Fertility preservation and minimizing reproductive damage in cancer survivors

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Recent advances in oncology have helped in the survival and cure of increasing numbers of childhood cancer patients and those during their reproductive age period. This has increased the need to improve the existing technology, and prompted the search for new technologies, to minimize the gonadotoxic effects of cancer treatment and preserve human fertility. Conservative surgical approaches for cancer treatment have been widely accepted following the progresses in early detection of cancer and accumulating long-term outcome safety data. Gonadal suppression to increase resistance to cancer treatment by gonadotropin analogues and sex hormones has been suggested. However, while the effectiveness is unlikely in the male, there is no general consensus on its success in the female. Fertility preservation options for both male and female patients include cryopreservation of embryos, gametes and gonads. While embryo cryopreservation is a well-established and successful technique, there are several obvious limitations. Gamete cryopreservation is very successful in the male (sperm freezing) while still experimental in the female (oocyte freezing), with growing evidence suggesting its potential success. Gonadal cryopreservation is still in its early stages of experimental development, both in the male (testicular tissue cryopreservation and *in vitro* spermatogenesis) and female (ovarian tissue cryopreservation and *in vitro* follicular maturation).

Expert Rev. Anticancer Ther. 7(7), xxx–xxx (2007)

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KEYWORDS:
cancer, chemotherapy, fertility, preservation, radiotherapy, reproduction

This is the second of a series of three articles discussing cancer and human reproduction from a clinical perspective. This article will present a general overview of two topics. The first: the different approaches to minimize the reproductive damage caused by cancer and its treatment, and the second: the various options for preserving human fertility in cancer survivors.

The previous article presented a general overview of how cancer and its treatment can adversely affect reproduction in humans, summarizing the nature and extent of those adverse effects in men, women and their offspring [1]. The third and last article of this series will present details on the management of reproductive issues in cancer patients, including fertility enhancement by ovarian stimulation and assisted reproduction, contraceptive needs, as well as management of reproductive and hormonal deficiency, in particular delayed or absent puberty, menopause and andropause.

In 2006, approximately 1,300,000 new cancer cases were expected in the USA with almost half of them women [2]. Extrapolating from previous reports, which found 8% of those women were younger than 40 years of age, more than 50,000 new cancer patients are expected to be women during their reproductive age group every year [3]. Moreover, by 2010, it is estimated that one in every 250 people in the adult population will be a childhood cancer survivor. These figures are due to the improved life expectancy and survival owing to advances in the diagnosis and treatment of childhood, adolescent and adult cancers. Cancer treatments, including aggressive chemotherapy and radiotherapy, impose a very high risk for gonadal dysfunction, leading to impairment or even termination of gonadal functions in most patients [4].

There are two main approaches to preserve fertility in cancer survivors. The first includes interventions to minimize the damaging effect

10.1586/14737140.7.7.xxx

ISSN 1473-7140

of cancer treatment on human reproduction, while the second includes fertility preservation, mainly by cryopreservation of gonadal tissues, gametes and embryos. In addition, the option of an oocyte donor is a viable one that is associated with high pregnancy rates for women with premature ovarian failure due to cancer treatment or other reasons. Obviously, the various options for fertility preservation are not only valid for cancer survivors, but also for individuals at high risk of premature cessation of reproductive functions, such as women with premature ovarian failure and patients receiving aggressive chemotherapy and/or radiotherapy treatment for noncancer indications. Such indications include benign hematological diseases, such as hemoglobinopathies (sickle cell anemia and thalassemias major) and aplastic anemia, as well as severe autoimmune diseases that did not respond to immunosuppressive therapy, such as systemic lupus erythematosus and autoimmune thrombocytopenia [5]. Other interesting indications include benign disorders, such as recurrent severe ovarian endometriosis and prophylactic oophorectomy, that protect against breast cancer. In addition, during recent years with the advances in the field of molecular genetics, increasing numbers of patients undergoing oophorectomy for prophylaxis against ovarian cancer (e.g., carriers of *BRCA* mutations) may also benefit from options of fertility preservations. Donnez and Bassil have summarized those indications in their comprehensive review [6].

Approaches to minimize reproductive damage caused by cancer treatment

The two main strategies to minimize reproductive damage caused by cancer treatment include the application of less damaging cancer treatment modalities and administering agents that increase the resistance of the gonads to damage by cancer treatment. BOX 1 summarizes the different options to minimize the reproductive-damaging effects of cancer treatment.

Minimizing reproductive damage caused by cancer treatment in females

The most important determining factors for the damaging effect of cancer treatment are the woman's age, followed by the nature and dose of treatment regimens and cancer diagnosis. It is also important to mention here that there may be some individual variability in the sensitivity to reproductive damage by cancer treatment, which adds to the complexity of the issue [1].

