impaired fertility as a direct effect of the disease or indirectly as a result of the mandatory cytotoxic treatment regimens. The deterioration in fertility potential may be temporary or permanent.

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

## References

- Agarwal A 2000 Semen banking in patients with cancer: 20-year experience. *International Journal of Andrology* **23**, 16–19.
- Agarwal A, Newton R 1991 The effect of cancer on semen quality after cryopreservation of sperm. *Andrologia* **23**, 329–332.
- Agarwal A, Said T 2003 Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update* **9**, 331–345.
- Agarwal A, Ranganathan P, Kattal N *et al.* 2004 Fertility after cancer: a prospective review of assisted reproductive outcome with banked semen specimens. *Fertility and Sterility* **81**, 342–348.
- ARSAC 1998 Administration of Radioactive Substances Advisory Committee (ARSAC). Notes for guidance on the clinical administration of radio-pharmaceuticals and use of sealed radioactive sources. *Nuclear Medicine Communications* **21**, S1–93.
- Aslam I, Fishel S, Moore H *et al.* 2000 Fertility preservation of boys undergoing anti-cancer therapy: a review of the existing situation

- and prospects for the future. *Human Reproduction* **15**, 2154–2159.
- Ataya K, Rao L, Lawrence E *et al.* 1995 Luteinizing hormone-releasing hormone agonist inhibits cyclophosphamide-induced ovarian follicular depletion in rhesus monkeys. *Biology of Reproduction* **52**, 365–372.
- Audrins P, Holden C, MacLachlan R *et al.* 1999 Semen storage for special purposes at Monash IVF from 1977 to 1997. *Fertility and Sterility* **72**, 179–181.
- Bahadur G, Whelan J, Davies MC *et al.* 2001 Cancer patients, gametes, gonadal tissue, and the UK legal status. *Reproductive BioMedicine Online* **2**, 8–10.
- Bahadur G, Ling K, Hart R *et al.* 2002 Semen quality and cryopreservation in adolescent cancer patients. *Human Reproduction* **17**, 3157–3161.
- Barr R, Clark D, Booth J 1993 Dyspermia in men with localized Hodgkin's disease. A potentially reversible, immune-mediated disorder. *Medical Hypotheses* **40**, 165–168.
- Bieri S, Rouzaud M, Miralbell R 1999 Seminoma of the testis: is scrotal shielding necessary when radiotherapy is limited to the para-aortic nodes. *Radiotherapy and Oncology* **50**, 349–353.
- Bines J, Oleske D, Cobleigh M 1996 Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer. *Journal of Clinical Oncology* **14**, 1718–1729.
- Blumenfeld Z 2002 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. *Molecular and Cellular Endocrinology* **187**, 93–105.
- Blumenfeld Z, Dann E, Avivi I *et al.* 2002 Fertility after treatment for Hodgkin's disease. *Annals of Oncology* **13**, 138–147.
- Brougham M, Kelnar C, Sharpe R *et al.* 2003 Male fertility following childhood cancer: current concepts and future therapies. *Asian Journal of Andrology* **5**, 325–337.
- Centola G, Keller J, Henzler M *et al.* 1994 Effect of low-dose testicular irradiation on sperm count and fertility in patients with testicular seminoma. *Journal of Andrology* **15**, 608–613.
- Chapman R, Sutcliffe S, Malpas J 1981 Male gonadal dysfunction in Hodgkin's disease. A prospective study. *Journal of the American Medical Association* **245**, 1323–1328.
- Chatterjee R, Goldstone A 1996 Gonadal damage and effects on fertility in adult patients with haematological malignancy undergoing stem cell transplantation. *Bone Marrow Transplantation* **17**, 5–11.
- Chen WY, Yang JG, Huang SH *et al.* 1998 Effects of cyclophosphamide on maturation and subsequent fertilizing capacity of pig oocytes in vitro. *Chinese Journal of Physiology* **41**, 75–83.
- Costabile RA, Spevak M 1998 Cancer and male factor infertility. *Oncology (Huntington)* **12**, 557–562, 565; discussion 566–568, 570.
- Critchley H 1999 Factors of importance for implantation and problems after treatment for childhood cancer. *Medical and Pediatric Oncology* **33**, 9–14.
- Critchley H, Bath L, Wallace W 2002 Radiation damage to the uterus – review of the effects of treatment of childhood cancer. *Human Fertility (Cambridge)* **5**, 61–66.
- Donnez J, Dolmans M, Demylle D *et al.* 2004 Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* **364**, 1–6.
- Fitoussi, Eghbali H, Tchen N *et al.* 2000 Semen analysis and cryoconservation before treatment in Hodgkin's disease. *Annals of Oncology* **11**, 679–684.
