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·*Review*·

Gonadal damage and options for fertility preservation in female and male cancer survivors

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Abstract

It is estimated that in 2010, 1 in every 250 adults will be a childhood cancer survivor. Today, oncological surgery, radiotherapy and chemotherapy achieve relatively high rates of remission and long-term survival, yet are often detrimental to fertility. Quality of life is increasingly important to long-term survivors of cancer, and one of the major quality-of-life issues is the ability to produce and raise normal children. Developments in the near future in the emerging field of fertility preservation in cancer survivors promise to be very exciting. This article reviews the published literature, discusses the effects of cancer treatment on fertility and presents the options available today thanks to advances in assisted-reproduction technology for maintaining fertility in male and female patients undergoing this type of treatment. The various diagnostic methods of assessing the fertility potential and the efficacy of *in vitro* fertilization (IVF) after cancer treatment are also presented. *(Asian J Androl 2006 Sep; 8: 515–533)*

Keywords: reproduction; cryopreservation; male infertility; semen preservation; fertility preservation; cancer treatment; ovarian tissue

1 Introduction

Today, approximately 1 in 700 young adults is a cancer survivor [1], whereas it is estimated that in 2010, 1 in every 250 adults will be a childhood cancer survivor [2]. In the USA alone, more than 20 000 children and young people of reproductive age are exposed to known mutagens in the form of chemotherapy and/or radiotherapy for cancer every year [3]. Women today are using better methods of contraception and are delaying childbearing for social or financial reasons, so that increasing numbers of women are anxious to preserve their fertility when early-stage cancers are discovered [4–6]. Also, patients with nonmalignant autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus and hematological diseases are being treated successfully with chemotherapy or radiotherapy [7].

Surgery, radiotherapy and chemotherapy might achieve relatively high rates of remission and long-term survival, but are often detrimental to fertility. Quality of life is increasingly important to long-term survivors of cancer, and one of the major quality-of-life issues is the ability to produce and raise normal children.

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This article reviews the published literature, discusses the effects of cancer treatment on fertility, and presents the options available for maintaining fertility in male and female patients undergoing such treatment. The various diagnostic methods of assessing the fertility potential and the efficacy of *in vitro* fertilization (IVF) after cancer treatment are also presented.

2 Ovarian anatomy and physiology

The peak number of oocytes in the ovary, approximately 6.8×10^6, occurs at 5 months' gestation. After this point, there is no further proliferation of germ cells, and progressive atresia occurs. At birth, the number of oocytes decreases to $1–2 \times 10^6$, and at puberty there are only 300 000 left. Approximately 300–500 of these follicles will develop into mature oocytes, whereas the rest will become atretic. In women aged 51 years, the average age of natural menopause in the developed countries, there are approximately 1 000 left [8].

In healthy women, accelerated atresia of the oocytes begins at approximately 37.5 years of age, associated with an increase in the level of follicle-stimulating hormone (FSH) [9]. As atresia continues, both the number and quality of oocytes fall below a critical level, and the rate of aneuploidy increases. This process leads to a greater risk of spontaneous abortion once pregnancy occurs. This central principle of age-dependent follicle depletion has been challenged by recent data suggesting that ovarian stem cells are present in mice and could presumably lead to the replenishment of follicles [10]: a theory that cannot be supported in humans, at least.

Premature ovarian failure, defined as menopause before the age of 40 years or hypergonadotropic amenorrhea, occurs in up to 0.9% of women in the general population.

There are three major developmental phases in the life cycle of the ovary [11]:

1 The phase of embryogenesis: In this phase, populations of primordial germ cells and somatic cells become an integrated ovary mass containing oocytes and granulosa cells located within primordial follicles. The first phase of oocyte maturation starts *in utero,* and is gonadotropin-independent.

2 The pubertal and adult phase: The gonadotropin-dependent folliculogenesis begins, in which oogenesis, granulogenesis and thecogenesis occur as a recruited primordial follicle grows and develops into the preovulatory follicle, or dies by atresia.

3 The postmenopausal phase: Despite the high circulation of gonadotropins the ovarian cortex is thin and usually devoid of follicles.

There are five phases of follicular development in the adult ovary [11]:

1 The preantral phase: The earliest phase of follicular growth is characterized by an increase in follicular diameter of the primary oocyte and the formation of a granulosa cells layer. The initiation of this phase is hormone independent.

2 The antral phase: The preantral follicles either die by the process of atresia or develop, when there are adequate levels of FSH and luteinizing hormone (LH) to the antral follicles. These are distinguished by a further increase in follicular size and the development of the follicular antrum. There is now a significant increase in androgen and estrogen production.

3 The preovulatory phase: This is the shortest and most dramatic phase of follicular growth. The antral follicles will die unless a brief surge of gonadotropin coincides with the appearance of LH receptors on the outer granulosa cells. This surge of LH causes terminal growth in both the follicle cells and the oocyte and the chromosomes progress from the arrested first meiotic prophase to the second metaphase.

4 The ovulation: The oocyte, triggered by the LH, transforms into a mature egg, which is secreted into the oviduct to await fertilization.

5 The corpus luteum: The follicle luteinizes into the corpus luteum, which dies by a process termed luteolysis if implantation does not occur.

3 Testicular anatomy and physiology

The testis consists of the seminiferous (or germinal) epithelium, arranged in tubules, and endocrine components (testosterone-producing Leydig cells) in the interstitial region between the tubules. The seminiferous tubules contain the germ cells, which consist of stem and differentiating spermatogonia, spermatocytes, spermatids and sperm, and the Sertoli cells, which support and regulate germ cell differentiation.

In men, germinal stem cells exist in the testicles from the time of birth, but do not develop into the haploid gametes (spermarche) until the boy reaches puberty. In the prepubertal testis, there is a steady turnover of early germ cells that undergo spontaneous degeneration before the haploid stage is reached, which is probably the

reason why the prepubertal testis is very vulnerable to cytotoxic therapy. In the mature testicle, the germinal stem cells undergo continual self-renewal and differentiation into mature spermatozoa within approximately 67 days throughout life. Therefore, there are always germ cells in various developmental stages in the testicles [12].

The loss of germ cells has secondary effects on the hypothalamic-pituitary-gonadal axis. Inhibin secretion by the Sertoli cells declines, and, consequently, serum FSH levels rise. Testicular blood flow is reduced, resulting in less testosterone being distributed in the circulation. Therefore, levels of LH increase to maintain constant serum testosterone levels [12].

The eventual recovery of sperm production after cancer treatment depends on the survival of the spermatogonial stem cells and their ability to differentiate. If treatment is limited to cytotoxic agents that do not kill stem spermatogonia, normospermia is usually restored within 3 months after cytotoxic therapy [13].

4 The effect of cancer treatment on female fertility

4.1 Radiotherapy-induced ovarian damage

The degree and persistence of radiation induced gonadal damage depends on the cumulative dose, the irradiation field and the patient's age, with older women being at greater risk of damage [14].

Exposure of the ovaries to high radiation doses, in the range of 30–700 Gy, as is the case for treatment of cervical and rectal cancer, and with craniospinal radiotherapy for central nervous malignancies can cause a mutagenic, embryotoxic, embryolethal and teratogenic effect [3]. This can also happen when the pelvic lymph nodes are irradiated for hematological malignancies, such as Hodgkin's disease, and with total body irradiation before bone marrow transplantation, so it is recommended that, when possible, the gonads should be shielded, the radiation field restricted, or when indicated the ovaries should be surgically relocated away from the radiation field (oophoropexy) [3].

Depletion of primordial follicles in mouse ovaries in a dose-related fashion using increasing radiation doses of 0.1, 0.2 and 0.3 Gy is demonstrated by Gosden *et al.* [15]. Exposure to high doses of radiotherapy caused sterilization, whereas lower doses that cause only partial depletion of the primordial follicle reserve resulted in premature ovarian failure [15].

The radiation dosage necessary for loss of ovarian function has been examined in many studies. Thibaud *et al.* [16] show that total body irradiation (TBI) of < 10 Gy administered in a single dose before puberty causes a high ovarian failure rate (55–80%), whereas fractionated TBI is less toxic to the ovaries even at higher doses. With fractionated TBI of > 15 Gy, ovarian failure is present in all cases. Socie *et al.* [17] note that that the incidence of pregnancy after TBI was less than 3% and that the outcome for recovery was more favorable if the patient was prepubertal and the radiation was delivered in several fractions.

Wallace *et al.* [18] estimate the dosage at which half of the follicles are lost in humans (LD_{50}) to be 4 Gy. Lashbaugh and Casarett [19] observe that women under 40 years of age are less sensitive to radiation-induced ovarian damage, with an estimated dose of 20 Gy being required to produce permanent ovarian failure in comparison with 6 Gy in older women.

Chiarelli *et al.* [20] observe a dose-dependent and distribution-dependent relationship between the risk of premature ovarian failure and the total dosage of abdominal pelvic irradiation: with doses < 20 Gy, the relative risk was 1.02; with irradiation of 20–35 Gy, the relative risk increased to 1.37; and with doses > 35 Gy, the relative risk of premature ovarian failure was 3.27. The percentage of women who suffered from infertility correlated with increasing dosages of abdominal pelvic irradiation: treatment doses of 20–35 Gy caused a 22% rate of infertility, and doses > 35 Gy caused a 32% rate of infertility.

Uterine irradiation is associated with infertility, spontaneous pregnancy loss and intrauterine growth retardation [21]. Direct effects on the uterus after irradiation include irreversible changes in the uterine musculature and blood flow, as well as hormonal-resistant endometrial insufficiency [22, 23]. A study by Holm *et al.* [24] shows that young women exposed to TBI also suffer impaired uterine growth and later require sex steroid replacement therapy. A review by Critchley and Wallace [25] indicates that physiological sex steroid replacement therapy might improve uterine characteristics in some patients after irradiation at a young age.

There are also increased rates of obstetric complications in patients who have undergone radiotherapy in comparison with the general population, including spontaneous abortions (38% *vs.* 12%), preterm labor (62% *vs.* 9%) and low-birthweight infants (62% *vs.* 6%).

However, as long as radiation is not administered during pregnancy, there is no risk of subsequent teratogenicity [26, 27]. These findings confirm research on women exposed to the atomic bomb and on offspring conceived and born to them after exposure, which show that the incidence of spontaneous abortion is greater, but that the children do not suffer a higher rate of mutations or major congenital anomalies in comparison with the normal population [28].