Applying less damaging cancer treatment modalities

Conservative surgery, less aggressive chemotherapy regimens, including agents with lower cytotoxicity to the gonads and applying lower doses of radiation with careful selection of schedule of implementation of radiotherapy, have all been suggested to minimize reproductive damage. It is important to stress the crucial rule that the priority in choosing the treatment modality should go first to the success in curing the malignancy or achieving the best chance of remission and that fertility preservation should come second. It is clear that the patient should never have her chance of survival and cure jeopardized for the sake of maintaining her fertility [7,8].

Conservative surgery

Conservative surgery aims to preserve the ovaries and uterus whenever possible without jeopardizing the patient's chance of cure and survival; for example, very early-stage ovarian cancer localized to one side and selected cases of early-stage cervical cancer. An important example for applying conservative surgery to preserve fertility in cancer survivors is performing radical vaginal trachelectomy to remove the uterine cervix while preserving the uterine body for future pregnancy in women with cervical cancer. The procedure was first performed more than 13 years ago with reports of a successful pregnancy achieved following radical vaginal trachelectomy and pelvic lymphadenectomy for the treatment of early-stage cervical cancer [9].

However, it is too early to generalize conclusions regarding the success of this procedure as it is still in its infancy, despite the preliminary data that suggest its success in providing a chance of fertility preservation while achieving excellent survival for a highly selected group of women with cervical cancer [10]. Gershenson has recently reviewed fertility-sparing surgery for malignancies in women, particularly those with ovarian, cervical and endometrial malignancies. The author reviews the accumulating data in the literature supporting the success and safety of such approaches that preserve ovaries and/or uterus, for example in early endometrial carcinoma [11]. Accumulating data supporting the success of the procedure from more recent reports are encouraging [12–14].

Box 1. Options to minimize the reproductive damaging effects of cancer treatment.

Options in female patients

- Applying less damaging cancer treatment modalities
  - Conservative surgery (effective)
  - Transposition of the ovaries away from the field of radiotherapy such as oophoropexy (effective, if only radiotherapy is implemented)
- Administering agents that increase the resistance of the ovaries to damage by cancer treatment
  - Gonadotropin-releasing hormone analogues (effectiveness is controversial, however, with the majority of data suggesting effectiveness)
  - Sex steroids (effectiveness is controversial)
  - Agents that antagonize the toxic effect of the cancer treatment on the ovaries (too few data available)

Options in male patients

- Testicular shielding during radiotherapy (effective)
- Hormonal testicular suppression (not effective)

Ovarian transposition to minimize damage by radiotherapy

Damage by pelvic radiotherapy to the ovaries can be reduced by surgical transposition of the ovaries (oophoropexy) out of the field of irradiation. The most common indications for oophoropexy are patients undergoing radiotherapy for Hodgkin's disease, cervical and vaginal cancer, and pelvic sarcomas. The technique was first described by McCall and colleagues approximately half a century ago [15]. Although oophoropexy was first described through a laparotomy approach, it can currently be performed laparoscopically [16], with several laparoscopic surgical techniques described successfully without significant complications [17]. The technique of lateral transposition of the ovaries is classically described by dividing the utero–ovarian ligament and tubes, then mobilizing the ovaries (with their infundibulo–pelvic ligaments that carry the blood supply) into the paracolic gutters so that they lie approximately 5–8 cm above the upper border of the radiation field, a safety margin that is believed to reduce ovarian radiation damage [18]. Laparoscopic dissection of the infundibulo–pelvic ligaments has been suggested to allow a medial oophoropexy to be performed [19].

While ovarian transposition is usually performed on both ovaries, recently, the combination of ovarian cryopreservation and ovarian transposition has been suggested to maximize future fertility options for women facing pelvic irradiation [20].

The laparoscopic approach enjoys the advantages of less invasiveness and fewer adhesions. However, the most important advantage is the significantly shortened recovery time compared with the laparotomy approach. Thus, unnecessary treatment delays are avoided as the radiotherapy can be initiated rapidly postoperatively.

Oophoropexy is believed to reduce the ovarian radiation dose to approximately 5–10% of that if the ovaries remain in their natural place [21]. Ovarian failure might result if the ovaries are not removed far enough or if they migrate back to their original position. Preservation of ovarian function by oophoropexy has been reported in 16–90% of patients [16,17]. The wide variability is thought to be secondary to the inability to calculate and prevent scatter radiation, concomitant use of chemotherapy and the different radiation dosages used [22].

Problems encountered in oophoropexy include rare complications, such as benign ovarian cysts, chronic abdominal pain, ovarian torsion, adhesions and the risk of ovarian metastasis, which appears to be extremely rare as only two cases of ovarian metastasis have been reported. To minimize the risk of metastasis, oophoropexy should be performed in patients with cervical cancer when the tumor is less than 3 cm and confined to the cervix in the absence of macroscopic extrauterine spread, irrespective to the histological cancer type [23]. Another problem associated with oophoropexy is that, in certain cases, the ovaries may need to be repositioned into or close to their original position. This is important for women undergoing *in vitro* fertilization (IVF) to facilitate oocyte pick up (transvaginal ultrasound-guided needle aspiration) and when surgical orthotopic retransposition is necessary to achieve a pregnancy.