- Fitzgerald D, Rowe J, Heal J 1986 Leukapheresis for control of chronic myelogenous leukemia during pregnancy. *American Journal of Hematology* **22**, 213–218.
- Fossa SD 2004 Long-term sequelae after cancer therapy – survivorship after treatment for testicular cancer. *Acta Oncologica* **43**, 134–141.
- Fossa SD, Aabyholm T, Vespestad S *et al.* 1993 Semen quality after treatment for testicular cancer. *European Urology* **23**, 172–176.
- Franchi-Rezgui P, Rousselot P, Espie M *et al.* 2003 Fertility in young women after chemotherapy with alkylating agents for Hodgkin and non-Hodgkin lymphomas. *Hematology Journal* **4**, 116–120.
- Frederickx V, Michiels A, Goossens E *et al.* 2004 Recovery, survival and functional evaluation by transplantation of frozen-thawed mouse germ cells. *Human Reproduction* **19**, 948–953.
- Ginsburg E, Yanushpolsky E, Jackson K 2001 In vitro fertilization for cancer patients and survivors. *Fertility and Sterility* **75**, 705–710.
- Gook D, Osborn S, Bourne H *et al.* 1994 Fertilization of human oocytes following cryopreservation; normal karyotypes and absence of stray chromosomes. *Human Reproduction* **9**, 684–691.
- Gosden RG 2002 Gonadal tissue cryopreservation and transplantation. *Reproductive BioMedicine Online* **4**, 64–67.
- Gradishar W, Schilsky R 1989 Ovarian function following radiation and chemotherapy for cancer. *Seminars in Oncology* **16**, 425–436.
- Griesshammer M, Bergmann L, Pearson T 1998 Fertility, pregnancy and the management of myeloproliferative disorders. *Bailliere's Clinical Haematology* **11**, 859–874.
- Grundy R, Larcher V, Gosden R *et al.* 2001 Fertility preservation for children treated for cancer (2): ethics of consent for gamete storage and experimentation. *Archives of Disease in Childhood* **84**, 360–362.
- Hallak J, Sharma R, Thomas A Jr *et al.* 1998 Why cancer patients request disposal of cryopreserved semen specimens posttherapy: a retrospective study. *Fertility and Sterility* **69**, 889–893.
- Hinterberger-Fischer M, Kier P, Kalhs P *et al.* 1991 Fertility, pregnancies and offspring complications after bone marrow transplantation. *Bone Marrow Transplantation* **7**, 5–9.
- Hyer S, Vini L, O'Connell M *et al.* 2002 Testicular dose and fertility in men following <sup>131</sup>I therapy for thyroid cancer. *Clinical Endocrinology* **56**, 755–758.
- Jahnukainen K, Hou M, Petersen C *et al.* 2001 Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia. *Cancer Research* **61**, 706–710.
- Kim S, Radford J, Harris M *et al.* 2001 Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. *Human Reproduction* **16**, 2056–2060.
- Kobayashi H, Larson K, Sharma R *et al.* 2001 DNA damage in patients with untreated cancer as measured by the sperm chromatin structure assay. *Fertility and Sterility* **75**, 469–475.
- Larsen E, Loft A, Holm K *et al.* 2000 Oocyte donation in women cured of cancer with bone marrow transplantation including total body irradiation in adolescence. *Human Reproduction* **15**, 1505–1508.
- Lass A, Akagbosu F, Brinsden P 2001 Sperm banking and assisted reproduction treatment for couples following cancer treatment of the male partner. *Human Reproduction Update* **7**, 370–377.
- Lockwood G 2003 Politics, ethics and economics: oocyte cryopreservation in the UK. *Reproductive BioMedicine Online* **6**, 151–153.
- Marmor D, Duyck F 1995 Male reproductive potential after MOPP therapy for Hodgkin's disease: a long-term survey. *Andrologia* **27**, 99–106.
- Marmor D, Elefant E, Dauchez C *et al.* 1986 Semen analysis in Hodgkin's disease before the onset of treatment. *Cancer* **57**, 1986–1987.
- Masala A, Faedda R, Alagna S *et al.* 1997 Use of testosterone to prevent cyclophosphamide-induced azoospermia. *Annals of Internal Medicine* **126**, 292–295.
- Meirow D, Nugent D 2001 The effects of radiotherapy and chemotherapy on female reproduction. *Human Reproduction Update* **7**, 535–543.
- Meistrich M 1993 Potential genetic risks of using semen collected during chemotherapy. *Human Reproduction* **8**, 8–10.
- Meistrich M, Shetty G 2003 Suppression of testosterone stimulates recovery of spermatogenesis after cancer treatment. *International Journal of Andrology* **26**, 141–146.
- Meistrich M, Wilson G, Brown B *et al.* 1992 Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing's and soft tissue sarcomas. *Cancer* **70**, 2703–2712.