Swerdlow *et al.* [29] confirm that there was no excess of stillbirths, low birth weight, congenital malformations, abnormal karyotypes or cancer in the offspring of women treated for Hodgkin's disease. However, Fenig *et al.* [30] report an increase in low birth weight and spontaneous abortions, especially if conception occurred less than a year after radiation exposure. They advise delaying pregnancy for a year after the completion of radiotherapy.

Radiation of the hypothalamic–pituitary area for brain tumors in excess of 30 Gy often causes early or even precocious puberty [31]. In addition, children who have received larger doses of cranial irradiation are at risk of developing hypogonadotropic hypogonadism in time [32].

4.2 Bone marrow transplantation

Bone marrow transplantation (BMT) has come into widespread use in last 30 years in the treatment of oncohematological malignancies. The conditioning regimens used for BMT include high-dose chemotherapy, with or without body irradiation. In a survey of 38 000 male and female patients who had received high-dose chemotherapy or TBI with allogeneic/autologous stem cell transplantation, the fecundity rate was found to be extremely low, with only 129 pregnancies reported [33]. Many studies have confirmed the extremely high risk of persistent ovarian failure in women who undergo BMT [34]. Growth and sexual development are impaired in children, and sterility is common in adults [14], so every effort to preserve fertility should be undertaken before BMT.

4.3 Chemotherapy-induced ovarian damage

Chemotherapeutic drugs act by interrupting vital cell processes and arresting the normal cellular proliferation cycle. The risk of chemotherapy-related amenorrhea is related to the patient's age, the specific chemotherapeutic agents used, and the cumulative dosage administered [35].

4.3.1 Age

Women over 38 years of age have a higher incidence of complete ovarian failure and permanent infertility in comparison with younger women, who have a larger primordial follicle reserve [14, 36].

4.3.2 Cytotoxic drugs

Alkylating agents These substances have a severe effect on human fertility. Known effects are ovarian fibrosis and follicular and oocyte depletion [37]. According to Meirow [38], alkylating agents, such as cyclophosphamide, involve the greatest risk of inducing ovarian failure among all the chemotherapeutic agents (odds ratio 3.98) in comparison with unexposed patients.

Cisplatin and analogs Meirow [38] estimates that cisplatin causes ovarian failure, with an odds ratio of 1.77. Studies of cisplatin treatment in female mice demonstrate the induction of different types of chromosomal damage [39].

Vinca alkaloids These substances are known aneuploidy inducers. According to Meirow, the odds ratio for ovarian failure is approximately 1.0 [38]. Many animal experiments have shown high levels of aneuploidy in oocytes exposed to vinblastine [40], which means that these damaged oocytes could produce malformed fetuses.

Antimetabolites Insufficient data are available on the effects of antimetabolites on female germ cells.

Anthracycline antibiotics Adriamycin and bleomycin are female-specific mutagens and have been shown to induce dominant lethal mutations in maturing/preovulatory oocytes in female mice [39].

Meirow *et al.* [41] demonstrate in animal experiments that regular menses and normal reproductive outcome after chemotherapy are not certain indicators of whether the ovarian follicular reserve has survived the treatment unaffected. They recommended that patients who recover from ovarian failure after high-dose chemotherapy or radiotherapy treatments should not delay childbearing for too many years. These patients should try to conceive after a disease-free interval of few years, but not < 6–12 months after treatment, because of the possible toxicity of the treatment for growing oocytes [38, 42].

Regarding the teratogenic effects of chemotherapy, studies that have monitored pregnancies in women exposed to chemotherapy before conception have not registered increased rates of miscarriage or congenital abnormalities in comparison with the general population. Because these pregnancies occurred long after treatment had ceased, it can be assumed that there are correction mechanisms within the oocyte or that there are undetec-

ted miscarriages as a result of dominant lethal mutations at a very early stage [42].

5 The effect of cancer and cancer treatment on male fertility

Infertility is a major concern for young men of reproductive age undergoing chemotherapy, radiotherapy or surgery, as most of these regimens can cause sterility. Several studies have indicated that some cancer patients have a reduced fertility potential even before starting treatment [43, 44]. This infertility can result from anatomical changes (e.g. hypogastric plexus damage leading to retrograde ejaculation), primary or secondary hormonal imbalance, or damage to germinal stem cells or supporting cells. These changes can result in compromised sperm numbers, motility, morphology or DNA integrity [4].

Malignancy is also associated with an increased catabolic state, malnutrition, an increase in stress hormones, and a decrease in pituitary gonadotropin levels, which can also have an impact on fertility [45].

5.1 Testicular cancer

Testicular cancer is the most common malignancy in young men, with a well-known association with abnormalities of spermatogenesis [43]. A statistically significantly lower sperm count was found in 83 patients with testicular germ cell cancer than in healthy men [46]. The exact mechanism responsible for this decreased sperm quality is unknown. One possible explanation might be a preexisting defect in germ cells, leading to both cancer and defective spermatogenesis, after bilateral undescended testis, genetic abnormalities or exposure to abnormal hormonal levels *in utero*, for example. Another explanation might be a local effect of the tumor itself, caused by the paracrine action of the tumor's secretory substances.

Cancer might also alter the process of spermatogenesis through a hormonal imbalance. The general systemic effects might lead to oversecretion or undersecretion of hormones by the endocrine glands, or the tumor might secrete its own hormones affecting spermatogenesis hormones, such as β-human chorionic gonadotropin and α-fetoprotein [47].

5.2 Hodgkin's lymphoma and leukemia

Hodgkin's lymphoma has also been associated with pretreatment impairment of spermatogenesis [48]. A study in 158 patients with Hodgkin's disease reports that elevated erythrocyte sedimentation rates and advanced disease stages are prognostic factors for severe fertility damage [49]. In another study, 70% of patients with Hodgkin's lymphoma had reduced levels of fertility before therapy, regardless of the disease stage or systemic symptoms [50].

Immunological processes that alter the balance between subpopulations of lymphocytes are also associated with spermatogenetic disorders induced by Hodgkin's disease [51].

Hallak *et al.* [52] found that the pretreatment semen quality (the median motile sperm count and motility) was poor in patients with acute and chronic leukemia.

5.3 Radiation effects

Ionizing radiation has adverse effects on gonadal function in men of all ages. The degree and persistence of the damage is dependent on the dose, the treatment field and the fractionation schedule [53]. Sperm production is susceptible to damage at very low doses of irradiation (> 1.2 Gy), but as Leydig cells are more resistant to damage from the radiotherapy than the germinal epithelium (function is usually preserved up to 20 Gy in prepubertal boys and 30 Gy in sexually mature men), progression through puberty with normal testosterone levels is common, despite a severe impairment of spermatogenesis [54]. Doses of more than 4 Gy can cause permanent damage to spermatogenesis [55]. Sperm counts are typically at their lowest 4–6 months after treatment is completed, and a return to pretreatment levels usually occurs in 10–24 months, with longer periods being required for recovery after higher doses [56]. TBI as a conditioning regimen for stem cell transplantation causes permanent gonadal failure in approximately 80% of men [17].

Recovery of spermatogenesis takes place from surviving stem cells (type A spermatogonia) and is dependent on the dose of radiation. Complete recovery, as indicated by a return to pre-irradiation sperm concentrations and germinal cell numbers, takes place within 9–18 months following radiation with 1 Gy or less, 30 months for 2–3 Gy, and 5 years or more for doses of 4 Gy and above [57].

In many cases, men who regain spermatogenesis after cancer treatment have low sperm counts and motility and an increased rate of chromosomal abnormalities [58]. These effects appear to be dose-dependent, with an apparent threshold [59], and persist for up to

3 years after radiotherapy, so that contraception for a period of 1–3 years is recommended after testicular irradiation.

5.4 Chemotherapy effects

Cytotoxic chemotherapy can cause gonadal injury, and the nature and extent of the damage depends on the drug administered, the dosage received, and the age of the patient [3]. In general, cytotoxic treatment targets rapidly dividing cells, and it is therefore not surprising that spermatogenesis can be impaired after treatment for cancer. The exact mechanism of the damage is uncertain, but it appears to involve depletion of the proliferating germ cell pool, by killing cells not only at the stage of differentiating spermatogonia but also stem cells themselves. In addition, stem spermatogonia that do survive fail to differentiate further [60]. Chemotherapy appears to lower healthy sperm counts in cancer survivors, but after an adequate period of therapy, small studies suggest that the DNA integrity of sperm is reestablished at a level similar to that in age-matched control individuals [61, 62]. Several studies report that most offspring of cancer survivors do not have any adverse effects resulting from preconception exposure to therapy [63, 64].

5.4.1 Alkylating agents

These cause depletion of the germinal epithelium in the testes and aplasia of germinal cells, resulting in severe oligospermia or azoospermia within 90–120 days of treatment [65], with poor long-term recovery [43]. In another study, most men had not regained spermatogenesis 4 years after cyclophosphamide treatment; those men who did regain spermatogenesis did so after an average interval of 31 months [66]. Alkylating agents are mutagenic in all stages of maturation of male human germ cells, but these agents do not cause transmissible chromosomal translocation or aneuploidy in stem cells [67]. The vast majority of men receiving procarbazine-containing regimens for the treatment of lymphomas are rendered permanently infertile [57].

5.4.2 Cisplatin and analogs

In studies in male mice, cisplatin induced chromosomal aberrations in spermatocytes, as well as differentiating spermatogonia immediately after the treatment. However, long after exposure, the transmission of such effects was found to have decreased substantially by the time the exposed spermatogonia matured [68].

5.4.3 Vinca alkaloids

In men, these agents arrest spermatogenesis and might also affect the motility of mature spermatozoa [3]. Vinblastine is cytotoxic to primary spermatocytes, while spermatogonia and preleptotene spermatocytes are relatively resistant [69].

5.4.4 Antimetabolites

These agents act on rapidly dividing cells (i.e. in the later stages of spermatogenesis) and induce dominant lethal mutations. 5-fluorouracil and 6-mercaptopurine cause chromosomal aberrations [70]. In Meirow [71], long-term administration of 6-mercaptopurine in low doses to male mice induced a high embryonic resorption rate in pregnant female mice mated with the exposed male mice.

5.4.5 Topoisomerase interactive agents

These agents are cytotoxic to all spermatogonial stages. The mutagenic effects of Adriamycin (doxorubicin) have been demonstrated in mice spermatocytes [72]. Administration of bleomycin to male mice also induces chromosomal anomalies in spermatogonia and spermatocytes [73].