Administering agents that increase the resistance of the ovaries to damage by cancer treatment

Several agents have been suggested to increase the resistance of the ovaries against the damaging effects of cancer treatment; this can be acheived through two mechanisms. The first, by putting the ovaries into a state of rest to reduce ovarian follicular cell division and growth, hence the follicles become less vulnerable to the antineoplasmic effect of cancer treatment, such as gonadotropin-releasing hormone (GnRH) agonists (GnRH-a). The second, by directly antagonizing the cytotoxic effect of the cancer treatment on the ovaries, such as antiapopototic agents. Unfortunately, until now there are no therapies that are capable of protecting the ovaries from the cytotoxic effects of chemotherapy or radiotherapy [24,25].

Agents that suppress ovarian follicular cell growth & division

Gonadotropin-releasing hormone analogues

GnRH analogues include GnRH-a and antagonists and result in temporary medical castration by suppressing pituitary gonadotropin production. GnRH-a have been suggested to be chemoprotective agents. However, the evidence for their benefit is controversial. Small studies reported that GnRH-a were well tolerated and might protect long-term ovarian function [24,25]. A much larger study by Blumenfeld confirmed the results of the smaller studies in a group of women (55 lymphoma patients) who received GnRH-a for 7–10 days before chemotherapy treatment. The authors found the rate of premature ovarian failure to be approximately 5% in the GnRH-a/chemotherapy group versus 55% in the group receiving chemotherapy alone, which had a different follow-up period [24].

However, contradictory results have been published on the effects of GnRH-a, with strong evidence against their benefit in protecting ovarian function from the cytotoxic effects of chemotherapy from a prospective randomized study that failed to find an improvement in outcome (ovarian function preservation) compared with placebo [26]. Moreover, some investigators even suggested a negative effect from the use of GnRH-a in breast cancer patients by arresting tumors cells in G_0 phase and making them less responsive to chemotherapy [27,28].

On the other hand, despite the controversy behind the use of GnRH-a to protect the ovaries from the cytotoxic effects of chemotherapy and contrary to the worries concerning reducing responsiveness of the breast cancer cells, GnRH-a are used today both in adjuvant treatment and in metastatic breast cancer for reversible medical castration to downregulate pituitary gonadotropin secretion, leading to suppressed ovarian estrogen production [29].

Clearly, the retrospective nature of most of the reports and small sample size significantly reduce the validity of the study findings. In addition, in these studies GnRH-a were used for variable durations before chemotherapy and the primary outcome was simply the return of menstruation rather than documenting ovulation, which are important shortcomings that explain, in a large part, such controversy. There is obviously a

need for adequately powered randomized trials to answer the question whether or not GnRH-a use is of benefit in reducing the cytotoxic effects of chemotherapy on the ovaries [8].

Currently, there are two important clinical trials underway to answer this question. The first, by the Southwest Oncology Group, is aimed at preventing early ovarian failure with GnRH-a among women with hormone receptor-negative breast cancer receiving chemotherapy. The second, conducted by the German Hodgkin's Lymphoma Study Group, is a randomized, Phase II trial evaluating GnRH-a and oral contraceptives to preserve fertility in women treated for advanced Hodgkin's lymphoma [30].

Most recently, a randomized trial including young women receiving chemotherapy for Hodgkin's disease investigated the value of the combined use of the GnRH-a triptorelin with hormonal add-back by tibolone cotreatment in preventing ovarian failure. The authors found the combined use of triptorelin and tibolone to be a useful tool for preserving ovarian function because all but three (10%) of the women in this treatment group returned to spontaneous ovulation and menses, in contrast to 23% of subjects in the control group ($p < 0.05$). The use of tibolone was successful in preventing any significant bone loss with triptorelin as there was no significant difference in bone mineral density between the study and control groups [31].

GnRH antagonists can suppress ovarian function by suppressing pituitary gonadotropin production through direct antagonism. Those agents can, at least theoretically, achieve comparable outcomes with GnRH-a in possible protection of ovarian function against deleterious effects induced by cancer treatment. However, they have not yet been studied for such indication in humans.

The available data on the possible protective role of GnRH antagonists comes only from animal studies, mostly in rodents, with contradictory results [32]. Moreover, a study even suggested a depletion of the ovarian follicles through a direct effect of the GnRH antagonist on the ovary in a murine model [33].