- Meistrich ML, Wilson G, Mathur K *et al.* 1997 Rapid recovery of spermatogenesis after mitoxantrone, vincristine, vinblastine, and prednisone chemotherapy for Hodgkin's disease. *Journal of Clinical Oncology* **15**, 3488–3495.
- Miller J 1976 Chronic myelocytic leukemia and the myeloproliferative diseases during the child-bearing years. *Journal of Reproductive Medicine* **17**, 217–224.
- Morice P, Juncker L, Rey A *et al.* 2000 Ovarian transposition for patients with cervical carcinoma treated by radiosurgical combination. *Fertility and Sterility* **74**, 743–748.
- Morita Y, Perez G, Paris F *et al.* 2000 Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. *Nature Medicine* **6**, 1109–1114.
- Mulder JE 1999 Benefits and risks of hormone replacement therapy in young adult cancer survivors with gonadal failure. *Medical and Pediatric Oncology* **33**, 46–52.
- Oktay K 2001 Ovarian tissue cryopreservation and transplantation: preliminary findings and implications for cancer patients. *Human Reproduction Update* **7**, 526–534.
- Porcu E 2001 Oocyte freezing. *Seminars in Reproductive Medicine* **19**, 221–230.
- Porcu E, Fabbri R, Damiano G *et al.* 2004 Oocyte cryopreservation in oncological patients. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **113**, S14–16.
- Puscheck E, Philip P, Jeyendran R 2004 Male fertility preservation and cancer treatment. *Cancer Treatment Reviews* **30**, 173.
- Radford J, Clark S, Crowther D *et al.* 1994 Male fertility after VAPEC-B chemotherapy for Hodgkin's disease and non-Hodgkin's lymphoma. *British Journal of Cancer* **69**, 379–381.
- Reed E, Sanger W, Armitage J 1986 Results of semen cryopreservation in young men with testicular carcinoma and lymphoma. *Journal of Clinical Oncology* **4**, 537–539.
- Rueffer U, Breuer K, Josting A *et al.* 2001 Male gonadal dysfunction in patients with Hodgkin's disease prior to treatment. *Annals of Oncology* **12**, 1307–1311.
- Sanger W, Olson J, Sherman J 1992 Semen cryobanking for men with cancer – criteria change. *Fertility and Sterility* **58**, 1024–1027.
- Schenker J, Meirou D, Schenker E 1992 Stress and human reproduction. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **45**, 1–8.
- Schover L, Rybicki L, Martin B *et al.* 1999 Having children after cancer. A pilot survey of survivors' attitudes and experiences. *Cancer* **86**, 697–709.
- Schrader M, Muller M, Sofikitis N *et al.* 2003 'Onco-tese': testicular sperm extraction in azoospermic cancer patients before chemotherapy – new guidelines? *Urology* **61**, 421–425.
- Shalet S, Tsatsoulis A, Whitehead E *et al.* 1989 Vulnerability of the human Leydig cell to radiation damage is dependent upon age. *Journal of Endocrinology* **120**, 161–165.
- Sy Ortin T, Shostak C, Donaldson S 1990 Gonadal status and reproductive function following treatment for Hodgkin's disease in childhood: the Stanford experience. *International Journal of Radiation Oncology, Biology, Physics* **19**, 873–880.
- Tesarik J, Mendoza C 2003 Using the male gamete for assisted reproduction: past, present, and future. *Journal of Andrology* **24**, 317–328.
- Thomson A, Campbell A, Irvine D *et al.* 2002 Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. *Lancet* **360**, 361–367.
- Tilly J 1998 Molecular and genetic basis of normal and toxicant-induced apoptosis in female germ cells. *Toxicology Letters* **102–3**, 497–501.
- Tilly J, Kolesnick R 2002 Sphingolipids, apoptosis, cancer treatments and the ovary: investigating a crime against female fertility. *Biochimica et Biophysica Acta* **1585**, 135–138.
- Vigersky R, Andersen A, Thompson R *et al.* 1977 Hypothalamic dysfunction in secondary amenorrhea associated with simple weight loss. *New England Journal of Medicine* **297**, 1141–1145.
- Viviani S, Santoro A, Ragni G *et al.* 1985 Gonadal toxicity after combination chemotherapy for Hodgkin's disease. Comparative results of MOPP vs. ABVD. *European Journal of Cancer and Clinical Oncology* **21**, 601–605.
- Viviani S, Ragni G, Santoro A *et al.* 1991 Testicular dysfunction in Hodgkin's disease before and after treatment. *European Journal of Cancer* **27**, 1389–1392.
- Wallace W, Walker D 2001 Conference consensus statement: ethical and research dilemmas for fertility preservation in children treated for cancer. *Human Fertility (Cambridge)* **4**, 69–76.
- Warne G, Fairley K, Hobbs J *et al.* 1973 Cyclophosphamide-induced ovarian failure. *New England Journal of Medicine* **289**, 1159–1162.
- Waxman J, Ahmed R, Smith D *et al.* 1987 Failure to preserve fertility in patients with Hodgkin's disease. *Cancer Chemotherapy and Pharmacology* **19**, 159–162.
- Williams R, Littell R, Mendenhall N 1999 Laparoscopic oophorectomy and ovarian function in the treatment of Hodgkin disease. *Cancer* **86**, 2138–2142.
- Witt K, Bishop J 1996 Mutagenicity of anticancer drugs in mammalian germ cells. *Mutation Research* **355**, 209–234.

Received 19 August 2004; refereed 21 September 2004; accepted 8 October 2004.