5.4.6 Combination chemotherapy

The MOPP regimen (mechlorethamine, oncovin/vincristine, procarbazine and prednisone), used for Hodgkin's disease, can cause azoospermia in 90% of men up to 4 years after therapy, as well as an increased frequency of aneuploidy for up to 18 years after treatment [74]. Sperm chromosomal anomalies were also assessed in testicular cancer patients before, during and after BEP (bleomycin, etoposide and cisplatin) chemotherapy [75]. Sperm aneuploidy was evaluated (using fluorescence *in situ* hybridization) in male patients with Hodgkin's disease who were treated with NOVP (novantrone/mitoxantrone, oncovin/vincristine, vinblastine and prednisone) chemotherapy [76]. Current treatment of Hodgkin's lymphoma in children includes chemotherapy with ABVD (adriamycin/doxorubicin, bleomycin, vinblastine and dacarbazine), which appears to be less gonadotoxic, and ChlVPP (chlorambucil, vinblastine, procarbazine and prednisone), a treatment that is known to cause gonadal damage, especially in men [21].

5.5 Effects of oncological surgery

Surgical intervention for cancer therapy, such as bladder neck or prostate resection, bilateral retroperitoneal

lymphadenectomy, or extensive pelvic surgery, might result in anejaculation as a result of retrograde flow of semen into the urinary bladder. Modified nerve-sparing surgical improvements have reduced this adverse outcome without compromising the efficacy of the procedure. Depending on the location of lymph nodes with metastases, retroperitoneal lymph-node dissection might involve a modified template of dissection (unilateral dissection below the inferior mesenteric artery, avoiding the lumbar sympathetic fibers and hypogastric plexus), preserving ejaculation in 50–85% of men. Similarly, improved surgical techniques in the treatment of bladder and prostate cancer avoid damaging the nerve fibers in the neurovascular bundles that innervate the penile corpora cavernosa. Consequently, 70–80% of men with radical prostatectomy or radical cystoprostatectomy maintain sexual function [77].

6 Fertility preservation options for female cancer patients

6.1 Ovarian transposition (oophoropexy)

Transposition of the ovaries out of the field of irradiation was described initially in 1958 [78]. The most common indications for this are Hodgkin's disease, cervical and vaginal cancer, and pelvic sarcomas. The ovarian dose after transposition is reduced to approximately 5–10% of that in the *in situ* ovaries [79]. Lateral ovarian transposition is typically carried out by laparotomy, with division of the utero-ovarian ligament and tubes and with the ovaries being removed to the paracolic gutters so that they lie 3 cm above the upper border of the field: a safety margin that maintains ovarian function [80]. Ovarian failure might result if the ovaries are not removed far enough, or if they migrate back to their original position.

Ovarian transposition is currently also being carried out laparoscopically [81], which offers the following advantages: there are fewer adhesions, radiotherapy can be initiated immediately postoperatively, and the laparoscopy can be repeated if postoperative assessment of the ovaries shows that the radiation dose is still likely to be significant. Various laparoscopic surgical procedures have been described [82].

Various degrees of preservation of ovarian function and the ability to conceive after radiation treatment and oophoropexy have been reported, ranging from 16 to 90% [81, 82]. The variations are a result of the inability to calculate and prevent scatter radiation, concomitant use of chemotherapy, and the different radiation dosages used [7].

6.2 GnRH analogs

Blumenfeld and other researchers, although in small studies, demonstrate that GnRH agonists are well tolerated and might protect long-term ovarian function [83, 84]. Blumenfeld reports on probably the largest group of women (55 lymphoma patients) who were started on GnRH analogs 7–10 days before chemotherapy treatment. The rate of premature ovarian failure was 5% in the GnRH analog/chemotherapy group *vs*. 55% in the group receiving chemotherapy alone [83].

However, contradictory results have been published on the effects of GnRH agonists, and there has been intensive debate on the existence of FSH receptors in primordial follicles and GnRH agonist receptors in the human ovary [85]. Meirow [86] did not observe a protective effect of GnRH after ablative chemotherapy and radiotherapy in patients undergoing bone marrow transplantation. Waxman *et al.* [87] found that buserelin was not effective for fertility preservation in humans. However, it is possible that complete pituitary ovarian suppression was not achieved, which might be a necessary condition for these drugs to work.

The role of GnRH agonists in the treatment of gynecological cancers has been the subject of intense investigation. Approximately 50% of breast cancers, 70% of ovarian cancers, and 80% of endometrial cancers express GnRH and its receptor [88]. Native GnRH and GnRH agonists inhibit the proliferation of human breast cancer, ovarian cancer, and endometrial cancer cell lines in a dose-dependent and time-dependent manner [89]. Emons *et al.* [88] suggest that GnRH and its receptor are part of a negative autocrine system that might be used therapeutically to inhibit cell proliferation by the administration of GnRH analogs.

GnRH agonists are used today both in adjuvant treatment and in metastatic breast cancer for reversible medical castration to downregulate pituitary gonadotropin secretion, leading to suppression of ovarian estrogen production [90].

These data show that GnRH agonists can be safely used for fertility purposes. Prospective and randomized studies are currently being conducted to investigate the not yet proven efficacy of GnRH agonists, and the initial results of these are expected in the near future.

6.3 Sex steroids

The oral contraceptive pill has been investigated as an agent for suppressing the ovaries during chemotherapy and providing protection against cytotoxic agents. Chapman and Sutcliffe [91] report more follicles in ovarian biopsies from 3 patients who received combination oral contraceptive pills during chemotherapy than in those who did not. By contrast, Whitehead *et al.* [92] found no protective effect of combination oral contraceptive pills in patients who received chemotherapy for Hodgkin's disease. One possible explanation for the varying results might be that the oral contraceptives do not manage to suppress the gonads completely.

6.4 Progesterone (P4)

In the rat, progesterone was found to have a protective effect when administered 1 week before the start of cyclophosphamide and during the treatment [93].

Familiari *et al.* [37] examined the protective effect of medroxyprogesterone acetate (MPA) on human primordial follicles exposed to cytotoxic drugs. Using electron microscopy, they showed that chemotherapy not only acutely damaged the ovary by reducing the number of follicles, but also chronically damaged the remaining follicular quality. MPA was unable to protect the ovary from early follicular atresia.

6.5 Apoptotic inhibitors

When mice oocytes were exposed to doxorubicin *in vitro*, they underwent a series of changes that produced apoptotic bodies [94]. Apoptosis also plays a significant role in the process of normal germ cell depletion [95], so that the existence of a genetic predetermined pathway has been suggested that can be aberrantly activated by chemotherapeutic drugs [96]. As a logical consequence, the use of apoptosis inhibitors could potentially stop the apoptotic process and protect the patient from premature ovarian failure.

The use of sphingosine-1-phosphate, a known apoptosis inhibitor, in mice treated with doxorubicin, was found to protect the oocytes from apoptosis. Also, the oocytes of mice that lacked the enzyme to generate ceramide and acid sphingomyelinase, early messengers in the apoptosis sequence, are more resistant to doxorubicin-induced apoptosis [97]. In Paris *et al.* [98] Sphingosine 1-phosphate preserved the fertility of irradiated female mice without any genomic damage for the offspring.

These studies show that these agents are promising but still at a very early experimental stage.

6.6 Cryopreservation of embryos

The most successful approach to fertility preservation is embryo cryopreservation, with delivery rates per embryo transfer using cryopreserved embryos reported to be in the range of 18–20% [99, 100]. However, this approach requires ovarian stimulation and consequent IVF and a participating male partner, although frozen sperm from a donor might also be used. Therefore, this option is not applicable to prepubertal adolescent girls.

In patients with estrogen-sensitive cancers (e.g. breast cancer) the use of the common stimulation agents for IVF purposes should be avoided. In such cases, antiestrogens, such as tamoxifen [101], or aromatase inhibitors [102] can be used, even if these regimens are less effective.

In a study by Oktay *et al.* [101] regarding the outcome of IVF in hormone sensitive breast cancer patients, tamoxifen (40–60 mg) was started on day 2 or 3 of the cycle and was administered daily for 5–12 weeks. The control group consisted of patients with an unstimulated IVF cycle. The tamoxifen group had a significantly higher number of mature oocytes, peak estradiol and embryos (mean of 1.6 embryos *vs.* 0.6 embryos) than the natural cycle group. They also reported the first pregnancy from cryopreserved embryos generated after tamoxifen stimulation.

Letrozole, an aromatase inhibitor, has been introduced as a promising ovulation induction agent [103]. Many groups are currently testing the feasibility of ovarian stimulation with aromatase inhibitors in breast and endometrial cancer patients. The patient is stimulated with gonadotropins, and an aromatase inhibitor is simultaneously introduced to reduce serum estradiol levels. Oocyte development is unaffected. A luteinizing hormone–releasing hormone antagonist is also used to prevent a premature luteinizing hormone surge [104]. Oktay *et al.* [105] compared the combination of tamoxifen or letrozole with FSH for stimulation in women with breast cancer, with very promising results.

Holzer *et al.* [106] report that aromatase inhibitors are as effective as, or superior to, clomiphene citrate for ovulation induction and in superovulation. However, their role in IVF remains to be determined.

6.7 Cryopreservation of oocytes

Oocyte banking is more problematic than cryopre-

servation of sperms or embryos. The first obstacle is the sensitivity of oocytes to chilling, probably because of the sensitivity of the spindle apparatus and the higher lipid content of the cells. Cooling and exposure to cryoprotective agents (CPA) affect the cytoskeleton and might aggravate the already high incidence of aneuploidy in human oocytes [107]. Exposure to CPA also causes "hardening" of the zona pellucida, so that all oocyte cryopreservation protocols involve intracytoplasmic sperm injection (ICSI) as a precaution. Fertilization has to be carried out approximately 3–5 hours after thawing while the oocyte remains fertile. Further disadvantages of this method are that cancer patients might not have more than one opportunity for oocyte harvesting before undergoing potentially sterilizing treatment, because a cycle of controlled stimulation requires several weeks and there is normally a delay of a few months before treatment cycles. The success of the method also depends on the total number of eggs harvested (< 10 oocytes means very slight chances of pregnancy). To date, more than 4 300 oocytes have been cryopreserved and more than 80 children have been born, mostly with the conventional slow-cooling method. The overall live birth rate per cryopreserved oocyte is approximately 2%, which is much lower than that with IVF using fresh oocytes [108].