Sex steroids

Different sex steroids (progesterone and combined estrogen/progesterone) have been tested at suppressing the ovaries, to protect them against the cytotoxic effects of cancer treatment. This has not been studied adequately and the available data are controversial. In a small study, Chapman and Sutcliffe found more follicles in ovarian biopsies from three patients who received combination oral contraceptive pills during chemotherapy than in those who did not [34]. However, Whitehead and colleagues failed to find a protective effect of combination oral contraceptive pills in patients who received chemotherapy for Hodgkin's disease [35]. Other investigators attempted the use of progesterone alone. In rat, progesterone was found to have a protective effect when administered 1 week before the start of cyclophosphamide and during treatment [36]; however, Familiari and colleagues could not find a protective effect of medroxyprogesterone acetate on human primordial follicles exposed to cytotoxic drugs [37].

As with the case of GnRH analogues, there is no consensus on the protective effect of sex steroids. Following findings by the German Hodgkin's Lymphoma Study Group, in a retrospective study, of a possible protective effect of oral contraceptives in younger women undergoing gonadotoxic chemotherapy, a randomized, Phase II trial was initiated with the aim of defining a standard cotreatment for the reduction of infertility rates in young female patients during chemotherapy for Hodgkin's lymphoma [37].

Agents that antagonize the toxic effect of the cancer treatment on the ovaries

Programmed cell death (apoptosis) is believed to play a significant role in the normal physiology of germ cell depletion (reduction of oocyte and follicular count throughout the female reproductive life from birth to menopause). Also, it is believed that the gonadotoxic effects of various cancer treatments are mediated through apoptosis [38–40]. Hence, it is logic to extrapolate that the use of antiapoptotic agents might help in protecting the ovaries against the cytotoxic effects of cancer treatments. On the other hand, it is also logical to extrapolate that those antiapoptotic agents might have a negative effect through interfering with the efficacy of cancer treatment agents by interfering with apoptosis in the tumor cells. In conclusion, it is still very early to draw any conclusions regarding these agents as research in this area is still in its very early experimental stages.

Minimizing reproductive damage caused by cancer treatment in males

The efficacy of applying protective agents to reduce the gonadotoxic effects of cancer treatment has not been adequately investigated. Applying the two concepts of gonadal suppression or administering protective agents (as discussed previously) does not apply in the situation of males as in females. Hormonal therapy in men is not successful in preserving fertility when highly gonadotoxic chemotherapy is administered, [26,41] nor did it speed recovery of spermatogenesis [42,43].

An important reason for the lack of significant research on the use of protective agents to reduce the gonadotoxic effect of cancer treatment in the male is the very high success of the practice of sperm cryopreservation and the success of pregnancies from cryopreserved human testicular tissue [44]. Obviously this option is not available for male patients before puberty, for whom the only option would be testicular tissue cryopreservation and reimplantation [45].

Options for fertility preservation in cancer survivors

There are three main options for fertility preservation, including cryopreservation of gonadal tissue, gametes and embryos. Clearly, the first option is the only option available before puberty, while the third option is available only for individuals with current partners who can provide their gametes for creation of embryos through IVF. In the absence of a current, readily available partner, donor sperm is still an option for single females. While gamete cryopreservation is very successful for

males (sperm cryopreservation), oocyte cryopreservation is still experimental, with variable degrees of success and limited information on long-term outcomes [7,8]. BOX 2 summarizes the different options for fertility preservation in patients undergoing cancer treatment.

Options for females

For female patients in their reproductive age period, age is the single most important determining factor (on the patient's side) of the outcome of fertility preservation after cancer treatment. Other important factors include cancer diagnosis and type of treatment, as well as the time available and the potential that cancer has metastasized to her ovaries. Another crucial factor is whether the patient has a male partner, with the hope that embryos can be produced by IVF and then cryopreserved. Obviously when that option is pursued, the two most important questions that arise are whether IVF treatment has a negative effect on the chance of cure by cancer treatment and whether such cancer treatment can be postponed until IVF treatment and embryo cryopreservation has been carried out. In their most recent recommendation on the fertility preservation options for cancer patients, the American Society of Clinical Oncology (ASCO) [8] and the American Society for Reproductive Medicine [7] concluded that the currently available options for fertility preservation in the female include one established technology (embryo cryopreservation) and two experimental ones (cryopreservation of oocytes and ovarian tissues) and that the choice of the most suitable strategy for preserving fertility depends on different parameters: the type and timing of chemotherapy, type of cancer, patient's age and partner status.

Cryopreservation of embryos

According to the Practice Committee of the American Society for Reproductive Medicine [7], the only established method of fertility preservation is embryo cryopreservation. Embryo cryopreservation is the most successful approach to fertility preservation, with delivery rates per embryo transfer using cryopreserved embryos reported to be in the range of approximately 10–40% per frozen embryo. The success depends on several factors, including the woman's age and presence of infertility factors, as well as the number, quality and stage of development at which the embryos were frozen [46,47]. However, this option requires the patient to be of postpubertal age, have a partner or use donor sperm and be able to undergo a cycle of ovarian stimulation that usually takes approximately 2–3 weeks. When the chemotherapy has to be initiated immediately or when stimulation is contraindicated, the option of embryo cryopreservation may not be feasible.