6.8 Cryopreservation of ovarian tissue

A promising method of preserving fertility is cryopreservation of ovarian tissue [109, 110]. The idea of cryopreserving ovarian tissue is based on the finding that the ovarian cortex harbors primordial follicles that are more resistant to cryoinjury than mature oocytes, because the oocytes they contain have a relatively inactive metabolism and lack a metaphase spindle, zona pellucida and cortical granules [111]. The clinical indications are almost identical to those for the oocytes, but there are fewer logistical restrictions and a greater fertility potential because of the far larger number of oocytes preserved.

Ovarian tissue cryopreservation might be the only acceptable method for any prepubertal or premenarchal female patients receiving chemotherapy or pelvic radiotherapy [4]. Follicular viability after cryopreservation and thawing is demonstrated in several studies [112]. Most of the follicles that survive cryopreservation are primordial [113]. There are three ways of getting these follicles to develop to maturity. The first is autografting: either orthotopic, which has yielded the first two pregnancies after cryopreservation of ovarian tissue [114, 115]; or heterotopic: for example, in the forearm s.c. [116]. The latter method requires the use of IVF to achieve a pregnancy.

The second option is *in vitro* follicular maturation and IVF: a method that has already yielded pregnancies in animal experiments [117]. This method is not applicable to the human species, owing to the long period necessary for the primordial follicle to reach the maturation stage [118].

The third method involves xenografting human ovarian tissue into immunodeficient animals (mice with severe combined immune deficiency) and stimulating it to full follicular maturation [119, 120].

The risks of ovarian tissue cryopreservation include reimplantation of the primary tumor and malignant transformation [121]. Shaw *et al*. [122] were the first to report the transmission of lymphoma from a donor to a graft recipient with fresh and cryopreserved mouse ovarian tissue samples. However, most of the malignant diseases encountered during the reproductive years in humans do not metastasize to the ovaries, with the following exceptions: blood-borne malignancies such as leukemias, neuroblastoma and Burkitt's lymphoma [121]. Kim [123] examined the risk of cancer relapse by transplanting frozen-thawed ovarian tissue from lymphoma patients into immunodeficient mice. None of the ovarian grafts from non-Hodgkin's or Hodgkin's lymphoma patients resulted in recurrences, whereas cancer spread was found in one of the five animals transplanted with lymph nodes from Hodgkin's disease. Therefore, a histological assessment for micrometastases should always be carried out on a small portion of the harvested tissue before cryopreservation. Another risk is the possibility of malignant transformation of the cryopreserved tissue after transplantation. In rats, heterotopic autotransplantation of cryopreserved ovarian tissue into the spleen resulted in the development of sex cord stromal tumors [124].

6.9 Construction of reconstituted human oocytes (artificial gametes)

The construction of artificial gametes might be made possible by transferring the nucleus of somatic cells into enucleated oocytes and inducing chromosomal halving (haploidization). The procedure involves nuclear transplantation of the cancer patient's somatic cell into an enucleated ooplast obtained from a donor. The chromosomes contained within this nucleus can be induced to

undergo meiosis, yielding a haploid cell: a functional oocyte. This reconstructed oocyte can then be fertilized by ICSI, creating a de novo individual and not a clone of the nuclear donor [125].

Although this method aims to ensure a normal genomic contribution from both parents, it has been observed that chromosomal aberrations are frequent, so that at this point this method has only experimental interest [126].

7 Fertility preservation options for male cancer patients

7.1 Sperm cryopreservation

Semen cryobanking before chemotherapy, radiotherapy or surgery affecting the reproductive system is a widely available and inexpensive option that yields good results and provides a reasonable chance of establishing a pregnancy after cancer therapy [127]. Traditionally, the banking of at least three semen samples, with an abstinence period of at least 48 hours between the samples, has been recommended. Completion of the process usually requires 5–8 days. Additional samples and longer abstinence periods (72–96 hours) to achieve higher total sperm counts might also be considered [128].

Various methods of semen collection are available, such as testicular sperm extraction [129], microsurgical epididymal sperm aspiration, penile vibratory stimulation, and electroejaculation even for younger adolescents [130]. Even a suboptimal sperm quality in not an insurmountable limitation, because ICSI allows successful fertilization even with a single sperm obtained from semen or by testicular sperm extraction [131, 132].

It is of interest that only a small percentage of patients (< 10%) who bank their spermatozoa before chemotherapy or radiotherapy return for assisted reproduction [133]. There are many possible reasons for this: recovery or waiting for possible resumption of spermatogenesis, a short period after the original illness, anxiety regarding potential risks for the children, and uncertainty about long-term health and suitability to be parents [134].

Statistics suggest that most men being treated for cancer do not participate in sperm banking and that most oncologists do not consistently discuss this option with their male patients. However, awareness of the option of sperm banking has increased in the past 4–5 years, coinciding with the advent of ICSI [45].

7.2 Hormonal therapy

Cytotoxic treatment acts principally on rapidly dividing cells. As the testis has high levels of cellular activity, it is, therefore, prone to this type of damage, and gonadotoxicity might result. In view of this, it has been postulated that inducing testicular quiescence could prevent gonadal toxicity by making germ cells less vulnerable to the cytotoxic effects. Spermatogenesis and fertility can be restored in rodents following treatment with radiation or some chemotherapeutic agents, by suppressing testosterone with GnRH agonists or antagonists either before or after the cytotoxic insult [13, 135].

However, there are species differences in the testicular response to radiation and GnRH antagonist therapy, and the rescue protocols that work in rodents have failed in clinical studies [136, 137]. Also in two recent studies conducted in non-human primates, hormone suppression by GnRH antagonists failed to enhance spermatogenic recovery after radiation, also indicating that there are important differences in this process between rats and primates [138, 139]. In addition, this approach might be ineffective in children, as the proliferation of germ cells in prepubertal primates might be gonadotropin-independent [140].

Therefore, hormonal manipulation based on the suppression of this axis is unlikely to be protective in such patients receiving gonadotoxic treatment [141].

7.3 Testicular tissue cryopreservation

Although the prepubertal testis does not produce mature spermatozoa, it does contain the diploid stem-germ cells from which haploid spermatozoa will ultimately be derived, so that testicular tissue could be harvested before chemotherapy and cryopreserved [141]. After the patient is cured, the tissue could be thawed and the stored germ cells could be reimplanted into the patient's own testes, where they would give rise to complete and normal spermatogenesis in the seminiferous tubules: a procedure known as germ cell transplantation [142]. The extent of spermatogenesis depends on the number of transplanted stem cells and the quantity and quality of stem cell niches in the transplanted testis. Each donor-derived colony of spermatogenesis arises from the clonogenic proliferation and differentiation from a single spermatogonial stem cell (SSC). This is the only technology that has the potential to restore natural fertility from a patient's own germ cells [143].

In a second procedure, testis tissue pieces could also be grafted to an ectopic site (e.g. under the skin) in cancer

survivors or in immunodeficient animals (xenografting). Therefore, in contrast to the transplantation technique, when SSC are removed from their cognate niches and transplanted to the new niches in the recipient testes, testis grafting involves the transplantation of SSC with their niches intact. The grafted testicular tissue is revascularized in the ectopic site and produces complete spermatogenesis [143]. Successful stem cell transplantation has been reported in many species: mice [144], rats, monkeys and humans [145].

Alternatively, the stored cells could be matured *in vitro* until fertilization can be achieved by the use of ICSI. Although the technique of testicular germ cell harvesting, cryopreservation, and transplantation are effective in mice [146], there are considerable differences in human spermatogenesis.

The most important issue with autotransplantation is the risk of reintroducing malignant cells after retransplantation. The risk is greater with hematological cancers, as the testes can act as sanctuary sites for leukemic cells [147]. The technique of *in vitro* maturation of stem cells circumvents the risk of reintroducing malignant cells, making this procedure potentially highly beneficial in this patient group.

7.4 In vitro spermatogenesis

Maturing germ cells *in vitro,* stimulating their differentiation into spermatozoa, would be particularly useful in patients who have received profoundly gonadotoxic therapy in whom the supporting Sertoli cells are unable to support spermatogenesis. Although restoration of fertility after *in vitro* spermatogenesis has been reported [148], it involved maturation of the later stages of spermatogenesis rather than stem cells; *in vitro* maturation of diploid stem cells into haploid spermatozoa appears unlikely to become technically possible in the near future [149].

8 Testing for ovarian reserve

The term "ovarian reserve" denotes the available pool of primordial follicles in the ovary and is a major determinant of female fertility potential. There is certainly a need to assess the functional ovarian reserve in premenopausal patients with cancer. This information is important for the choice of the appropriate chemotherapy regimen and the correct strategy for fertility preservation before the cancer treatment, and can also serve as a guide for the prediction of premature menopause after cancer treatment. In general, ovarian reserve tests are either biochemical (basal or dynamic) or biophysical [150].

8.1 Biochemical (basal) ovarian reserve tests

8.1.1 Serum follicle-stimulating hormone

Basal serum FSH assessment is one of the longest-established parameters for estimating ovarian reserve. On day 3 of the menstrual cycle, serum FSH levels are usually less than 10 mIU/mL in most assays. FSH levels that are over 15 mIU/mL on day 3 suggest a decreased ovarian reserve and a reduced probability of pregnancy; if values exceed 20 mIU/mL, the probability of pregnancy is close to nil [8, 9]. Van Rooij *et al.* [151] observed an ongoing pregnancy in as many as 28% of women with regular cycles who had FSH levels of 15–20 IU/L, and there was a clear fall in the pregnancy rate regardless of age only when the FSH level was > 20 IU/L.

8.1.2 Estradiol

With the decline of the follicle pool, serum levels of E2 decrease [152]. However, the condensed follicular phase length in older women might be the result of an advanced follicular recruitment by cycle day 3. This early dominant follicle selection is expressed by high serum concentrations of estradiol. It has been shown in a population receiving assisted-reproduction treatment (in whom GnRH analogs were not administered) that increasing day 3 estradiol concentrations are associated with decreasing oocyte numbers and pregnancy rates [153]. Estradiol measurement is also useful when obtained concurrently with FSH levels, because values > 80 pg/mL indicate disrupted folliculogenesis, which does not allow accurate interpretation of FSH measurements [153]. Other authors have not found any correlation between estradiol concentrations and the ovarian reserve [154].

8.1.3 Inhibin B

Inhibins and activins are glycoproteins produced by the granulosa cells that belong to the transforming growth factor-β family. Inhibins have an inhibitory effect on pituitary FSH synthesis and secretion. Activins act as functional antagonists of inhibin to stimulate FSH synthesis and secretion [155]. Serum dimeric inhibin B is regarded as a direct measure of ovarian reserve, as it is mainly secreted by the granulosa cells of pre-antral follicles [156]. Low levels of both inhibin A and inhibin B are typical in women with premature ovarian failure and postmenopausal women [157]. Seifer *et al.* [158] sug-

gest that a fall in the inhibin B concentration might be an earlier marker for limited ovarian reserve than an elevated FSH concentration.

8.1.4 Anti-Müllerian hormone

Another test of ovarian reserve is measurement of the level of anti-Müllerian hormone (AMH), which reflects the health of granulosa cells [159] and decreases with age in postmenopausal women [160]. AMH inhibits the recruitment of primordial follicles into the pool of growing follicles and also decreases the responsiveness of growing follicles to FSH [161]. There are findings that suggest that AMH is produced solely by antral follicles capable of growing and is independent of the gonadotropic status; serum levels of AMH might, therefore, represent both the quantity and quality of the ovarian follicle pool [162].

8.2 Ovarian stimulation tests (dynamic)

8.2.1 Clomiphene citrate challenge test

This test involves the administration of 100 mg clomiphene citrate on cycle days 5–9 and measurement of FSH concentrations on days 3 and 10 [163]. In women with a normal ovarian reserve, the increase in estradiol and inhibin production by the developing follicles should be able to overcome the estrogen antagonist effect of clomiphene on the hypothalamic-pituitary axis and suppress FSH levels back into the normal range by day 10. An exaggerated FSH response and/or an elevated basal FSH value are considered to be signs of diminished ovarian reserve. A recent comparison of the clomiphene citrate challenge test (CCCT) with basal markers (FSH, inhibin B, antral follicle count and estradiol) in 63 patients concluded that the increased burden placed on both patients and physicians by the CCCT is not justified [164].

8.2.2 Gonadotropin-releasing hormone agonist stimulation test

This test evaluates the estradiol serum concentration change from cycle day 2 to 3 after administration of a GnRH agonist, which causes a temporary increase in the pituitary secretion of FSH and LH, which, as a consequence, stimulates ovarian estradiol production [165]. However, the gonadotropin-releasing hormone agonist stimulation test has not been evaluated outside of groups receiving assisted-reproduction treatment and is relatively costly, and two early studies show only a limited ability of this test to differentiate between normal and diminished ovarian reserve [166, 167].

8.2.3 Human menopausal gonadotropin test

The most recent study using this test compared basal values of FSH, E2 and inhibin with hormonal and ultrasound parameters after 5 days' stimulation with human menopausal gonadotropin [168]. However, the predictive value of the test is not high and it is rather expensive, so it has not become established in routine practice.

8.2.4 Exogenous follicle-stimulating hormone (FSH) ovarian reserve test

This test determines the increase in day 3 FSH and E2 serum concentrations over 24 h after administration of a standardized dose (300 IU) of purified FSH on day 3 [169]. A recent randomized and prospective study comparing basal and dynamic values found that the exogenous follicle-stimulating hormone ovarian reserve test was the best predictor for ovarian reserve [170].

8.3 Biophysical ovarian reserve tests

8.3.1 Baseline assessment of the number of antral follicles by vaginal ultrasonography

The antral follicle count is defined as the ultrasound-detected number of antral follicles < 10 mm in diameter in the early follicular phase and is a reasonably good predictor of ovarian reserve [171]. An antral follicle count of less than five usually signifies a poorer prognosis [172].

8.3.2 Ultrasound measurement of ovarian volume

The ovary is a dynamic organ that changes in size during a woman's life. From 0.7 cm^3 at age 10 years, the ovarian volume increases to 5.8 cm^3 at 17 years [173]; at the age of 40 years, the ovaries tend to decrease in size and they decrease even further after menopause [174]. Recently, a model has been proposed using the ovarian volume to predict reproductive age [175]. The main limitation of this method is the lack of data on age-dependent ovarian volume measurements in the general population.

8.3.3 Ovarian biopsy

There is contradictory evidence on the use of ovarian biopsy as an ovarian reserve test, as the follicular distribution is extremely heterogeneous [176]. Other authors draw attention to the use of the antral follicle count as the most predictive test of ovarian reserve [177].

9 Fertility potential in men after cancer treatment

In male patients, the assessment of gonadal function includes clinical assessment of pubertal progression, biochemical analysis of plasma gonadotropins and sex steroids, and, most important of all, a semen analysis. Testicular enlargement is the first sign of puberty in boys, followed by penis enlargement and the development of pubic hair. Many patients will have preserved Leydig cell function after gonadotoxic treatment and will, therefore, develop healthy secondary sexual characteristics. However, their testes might be of reduced size and consistency, with a loss of tubular space suggestive of diminished sperm production [178]. Men with mildly compromised Leydig cell function have been found to have normal plasma testosterone levels, but with slightly increased amounts of LH [139]. Inhibin B is secreted mainly from Sertoli cells in men and might be reduced after gonadotoxic chemotherapy, indicating reduced sperm production [179].

10 Efficacy of *in vitro* fertilization after chemotherapy

Little is known about the efficacy and safety of IVF in female patients who have been treated for cancer. In 2001, Ginsburg *et al.* [180] retrospectively examined 15 patients and found a poorer response to gonadotropins than in women with locally treated cancers, as well as a significantly diminished ovarian response to ovulation induction in comparison with patients who underwent IVF before cancer treatment. In a retrospective study, Dolmans *et al.* [181] examined the effect of chemotherapy directly before IVF treatment in a small number of patients (4 patients who underwent IVF in an interval between two chemotherapy regimens *vs*.7 patients who underwent IVF before starting chemotherapy) and report a dramatic reduction in the efficacy of IVF even after only one regimen. They recommend that in women whose cancer therapy can be delayed, IVF with embryo cryopreservation should be offered before chemotherapy rather than after it.

With regard to male cancer survivors, Sanger *et al.* [127] report live births after fertilization from cryopreserved semen. Agarwal *et al.* [182] report the outcome of assisted-reproduction technology in 29 male cancer survivors, in all cases with cryopreserved semen. A total of 87 cycles were carried out, with a mean pregnancy rate of 18.3% per cycle (7% after intrauterine insemination, 23% after IVF and 37% after ICSI) [182]. Schmidt *et al.* [183] examined the fertility outcome in 67 patients and found pregnancy rates of 14.8% after intrauterine insemination and 38.6% after ICSI.

11 Conclusion

Young cancer patients are still being poorly counseled with regard to the negative impact of the treatment on their fertility, as well as on their options for fertility preservation. This review focuses both on the effect of cancer treatments on fertility and on the various surgical and assisted-reproduction innovations that provide the patient with the possibility of future potential fertility. As the emerging discipline of fertility preservation is attracting steadily increasing interest, developments in the near future promise to be very exciting. However, in everyday routine work, better interdisciplinary cooperation between patients and pediatric oncologists, surgeons, immunologists and endocrinologists is necessary to provide individualized options for fertility preservation before surgical procedures or cancer treatment.

References

1 Muller J. Impact of cancer therapy on the reproductive axis. Horm Res 2003; 59: 12–20.
2 Blatt J. Pregnancy outcome in long-term survivors of childhood cancer. Med Pediatr Oncol 1999; 33: 29–33.
3 Arnon J, Meirow D, Lewis-Roness H, Ornoy A. Genetic and teratogenic effects of cancer treatments on gametes and embryos. Hum Reprod Update 2001; 7: 394–403.
4 Simon B, Lee SJ, Partridge AH, Runowicz CD. Preserving fertility after cancer. CA Cancer J Clin 2005; 55: 211–28.
5 Stern CJ, Toledo MG, Gook DA, Seymour JF. Fertility preservation in female oncology patients. Aust N Z J Obstet Gynaecol 2006; 46: 15–23.
6 Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, *et al*. American society of clinical oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006; 24: 2917–31.
7 Sonmezer M, Oktay K. Fertility preservation in female patients. Hum Reprod Update 2004; 10: 251–66.
8 Lobo RA. Potential options for preservation of fertility in women. N Engl J Med 2005; 353: 64–73.
9 Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. Follicle-stimulating hormone levels on cycle day 3 are predictive of *in vitro* fertilization outcome. Fertil Steril 1989; 51: 651–4.
10 Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428: 145–50.
11 Johnson M, Everitt BJ. Ovarian Function. In: Essential Reproduction. Oxford: Blackwell Scientific Publications;1988:

p75–100.
12 Thomson AB, Critchley HO, Kelnar CJ, Wallace WH. Late reproductive sequelae following treatment of childhood cancer and options for fertility preservation. Best Pract Res Clin Endocrinol Metab 2002; 16: 311–34.
13 Shetty G, Meistrich ML. Hormonal approaches to preservation and restoration of male fertility after cancer treatment. J Natl Cancer Inst Monogr 2005; 34: 36–9.
14 Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update 2001; 7: 535–43.
15 Gosden RG, Wade JC, Fraser HM, Sandow J, Faddy MJ. Impact of congenital or experimental hypogonadotrophism on the radiation sensitivity of the mouse ovary. Hum Reprod 1997; 12: 2483–8.
16 Thibaud E, Rodriguez-Macias K, Trivin C, Esperou H, Michon J, Brauner R. Ovarian function after bone marrow transplantation during childhood. Bone Marrow Transplant 1998; 21: 287–90.
17 Socie G, Salooja N, Cohen A, Rovelli A, Carreras E, Locasciulli A, *et al*. Nonmalignant late effects after allogeneic stem cell transplantation. Blood 2003; 101: 3373–85.
18 Wallace WH, Shalet SM, Hendry JH, Morris-Jones PH, Gattamaneni HR. Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte. Br J Radiol 1989; 62: 995–8.
19 Lushbaugh CC, Casarett GW. The effects of gonadal irradiation in clinical radiation therapy: a review. Cancer 1976; 37 (Suppl 2): 1111–25.
20 Chiarelli AM, Marrett LD, Darlington G. Early menopause and infertility in females after treatment for childhood cancer diagnosed in 1964–1988 in Ontario, Canada. Am J Epidemiol 1999; 150: 245–54.
21 Wallace WH, Thomson AB. Preservation of fertility in children treated for cancer. Arch Dis Child 2003; 88: 493–6.
22 Critchley HO, Bath LE, Wallace WH. Radiation damage to the uterus–review of the effects of treatment of childhood cancer. Hum Fertil (Camb) 2002; 5: 61–6.
23 Larsen EC, Schmiegelow K, Rechnitzer C, Loft A, Muller J, Andersen AN. Radiotherapy at a young age reduces uterine volume of childhood cancer survivors. Acta Obstet Gynecol Scand 2004; 83: 96–102.
24 Holm K, Nysom K, Brocks V, Hertz H, Jacobsen N, Muller J. Ultrasound B-mode changes in the uterus and ovaries and Doppler changes in the uterus after total body irradiation and allogeneic bone marrow transplantation in childhood. Bone Marrow Transplant 1999; 23: 259–63.
25 Critchley HO, Wallace WH. Impact of cancer treatment on uterine function. J Natl Cancer Inst Monogr 2005; 34: 64–8.
26 Li FP, Gimbrere K, Gelber RD, Sallan SE, Flamant F, Green DM, *et al*. Outcome of pregnancy in survivors of Wilms' tumor. JAMA 1987; 257: 216–9.
27 Hawkins MM, Smith RA. Pregnancy outcomes in childhood cancer survivors: probable effects of abdominal irradiation. Int J Cancer 1989; 43: 399–402.
28 Damewood MD, Grochow LB. Prospects for fertility after chemotherapy or radiation for neoplastic disease. Fertil Steril 1986; 45: 443–59.
29 Swerdlow AJ, Jacobs PA, Marks A, Maher EJ, Young T, Barber JC, *et al*. Fertility, reproductive outcomes, and health of offspring, of patients treated for Hodgkin's disease: an investigation including chromosome examinations. Br J Cancer 1996; 74: 291–6.
30 Fenig E, Mishaeli M, Kalish Y, Lishner M. Pregnancy and radiation. Cancer Treat Rev 2001; 27: 1–7.
31 Ogilvy-Stuart AL, Clayton PE, Shalet SM. Cranial irradiation and early puberty. J Clin Endocrinol Metab 1994; 78: 1282–6.
32 Constine LS, Woolf PD, Cann D, Mick G, McCormick K, Raubertas RF, *et al*. Hypothalamic–pituitary dysfunction after radiation for brain tumors. N Engl J Med 1993; 328: 87–94.
33 Apperley JF, Reddy N. Mechanism and management of treatment-related gonadal failure in recipients of high dose chemoradiotherapy. Blood Rev 1995; 9: 93–116.
34 Teinturier C, Hartmann O, Valteau-Couanet D, Benhamou E, Bougneres PF. Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulfan is a major cause of ovarian failure. Bone Marrow Transplant 1998; 22: 989–94.
35 Minton SE, Munster PN. Chemotherapy-induced amenorrhea and fertility in women undergoing adjuvant treatment for breast cancer. Cancer Control 2002; 9: 466–72.
36 Behringer K, Breuer K, Reineke T, May M, Nogova L, Klimm B, *et al*. Secondary amenorrhea after Hodgkin's lymphoma is influenced by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives during therapy: a report from the German Hodgkin's Lymphoma Study Group. J Clin Oncol 2005; 23: 7555–64
37 Familiari G, Caggiati A, Nottola SA, Ermini M, Di Benedetto MR, Motta PM. Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. Hum Reprod 1993; 8: 2080–7.
38 Meirow D. Ovarian injury and modern options to preserve fertility in female cancer patients treated with high dose radiochemotherapy for hemato-oncological neoplasias and other cancers. Leuk Lymphoma 1999; 33: 65–76.
39 Katoh MA, Cain KT, Hughes LA, Foxworth LB, Bishop JB, Generoso WM. Female-specific dominant lethal effects in mice. Mutat Res 1990; 230: 205–17.
40 Mailhes JB. Important biological variables that can influence the degree of chemical-induced aneuploidy in mammalian oocyte and zygotes. Mutat Res 1995; 339: 155–76.
41 Meirow D, Epstein M, Lewis H, Nugent D, Gosden RG. Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and fetal malformations. Hum Reprod 2001; 16: 632–7.
42 Meirow D, Schiff E. Appraisal of chemotherapy effects on reproductive outcome according to animal studies and clinical data. J Natl Cancer Inst Monogr 2005; 34: 21–5.
43 Meirow D, Schenker JG. Cancer and male infertility. Hum Reprod 1995; 10: 2017–22.
44 Hallak J, Kolettis PN, Sekhon VS, Thomas AJ Jr, Agarwal A. Sperm cryopreservation in patients with testicular cancer. Urology 1999; 54: 894–9.
45 Agarwal A, Said TM. Implications of systemic malignancies

on human fertility. Reprod Biomed Online 2004; 9: 673–9.
46 Petersen PM, Skakkebaek NE, Vistisen K, Rorth M, Giwercman A. Semen quality and reproductive hormones before orchiectomy in men with testicular cancer. J Clin Oncol 1999; 17: 941–7.
47 Agarwal A, Allamaneni SS. Disruption of spermatogenesis by the cancer disease process. J Natl Cancer Inst Monogr 2005; 34: 9–12.
48 Chapman RM, Sutcliffe SB, Malpas JS. Male gonadal dysfunction in Hodgkin's disease. a prospective study. JAMA 1981; 245: 1323–8.
49 Rueffer U, Breuer K, Josting A, Lathan B, Sieber M, Manzke O, *et al*. Male gonadal dysfunction in patients with Hodgkin's disease prior to treatment. Ann Oncol 2001; 12: 1307–11.
50 Viviani S, Ragni G, Santoro A, Perotti L, Caccamo E, Negretti E, *et al*. Testicular dysfunction in Hodgkin's disease before and after treatment. Eur J Cancer 1991; 27: 1389–92.
51 Barr RD, Clark DA, Booth JD. Dyspermia in men with localized Hodgkin's disease. a potentially reversible, immune-mediated disorder. Med Hypotheses 1993; 40: 165–8.
52 Hallak J, Sharma RK, Thomas AJ Jr, Agarwal A. Why cancer patients request disposal of cryopreserved semen specimens posttherapy: a retrospective study. Fertil Steril 1998; 69: 889–93.
53 Wallace WH, Shalet SM, Crowne EC, Morris-Jones PH, Gattamaneni HR. Ovarian failure following abdominal irradiation in childhood: natural history and prognosis. Clin Oncol (R Coll Radiol) 1989; 1: 75–9.
54 Shalet SM, Didi M, Ogilvy-Stuart AL, Schulga J, Donaldson MD. Growth and endocrine function after bone marrow transplantation. Clin Endocrinol (Oxf) 1995; 42: 333–9.
55 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: 608–13.
56 Gordon W Jr, Siegmund K, Stanisic TH, McKnight B, Harris IT, Carroll PR, *et al*. A study of reproductive function in patients with seminoma treated with radiotherapy and orchidectomy (SWOG-8711). Southwest Oncology Group. Int J Radiat Oncol Biol Phys 1997; 38: 83–94.
57 Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. J Natl Cancer Inst Monogr 2005; 34: 12–7.
58 Martin RH, Hildebrand K, Yamamoto J, Rademaker A, Barnes M, Douglas G, *et al*. An increased frequency of human sperm chromosomal abnormalities after radiotherapy. Mutat Res 1986; 174: 219–25.
59 Fattibene P, Mazzei F, Nuccetelli C, Risica S. Prenatal exposure to ionizing radiation: sources, effects and regulatory aspects. Acta Paediatr 1999; 88: 693–702.
60 Brougham MF, Kelnar CJ, Sharpe RM, Wallace HB. Male fertility following childhood cancer: current concepts and future therapies. Asian J Androl 2003; 5: 325–37.
61 Thomson AB, Campbell AJ, Irvine DC, Anderson RA, Kelnar CJ, Wallace WH. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case–control study. Lancet 2002; 360: 361–7.
62 Chatterjee R, Haines GA, Perera DM, Goldstone A, Morris ID. Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukaemia. Hum Reprod 2000; 15: 762–6.
63 Li FP, Fine W, Jaffe N, Holmes GE, Holmes FF. Offspring of patients treated for cancer in childhood. J Natl Cancer Inst 1979; 62: 1193–7.
64 Hawkins MM, Smith RA, Curtice LJ. Childhood cancer survivors and their offspring studied through a postal survey of general practitioners: preliminary results. J R Coll Gen Pract 1988; 38: 102–5.
65 Byrne J, Mulvihill JJ, Myers MH, Connelly RR, Naughton MD, Krauss MR, *et al*. Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. N Engl J Med 1987; 317: 1315–21.
66 Buchanan JD, Fairley KF, Barrie JU. Return of spermatogenesis after stopping cyclophosphamide therapy. Lancet 1975; 2: 156–7.
67 Witt KL, Bishop JB. Mutagenicity of anticancer drugs in mammalian germ cells. Mutat Res 1996; 355: 209–34.
68 Choudhury RC, Jagdale MB, Misra S. Potential transmission of the cytogenetic effects of cisplatin in the male germline cells of Swiss mice. J Chemother 2000; 12: 352–9.
69 Sjoblom T, Parvinen M, Lahdetie J. Stage-specific DNA synthesis of rat spermatogenesis as an indicator of genotoxic effects of vinblastine, mitomycin C and ionizing radiation on rat spermatogonia and spermatocytes. Mutat Res 1995; 331: 181–90.
70 Albanese R. Mammalian male germ cell cytogenetics. Mutagenesis 1987; 2: 79–85.
71 Meirow D. Reproduction post-chemotherapy in young cancer patients. Mol Cell Endocrinol 2000; 169: 123–31.
72 Liang JC, Sherron DA, Johnston D. Lack of correlation between mutagen-induced chromosomal univalency and aneuploidy in mouse spermatocytes. Mutat Res 1986; 163: 285–97.
73 van Buul PP, Goudzwaard JH. Bleomycin-induced structural chromosomal aberrations in spermatogonia and bone-marrow cells of mice. Mutat Res 1980; 69: 319–24.
74 Genesca A, Caballin MR, Miro R, Benet J, Bonfill X, Egozcue J. Human sperm chromosomes. long-term effect of cancer treatment. Cancer Genet Cytogenet 1990; 46: 251–60.
75 Martin RH, Ernst S, Rademaker A, Barclay L, Ko E, Summers N. Analysis of sperm chromosome complements before, during, and after chemotherapy. Cancer Genet Cytogenet 1999; 108: 133–6.
76 Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier HU, Cassel MJ, *et al*. Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. Nat Genet 1997; 16: 74–8.
77 Puscheck E, Philip PA, Jeyendran RS. Male fertility preservation and cancer treatment. Cancer Treat Rev 2004; 30: 173–80.
78 McCall ML, Keaty EC, Thompson JD. Conservation of ovarian tissue in the treatment of carcinoma of the cervix with radical surgery. Am J Obstet Gynecol 1958; 75: 590–600.
79 Morice P, Castaigne D, Haie-Meder C, Pautier P, El Hassan J, Duvillard P, *et al*. Laparoscopic ovarian transposition for pelvic malignancies: indications and functional outcomes. Fertil