In patients with estrogen-sensitive cancers (e.g., breast cancer), stimulating the development of multiple follicles (controlled ovarian hyperstimulation) for IVF is associated with high levels of estrogen, which should be avoided. Interestingly, aromatase inhibitors have recently been reported to be successful in ovarian stimulation. Aromatase inhibitors reduce estrogen production through blockade of the aromatase enzyme. Ovarian stimulation with aromatase inhibitors has been found to be associated with significantly lower estrogen production per growing follicle than other ovarian stimulation agents, such as clomiphene citrate and gonadotropins [48–50]. Those agents are particularly promising in light of the recent safety data regarding the outcome of pregnancies achieved after their use in infertile women [51]. Oktay and colleagues compared the combination of tamoxifen or letrozole with follicle-stimulating hormone (FSH) for stimulation in women with breast cancer, with very promising results [52]. In a more recent study, Oktay and colleagues reported that ovarian stimulation with letrozole and gonadotropins was a cost-effective alternative for fertility preservation in breast cancer patients [53]. Such an approach was found to be associated with reduced estrogen exposure compared with standard IVF.

Cryopreservation of oocytes

While embryo cryopreservation is an established technique, oocyte cryopreservation is still an experimental option for female fertility preservation. Oocyte cryopreservation is an attractive strategy as it does not require surgery to harvest ovarian tissue and well-tested stimulation protocols for IVF can be used [7]. Oocyte cryopreservation is a particularly viable option for fertility preservation in patients for whom a partner is unavailable or who have religious or ethical objections to embryo cryopreservation. The oocytes can be thawed later and fertilized *in vitro*. Ovarian stimulation and harvesting requirements are identical to those applied during the IVF treatment cycle for embryo cryopreservation. For this reason, oocyte cryopreservation is associated with similar concerns regarding delays in therapy and potential risks of short-term exposure to high hormonal levels. Further research is needed to delineate the current success rates and safety, as well as to improve the efficiency of this procedure before establishing recommendations on the technique. ASCO recommends that oocyte cryopreservation

Box 2. Options for fertility preservation in patients undergoing cancer treatment.

Options in female patients

- Embryo cryopreservation (effective with proven success)
- Oocyte cryopreservation (experimental with recent promising results)
- Ovarian tissue cryopreservation (experimental with recent promising results)

Options in male patients

- Sperm cryopreservation (effective with proven success)
- Testicular tissue cryopreservation and *in vitro* spermatogenesis (experimental)

should only be performed in centers with the necessary expertise, with patients participating in institutional review board (IRB)-approved protocols [8].

Currently, the effectiveness of this technique seems to be very low, with pregnancy and delivery rates ranging from 1 to 5% per frozen oocyte [54,55].

The very low success rate of pregnancy per frozen oocyte (compared with frozen embryo) is due to several problems associated with oocyte cryopreservation compared with cryopreservation of sperms or embryos. The mature oocyte (metaphase II oocyte) is a very large cell that is surrounded by an important protective layer called the zona pellucida. The metaphase II oocyte is extremely fragile owing to its large size, high water content and chromosomal arrangement. In the mature oocyte, the metaphase chromosomes are lined up by the meiotic spindle along the equatorial plate. The spindle apparatus is documented to be very easily damaged by intracellular ice formation during the freezing or thawing process [56,57]. This is particularly true with the high water content of the oocyte. Thus, cooling and exposure to cryoprotective agents affect the cytoskeleton and might aggravate the already high incidence of aneuploidy in human oocytes [58]. In addition, hardening of the zona pellucida can adversely affect the normal fertilization process and the need to fertilize cryopreserved oocytes with intracytoplasmic sperm injection (ICSI) [59].

Cryopreservation of ovarian tissue

Cryopreservation of ovarian tissue is another experimental technique for fertility preservation. This is the only option available for prepubertal girls and for woman who cannot delay cancer treatment to receive ovarian stimulation and harvest oocytes. In addition, theoretically, cryopreservation of ovarian tissue is the only experimental technology to reverse menopause.

There are three different techniques that have been reported for freezing ovarian tissue as fragments of the ovarian cortex, the entire ovary with its vascular pedicle or as isolated follicles [60–61]. Most data in the literature involve the first option (ovarian cortical cryopreservation). However, there are emerging promising results and improvements in the technique of the second approach (whole-ovary cryopreservation) [62–64].

This promising method of preserving fertility relies on the idea of cryopreserving ovarian cortical tissue. 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 [65].