Steril 1998; 70: 956–60.
80 Bidzinski M, Lemieszczuk B, Zielinski J. Evaluation of the hormonal function and features of the ultrasound picture of transposed ovary in cervical cancer patients after surgery and pelvic irradiation. Eur J Gynaecol Oncol 1993; 14 (Suppl): 77–80.
81 Morice P, Thiam-Ba R, Castaigne D, Haie-Meder C, Gerbaulet A, Pautier P, *et al.* Fertility results after ovarian transposition for pelvic malignancies treated by external irradiation or brachytherapy. Hum Reprod 1998; 13: 660–3.
82 Bisharah M, Tulandi T. Laparoscopic preservation of ovarian function: an underused procedure. Am J Obstet Gynecol 2003; 188: 367–70.
83 Blumenfeld Z. Preservation of fertility and ovarian function and minimalization of chemotherapy associated gonadotoxicity and premature ovarian failure: the role of inhibin-A and -B as markers. Mol Cell Endocrinol 2002; 187: 93–105.
84 Pereyra Pacheco B, Mendez Ribas JM, Milone G, Fernandez I, Kvicala R, Mila T, *et al*. Use of GnRH analogs for functional protection of the ovary and preservation of fertility during cancer treatment in adolescents: a preliminary report. Gynecol Oncol 2001; 81: 391–7.
85 Oktay K, Sonmezer M, Oktem O. 'Ovarian cryopreservation versus ovarian suppression by GnRH analogues: *primum non nocere*'– reply. Hum Reprod 2004; 19: 1681–3.
86 Meirow D. Reproduction post-chemotherapy in young cancer patients. Mol Cell Endocrinol 2000; 169: 123–31.
87 Waxman JH, Ahmed R, Smith D, Wrigley PF, Gregory W, Shalet S, *et al*. Failure to preserve fertility in patients with Hodgkin's disease. Cancer Chemother Pharmacol 1987; 19: 159–62.
88 Emons G, Grundker C, Gunthert AR, Westphalen S, Kavanagh J, Verschraegen C. GnRH antagonists in the treatment of gynecological and breast cancers. Endocr Relat Cancer 2003; 10: 291–9.
89 Grundker C, Gunthert AR, Westphalen S, Emons G. Biology of the gonadotropin-releasing hormone system in gynecological cancers. Eur J Endocrinol 2002; 146: 1–14.
90 Jonat W. The role of LHRH analogs in premenopausal breast cancer. In: Lunenfeld B, editor. GnRH analogues: the state of the art 2001. A summary of the 6th International Symposium on GnRH Analogues in Cancer and Human Reproduction, Geneva, February 2001. New York: Parthenon; 2002
91 Chapman RM, Sutcliffe SB. Protection of ovarian function by oral contraceptives in women receiving chemotherapy for Hodgkin's disease. Blood 1981; 58: 849–51.
92 Whitehead E, Shalet SM, Blackledge G, Todd I, Crowther D, Beardwell CG. The effect of combination chemotherapy on ovarian function in women treated for Hodgkin's disease. Cancer 1983; 52: 988–93.
93 Montz FJ, Wolff AJ, Gambone JC. Gonadal protection and fecundity rates in cyclophosphamide-treated rats. Cancer Res 1991; 51: 2124–6.
94 Tilly JL. Molecular and genetic basis of normal and toxicant-induced apoptosis in female germ cells. Toxicol Lett 1998; 102–3: 497–501.
95 Tilly JL. Apoptosis and ovarian function. Rev Reprod 1996; 1: 162–72.
96 Morita Y, Tilly JL. Oocyte apoptosis: like sand through an hourglass. Dev Biol 1999; 213: 1–17.
97 Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, *et al*. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. Nat Med 2000; 6: 1109–14.
98 Paris F, Perez GI, Fuks Z, Haimovitz-Friedman A, Nguyen H, Bose M, *et al*. Sphingosine 1-phosphate preserves fertility in irradiated female mice without propagating genomic damage in offspring. Nat Med 2002; 8: 901–2.
99 Son WY, Yoon SH, Yoon HJ, Lee SM, Lim JH. Pregnancy outcome following transfer of human blastocysts vitrified on electron microscopy grids after induced collapse of the blastocoele. Hum Reprod 2003; 18: 137–9.
100 Seli E, Tangir J. Fertility preservation options for female patients with malignancies. Curr Opin Obstet Gynecol 2005; 17: 299–308.
101 Oktay K, Buyuk E, Davis O, Yermakova I, Veeck L, Rosenwaks Z. Fertility preservation in breast cancer patients: IVF and embryo cryopreservation after ovarian stimulation with tamoxifen. Hum Reprod 2003; 18: 90–5.
102 Al-Fozan H, Al-Khadouri M, Tan SL, Tulandi T. A randomized trial of letrozole versus clomiphene citrate in women undergoing superovulation. Fertil Steril 2004; 82: 1561–3.
103 Mitwally MF, Casper RF. Aromatase inhibition reduces gonadotrophin dose required for controlled ovarian stimulation in women with unexplained infertility. Hum Reprod 2003; 18: 1588–97.
104 Falcone T, Bedaiwy MA. Fertility preservation and pregnancy outcome after malignancy. Curr Opin Obstet Gynecol 2005; 17: 21–6.
105 Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol 2005; 23: 4347–53.
106 Holzer H, Casper R, Tulandi T. A new era in ovulation induction. Fertil Steril 2006; 85: 277–84.
107 Pickering SJ, Braude PR, Johnson MH, Cant A, Currie J. Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. Fertil Steril 1990; 54: 102–8.
108 Gosden RG. Prospects for oocyte banking and *in vitro* maturation. J Natl Cancer Inst Monogr 2005; 34: 60–3.
109 Dittrich R, Maltaris T. A simple freezing protocol for the use of an open freezing system for cryopreservation of ovarian tissue. Cryobiology 2006; 52: 166.
110 Maltaris T, Dimmler A, Muller A, Binder H, Hoffmann I, Kohl J, *et al*. The use of an open-freezing system with self-seeding for cryopreservation of mouse ovarian tissue. Reprod Domest Anim 2005; 40: 250–4.
111 Oktay K, Newton H, Aubard Y, Salha O, Gosden RG. Cryopreservation of immature human oocytes and ovarian tissue: an emerging technology? Fertil Steril 1998; 69: 1–7.
112 Maltaris T, Dimmler A, Muller A, Hoffmann I, Beckmann MW, Dittrich R. Comparison of two freezing protocols in an

open freezing system for cryopreservation of rat ovarian tissue. J Obstet Gynaecol Res 2006; 32: 273–9.
113 Newton H, Aubard Y, Rutherford A, Sharma V, Gosden R. Low temperature storage and grafting of human ovarian tissue. Hum Reprod 1996; 11: 1487–91.
114 Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, *et al*. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004; 364: 1405–10.
115 Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, *et al*. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005; 353: 318–21.
116 Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, *et al*. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet 2004; 363: 837–40.
117 Eppig JJ, O'Brien MJ. Development *in vitro* of mouse oocytes from primordial follicles. Biol Reprod 1996; 54: 197–207.
118 Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. Hum Reprod 1986; 1: 81–7.
119 Kim SS, Soules MR, Battaglia DE. Follicular development, ovulation, and corpus luteum formation in cryopreserved human ovarian tissue after xenotransplantation. Fertil Steril 2002; 78: 77–82.
120 Maltaris T, Kaya H, Hoffmann I, Mueller A, Beckmann MW, Dittrich R. Comparison of xenografting in SCID mice and LIVE/DEAD assay as a predictor of the developmental potential of cryopreserved ovarian tissue. In Vivo 2006; 20: 11–6.
121 Sonmezer M, Shamonki MI, Oktay K. Ovarian tissue cryopreservation: benefits and risks. Cell Tissue Res 2005; 322: 125–32.
122 Shaw JM, Bowles J, Koopman P, Wood EC, Trounson AO. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. Hum Reprod 1996; 11: 1668–73.
123 Kim SS. Fertility preservation in female cancer patients: current developments and future directions. Fertil Steril 2006; 85: 1–11.
124 Mueller A, Maltaris T, Dimmler A, Hoffmann I, Beckmann MW, Dittrich R. Development of sex cord stromal tumors after heterotopic transplantation of cryopreserved ovarian tissue in rats. Anticancer Res 2005; 25: 4107–11.
125 Go KJ. Better by half: the pursuit of haploidization. Embryol Newsl 2003; 6: 1–9.
126 Nagy ZP, Chang CC. Current advances in artificial gametes. Reprod Biomed Online 2005; 11: 332–9.
127 Sanger WG, Olson JH, Sherman JK. Semen cryobanking for men with cancer–criteria change. Fertil Steril 1992; 58: 1024–7.
128 Shin D, Lo KC, Lipshultz LI. Treatment options for the infertile male with cancer. J Natl Cancer Inst Monogr 2005; 34: 48–50.
129 Schrader M, Muller M, Straub B, Miller K. Testicular sperm extraction in azoospermic patients with gonadal germ cell tumors prior to chemotherapy–a new therapy option. Asian J Androl 2002; 4: 9–15.
130 Schmiegelow ML, Sommer P, Carlsen E, Sonksen JO, Schmiegelow K, Muller JR. Penile vibratory stimulation and electroejaculation before anticancer therapy in two pubertal boys. J Pediatr Hematol Oncol 1998; 20: 429–30.
131 Brougham MF, Kelnar CJ, Sharpe RM, Wallace WH. Male fertility following childhood cancer: current concepts and future therapies. Asian J Androl 2003; 5: 325–37.
132 Park YS, Lee SH, Song SJ, Jun JH, Koong MK, Seo JT. Influence of motility on the outcome of *in vitro* fertilization/intracytoplasmic sperm injection with fresh vs. frozen testicular sperm from men with obstructive azoospermia. Fertil Steril 2003; 80: 526–30.
133 Lass A, Akagbosu F, Brinsden P. Sperm banking and assisted reproduction treatment for couples following cancer treatment of the male partner. Hum Reprod Update 2001; 7: 370–7.
134 Hallak J, Sharma RK, Thomas AJ Jr, Agarwal A. Why cancer patients request disposal of cryopreserved semen specimens posttherapy: a retrospective study. Fertil Steril 1998; 69: 889–93.
135 Kurdoglu B, Wilson G, Parchuri N, Ye WS, Meistrich ML. Protection from radiation-induced damage to spermatogenesis by hormone treatment. Radiat Res 1994; 139: 97–102.
136 Waxman JH, Ahmed R, Smith D, Wrigley PF, Gregory W, Shalet S, *et al*. Failure to preserve fertility in patients with Hodgkin's disease. Cancer Chemother Pharmacol 1987; 19: 159–62.
137 Thomson AB, Anderson RA, Irvine DS, Kelnar CJ, Sharpe RM, Wallace WH. Investigation of suppression of the hypothalamic–pituitary–gonadal axis to restore spermatogenesis in azoospermic men treated for childhood cancer. Hum Reprod 2002; 17: 1715–23.
138 Kamischke A, Kuhlmann M, Weinbauer GF, Luetjens M, Yeung CH, Kronholz HL, *et al*. Gonadal protection from radiation by GnRH antagonist or recombinant human FSH: a controlled trial in a male nonhuman primate (Macaca fascicularis). J Endocrinol 2003; 179: 183–94.
139 Boekelheide K, Schoenfeld HA, Hall SJ, Weng CC, Shetty G, Leith J, *et al*. Gonadotropin-releasing hormone antagonist (Cetrorelix) therapy fails to protect nonhuman primates (Macaca arctoides) from radiation-induced spermatogenic failure. J Androl 2005; 26: 222–34.
140 Kelnar CJ, McKinnell C, Walker M, Morris KD, Wallace WH, Saunders PT, *et al*. Testicular changes during infantile 'quiescence' in the marmoset and their gonadotrophin dependence: a model for investigating susceptibility of the prepubertal human testis to cancer therapy? Hum Reprod 2002; 17: 1367–78.
141 Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol 2005; 6: 209–18.
142 Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A 1994; 91: 11298–302.
143 Orwig KE, Schlatt S. Cryopreservation and transplantation of spermatogonia and testicular tissue for preservation of male fertility. J Natl Cancer Inst Monogr 2005; 34: 51–6.
144 Brinster CJ, Ryu BY, Avarbock MR, Karagenc L, Brinster

RL, Orwig KE. Restoration of fertility by germ cell transplantation requires effective recipient preparation. Biol Reprod 2003; 69: 412–20.
145 Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF, Nieschlag E. Germ cell transfer into rat, bovine, monkey and human testes. Hum Reprod 1999; 14: 144–50.
146 Frederickx V, Michiels A, Goossens E, De Block G, Van Steirteghem AC, Tournaye H. Recovery, survival and functional evaluation by transplantation of frozen-thawed mouse germ cells. Hum Reprod 2004; 19: 948–53.
147 Jahnukainen K, Hou M, Petersen C, Setchell B, Soder O. Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia. Cancer Res 2001; 61: 706–10.
148 Tesarik J, Bahceci M, Ozcan C, Greco E, Mendoza C. Restoration of fertility by *in vitro* spermatogenesis. Lancet 1999; 353: 555–6.
149 Lee DR, Kim KS, Yang YH, Oh HS, Lee SH, Chung TG, *et al*. Isolation of male germ stem cell-like cells from testicular tissue of non-obstructive azoospermic patients and differentiation into haploid male germ cells *in vitro*. Hum Reprod 2006; 21: 471–6.
150 Lutchman Singh K, Davies M, Chatterjee R. Fertility in female cancer survivors: pathophysiology, preservation and the role of ovarian reserve testing. Hum Reprod Update 2005; 11: 69–89.
151 van Rooij IA, Bancsi LF, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in *in vitro* fertilization. Fertil Steril 2003; 79: 482–8.
152 Burger HG, Dudley EC, Hopper JL, Shelley JM, Green A, Smith A, *et al*. The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. J Clin Endocrinol Metab 1995; 80: 3537–45.
153 Licciardi FL, Liu HC, Rosenwaks Z. Day 3 estradiol serum concentrations as prognosticators of ovarian stimulation response and pregnancy outcome in patients undergoing *in vitro* fertilization. Fertil Steril 1995; 64: 991–4.
154 Lee SJ, Lenton EA, Sexton L, Cooke ID. The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. Hum Reprod 1988; 3: 851–5.
155 Muttukrishna S, Knight PG. Inverse effects of activin and inhibin on the synthesis and secretion of FSH and LH by ovine pituitary cells *in vitro*. J Mol Endocrinol 1991; 6: 171–8.
156 Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia DE, Soules MR. Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. J Clin Endocrinol Metab 1996; 81: 2742–5.
157 Munz W, Hammadeh ME, Seufert R, Schaffrath M, Schmidt W, Pollow K. Serum inhibin A, inhibin B, pro-alphaC, and activin A levels in women with idiopathic premature ovarian failure. Fertil Steril 2004; 82: 760–2.
158 Seifer DB, Scott RT Jr, Bergh PA, Abrogast LK, Friedman CI, Mack CK, *et al*. Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. Fertil Steril 1999; 72: 63–5.
159 van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, *et al*. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002; 17: 3065–71.
160 Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Müllerian hormone: a new marker for ovarian function. Reproduction 2006; 131: 1–9.
161 Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction 2002; 124: 601–9.
162 te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002; 8: 141–54.
163 Navot D, Rosenwaks Z, Margalioth EJ. Prognostic assessment of female fecundity. Lancet 1987; 2: 645–7.
164 Hendriks DJ, Broekmans FJ, Bancsi LF, de Jong FH, Looman CW, Te Velde ER. Repeated clomiphene citrate challenge testing in the prediction of outcome in IVF: a comparison with basal markers for ovarian reserve. Hum Reprod 2005; 20: 163–9.
165 Sharara FI, Scott RT Jr, Seifer DB. The detection of diminished ovarian reserve in infertile women. Am J Obstet Gynecol 1998; 179: 804–12.
166 Padilla SL, Bayati J, Garcia JE. Prognostic value of the early serum estradiol response to leuprolide acetate in *in vitro* fertilization. Fertil Steril 1990; 53: 288–94.
167 Winslow KL, Toner JP, Brzyski RG, Oehninger SC, Acosta AA, Muasher SJ. The gonadotropin-releasing hormone agonist stimulation test–a sensitive predictor of performance in the flare-up *in vitro* fertilization cycle. Fertil Steril 1991; 56: 711–7.
168 Fabregues F, Balasch J, Creus M, Carmona F, Puerto B, Quinto L, *et al*. Ovarian reserve test with human menopausal gonadotropin as a predictor of *in vitro* fertilization outcome. J Assist Reprod Genet 2000; 17: 13–9.
169 Fanchin R, de Ziegler D, Olivennes F, Taieb J, Dzik A, Frydman R. Exogenous follicle stimulating hormone ovarian reserve test (EFORT): a simple and reliable screening test for detecting 'poor responders' in *in vitro* fertilization. Hum Reprod 1994; 9: 1607–11.
170 Kwee J, Elting MW, Schats R, Bezemer PD, Lambalk CB, Schoemaker J. Comparison of endocrine tests with respect to their predictive value on the outcome of ovarian hyperstimulation in IVF treatment: results of a prospective randomized study. Hum Reprod 2003; 18: 1422–7.
171 Chang MY, Chiang CH, Hsieh TT, Soong YK, Hsu KH. Use of the antral follicle count to predict the outcome of assisted reproductive technologies. Fertil Steril 1998; 69: 505–10.
172 Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. Hum Reprod 2005; 20: 611–5.
173 Ivarsson SA, Nilsson KO, Persson PH. Ultrasonography of the pelvic organs in prepubertal and postpubertal girls. Arch Dis Child 1983; 58: 352–4.

174 Andolf E, Jorgensen C, Svalenius E, Sunden B. Ultrasound measurement of the ovarian volume. Acta Obstet Gynecol Scand 1987; 66: 387–9.
175 Wallace WH, Kelsey TW. Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography. Hum Reprod 2004; 19: 1612–7.
176 Schmidt KL, Byskov AG, Nyboe Andersen A, Muller J, Yding Andersen C. Density and distribution of primordial follicles in single pieces of cortex from 21 patients and in individual pieces of cortex from three entire human ovaries. Hum Reprod 2003; 18: 1158–64.
177 Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in *in vitro* fertilization: a prospective study comparing basal markers of ovarian reserve. Fertil Steril 2002; 77: 328–36.
178 Siimes MA, Rautonen J. Small testicles with impaired production of sperm in adult male survivors of childhood malignancies. Cancer 1990; 65: 1303–6.
179 Wallace EM, Groome NP, Riley SC, Parker AC, Wu FC. Effects of chemotherapy-induced testicular damage on inhibin, gonadotropin, and testosterone secretion: a prospective longitudinal study. J Clin Endocrinol Metab 1997; 82: 3111–5.
180 Ginsburg ES, Yanushpolsky EH, Jackson KV. *In vitro* fertilization for cancer patients and survivors. Fertil Steril 2001; 75: 705–10.
181 Dolmans MM, Demylle D, Martinez-Madrid B, Donnez J. Efficacy of *in vitro* fertilization after chemotherapy. Fertil Steril 2005; 83: 897–901.
182 Agarwal A, Ranganathan P, Kattal N, Pasqualotto F, Hallak J, Khayal S, *et al*. Fertility after cancer: a prospective review of assisted reproductive outcome with banked semen specimens. Fertil Steril 2004; 81: 342–8.
183 Schmidt KL, Larsen E, Bangsboll S, Meinertz H, Carlsen E, Andersen AN. Assisted reproduction in male cancer survivors: fertility treatment and outcome in 67 couples. Hum Reprod 2004; 19: 2806–10.

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Erratum

Erectile dysfunction in Fragile X patients

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The editorial office wishes to apologise for an error in the above paper. This paper should belong to Clinical Experience instead of Original Article.