The follicular viability after cryopreservation and thawing of ovarian tissue has been demonstrated in several studies with most of the follicles that survived cryopreservation being primordial in type [66,67]. It has been suggested that maturing those primordial follicles and obtaining mature oocytes from them can be performed by three options. The first option is transferring the ovarian tissue back to the patient (autografting). Autografting can be done by placing the ovarian tissue back to its original, normal place in the pelvis (orthotopic autografting). This method has yielded the first pregnancies after cryopreservation of ovarian tissue [68–70]. Autografting can also be performed by placing the ovarian tissue back into the patient at a site away from the pelvis, such as the subcutaneous tissue of the patient's forearm (heterotopic autografting) [71]. Obviously hetertopic autografting requires the use of IVF to achieve a pregnancy.

The second option is *in vitro* follicular maturation and IVF. This method has already yielded pregnancies in animal experiments [72]. However, this method is very difficult to apply in humans because of the long period necessary for the primordial follicle to reach the maturation stage, which is believed to be approximately 3 months [73].

The third method involves transplanting the human ovarian tissue into immunodeficient animals, such as mice with severe combined immune deficiency (xenografting), followed by stimulating the animal to full follicular maturation [3,74,75]. This method has the major advantages of avoiding the risk of reimplantation of the primary tumor back to the patient with autotransplantation and of exposing the animal instead of the patient to ovarian stimulation. However, there remain the major problems of the long period needed to grow human follicles to maturity and the theoretical risk of transferring infection from the animal to the patient, particularly the risk of transspecies retroviral infection.

There are important risks associated with ovarian tissue cryopreservation, including reimplantation of the primary tumor and malignant transformation [76]. Shaw and colleagues were the first to report the transmission of lymphoma from a donor to a graft recipient with fresh and cryopreserved mouse ovarian tissue samples [77]. However, it is important to note that most cancers encountered during the reproductive years in humans tend not to metastasize to the ovaries, with the exceptions of bloodborne malignancies, such as leukemias, neuroblastoma and Burkitt's lymphoma [76]. In addition, a recent study on the risk of ovarian tissue cryopreservation in patients with lymphoma found no evidence of disease contamination in ovarian tissue harvested for cryopreservation from patients with Hodgkin's lymphoma [78]. Another important risk in patients undergoing ovarian cryopreservation for carrying the mutations of *BRCA1* and *2* is transmission of the mutations to the offspring. To reduce the risk of retransplanting cancer back to the patient, a histological assessment for micrometastases should always be carried out on a small portion of the harvested tissue before cryopreservation. Evidence for the risk of malignant transformation of the cryopreserved tissue after transplantation comes from experiments in rats, in which heterotopic autotransplantation of cryopreserved ovarian tissue into the spleen resulted in the development of sex cord stromal tumors [79]. Currently, there are no reports of cancer recurrence after ovarian transplantation, although fewer than 20 procedures have been reported thus far. Similar to oocyte cryopreservation, ovarian cryopreservation and transplantation procedures should only be performed in centers with the necessary expertise under IRB-approved protocols that include follow-up for recurrent cancer [8].

Options for males

The two main options for fertility preservation in males include cryopreservation of the sperm and testicular tissue. Contrary to females, the option of fertility preservation by sperm cryopreservation is much more successful than oocyte or even embryo cryopreservation. Moreover, obtaining sperm is much less inconvenient, less time consuming and less expensive when compared with obtaining oocytes or embryos. However, this option is obviously only available to postpubertal male patients, while for prepubertal boys the only available option is testicular cryopreservation. Before the era of assisted reproductive technology and ICSI, sperm quality (count, motility and morphology) were very important factors in determining the success of fertility preservation and achievement of pregnancy following cancer treatment. Currently, even very poor quality preserved sperm samples can result in promising outcome (achievement of pregnancy and live birth) when ICSI is applied.

Recently a scientific panel from ASCO reviewed the available information supporting sperm and testicular tissue cryopreservation. The panel concluded that the available evidence suggested that sperm cryopreservation is an effective method of fertility preservation in males treated for cancer. On the other hand, testicular tissue or spermatogonial cryopreservation and transplantation or testis xenografting are in the early phases of experimentation and have not yet been successfully tested in humans. The panel further noted that available interventions are unlikely to delay the initiation of cancer treatment once a patient is successfully referred [7].

Cryopreservation of human sperm

Before discussing the available options for fertility preservation in the male further, there is an important issue to be addressed. This issue is the significant underutilization of fertility preservation options for males who are affected by cancer. Epidemiological studies confirm that most young male patients with cancer are not referred for sperm banking [80–82]. In most cases, physicians may not discuss or emphasize the available opportunities for preserving fertility before cancer treatment [83].

There are several reasons for this apparent underutilization, including the psychological and logistic factors, as well as financial constraints on patients that may further limit sperm banking [8]. This situation is encountered even more with other approaches of fertility preservation, particularly ovarian cryopreservation. Regarding the psychological factors, while men may be traumatized regarding their diagnosis or show a lack of interest in fertility preservation at the time of diagnosis, two recent surveys suggested that for men who desire children in the future, lack of timely information is the most common reason for not banking sperm [84,85]. With regards to the financial constraints, unfortunately most insurance companies in the USA do not cover sperm cryopreservation. Even when the service is partially covered, as in the UK, where the national health system subsidizes sperm banking for young cancer patients, many young men are not given referrals [86]. Moreover, when sperm is banked, most studies suggest that only a minority (up to 30%, but <10% in most cohorts) of men return to use their stored specimens [87–89].

Semen cryopreservation before chemotherapy, radiotherapy or surgery affecting the male reproductive system is a widely available and inexpensive option [90]. Traditionally, the clinical recommendations include the banking of at least three semen samples, each collected following an abstinence period of at least 48 h. The collection period can be completed in approximately 1 week. However, more samples and longer abstinence periods (3–4 days), to achieve higher total sperm concentration, are recommended when circumstances allow [90].

It is strongly recommended to start collecting sperm before the initiation of cancer therapy because the quality of the sample and sperm DNA integrity may be compromised, even after a single treatment session [82,91]. However, many patients may have to start chemotherapy immediately or soon enough to limit the number of semen samples that can be collected. In these instances, it is reasonable to make every effort to bank sperm even after starting treatment [83], since recent progress in andrology laboratories and the use of assisted reproductive techniques, particularly the technique of ICSI, allows the successful freezing and future use of a very limited amount of sperm. In support of this recommendation, several case reports and small case series have reported the success of collecting sperm from a postmasturbation urine sample, rectal electroejaculation under anesthesia [92,93] and testicular sperm aspiration [94].

Sperm cryopreservation in boys and young patients involves additional considerations as boys start forming sperm (spermarche) at approximately 13–14 years. Interestingly, once sperm are present, the patient's age does not seem to significantly affect the quality of sperm produced [95]. However, in young patients, collection of semen through masturbation may be compromised by embarrassment and issues of informed consent, with data suggesting that adolescent boys may be more successful in collecting samples if a parent does not accompany them to the sperm bank [96].

There are various methods for semen collection, including masturbation, stimulated ejaculation and even sperm aspiration from the epididymis and/or testicles [97]. Sperm aspiration, penile vibratory stimulation and electroejaculation are viable options for younger adolescents or patients not capable of ejaculation [98].

In cases of nonheritable cancer syndromes, there is no evidence that a prior history of cancer increases the rate of congenital abnormalities or cancer in a man's offspring [99]. However, recent studies suggested that cryopreserved sperm of untreated men with cancer may have poor DNA integrity even before treatment [100], with transiently higher rates of aneuploidy after chemotherapy and radiotherapy [101], although DNA integrity of sperm seemed similar to age-matched controls in one cohort of pediatric cancer survivors [102]. For this reason, men should be advised of a possible, not yet quantifiable, higher risk of genetic damage in sperm stored after diagnosis of cancer or initiation of cancer therapy. It is important to mention here that such risks are not known for noncancer populations [8].

Cryopreservation of testicular tissue & *in vitro* spermatogenesis

This option is available for prepupertal patients. However, the technology is still experimental. The prepubertal testis does not produce mature spermatozoa. However, it contains the diploid stem germ cells from which the haploid spermatozoa will ultimately be derived. The testicular tissue can be harvested before chemotherapy and cryopreserved [81], then, when the patient is cured, the tissue can be thawed and the stored germ cells reimplanted into the patient's own testes. Theoretically, the germ cells would give rise to complete and normal spermatogenesis in the seminiferous tubules, a procedure known as germ cell transplantation [103].

Alternatively, as with ovarian tissue cryopreservation discussed previously, the harvested testicular tissue pieces could be grafted to an ectopic site (e.g., subcutaneous tissue) in cancer survivors or in immunodeficient animals (xenografting). The grafted testicular tissue can be revascularized in the ectopic site and produce sperm [104]. Also, successful stem cell transplantation has been reported in many species, including mice [105], rats, monkeys and humans [106].

As with ovarian tissue cryopreservation, the most important risk associated with autotransplantation of cryopreserved testicular tissue is that of reintroducing malignant cells after retransplantation. The risk is greater with hematological cancers, as the testes can act as sanctuary sites for leukemic cells [107]. To avoid such a risk, the technique of *in vitro* maturation of stem cells has been suggested. The stored cells could be matured *in vitro* and fertilization can be achieved by the use of ICSI. Although the technique of testicular germ cell harvesting, cryopreservation and transplantation are effective in mice [108], there are considerable differences in human spermatogenesis. Alternatively, maturation of the later stages of spermatogenesis rather than stem cells, *in vitro* maturation of diploid stem cells into haploid spermatozoa, has been suggested. However, the technique is still at the very beginning of its development and it appears unlikely to become technically possible in the near future [109,110].

Recently, Kvist and colleagues found that intact testicular tissue from young boys with nondescended testes tolerated cryopreservation with preservation of the ability to produce testis-specific hormones *in vitro*. These nondescended testes are surgically removed because of the increased risk of developing malignant tumors. Those results encourage the approach of preserving the removed testicular tissue for fertility preservation in adult life [111]. More recently, reports have presented encouraging data on the success of cryopreservation of the tesicular tissues. Prepubertal testicular tissue from boys was successfully cryopreserved by slow, programmed freezing with dimethyl sulfoxide as a cryoprotectant. Keros and colleagues found this slow, programmed freezing efficient in maintaining the spermatogonia, Sertoli cells and stromal compartment during freezing, thawing and tissue culture [112]. Orthotopic xenografting was demonstrated by Wyns and colleagues to be associated with the survival and presence of proliferative activity of spermatogonia and Sertoli cells from cryopreserved immature human cryptorchid tissue [113].

Conclusion

The two established technologies of cryopreservation of embryos and sperm, as well as the emerging methods, including cryopreservation of oocytes, ovarian tissues and testicular tissue, provide hope to increasing numbers of cancer survivors in their reproductive age period. While embryo and sperm cryopreservation are considered accepted standard clinical practices, other fertility-preservation technologies should be offered within IRB-approved clinical protocols after thorough counseling regarding the limited data, particularly on long-term outcomes. As a golden rule, patients safety regarding achievement of the best chance of cure from cancer treatment should come first, ahead of any other consideration for fertility preservation. This is to avoid reducing the chance of the success of cancer treatment by any delays or exposure to fertility medications for the sake of fertility preservation. Modern practice of clinical oncology should include a collaborative bridge with reproductive endocrinologists to provide cancer patients with adequate counseling regarding options of preserving their fertility.

Expert commentary

There are still unmet needs for providing adequate reproductive care for patients undergoing cancer treatment during their childhood and reproductive period. Several factors could explain the deficiency in providing such reproductive care, including a lack of adequate patient counseling, failure to arrange timely consultation with a reproductive endocrinologist and financial constraints. It is unfair for patients who are candidates for benefit from the established technologies of sperm and embryo cryopreservation to forfeit their chances of fertility preservation. Even though the practices of cryopreservation of oocytes, ovarian and testicular tissue remain experimental and should be performed within IRB-approved protocols, these options should be discussed with the patients, particularly when causing no delays in cancer treatment. Bridging the gap between oncologists and reproductive endocrinologists by creating a hot referral line, as well as adequate health education of the community and better understanding and support on the side of the authorities providing financial care for the health system, should all help in providing more satisfactory reproductive care for cancer survivors.

Five-year view

The fast-growing reproductive technologies, along with an increased awareness of the fertility preservation options avaliable, will both improve the availability, utilization and success of providing adequate reproductive care for cancer survivors. I expect the recent legislations and restrictions imposed on the practice of assisted reproduction in Europe, and other places in the world, regarding restricting the number of oocytes that can be fertilized during IVF, as well as the restrictions on embryo freezing, to lead to a vast, growing advancement in the technology of oocyte cryopreservation. This will inevitably help to enhance the success of that technology and make it readily available for cancer survivors. The emerging success of modest

ovarian stimulation protocols needed for IVF and for harvesting multiple oocytes, for example applying aromatase inhibitors, can help to reduce the risk of exposure to high hormonal levels. In addition, the low cost of those oral medications (aromatase inhibitors) should also help in reducing the overall cost of reproductive technologies for cancer patients. However, those agents are still awaiting more research for their approval for the indication of ovarian stimulation. The approaches of spermatogonial stem cell transplantation and ovarian cryopreservation with *in vitro* maturation of primordial follicles are exciting approaches. However, these technologies are still in their infancy of development, with several obstacles remaining. It may take more than 5 years before we see those technologies coming close to clinical application.

Key issues

- The success of treatment of cancers that affect individuals during their reproductive period and childhood leads to a growing number of patients seeking reproductive care, including fertility preservation.
- There is still a wide gap between oncologists and reproductive endocrinologists concerning which patients might benefit from fertility preservation. This results in an increasing number of cancer patients who miss the chance of preserving their fertility.
- Cancer cure should be the ultimate primary goal of cancer treatment, while fertility preservation should come next. However, no effort should be spared in trying to preserve reproductive functions by adopting conservative treatment approaches that do not jeopardize the chance of cure from cancer.
- The two main strategies to preserve reproductive functions in cancer patients include approaches to reduce the reproductive damage caused by cancer treatment and fertility preservation by cryopreservation of embryos, gametes and gonadal tissues.
- With regards to the strategy of reducing reproductive damage caused by cancer treatment, conservative surgery in selected candidates is an emerging interesting concept that is more widely accepted, with data accumulating on long-term safety. On the other hand, while ovarian surgical mobilization and testicular shielding away from the field of radiotherapy have proven effective, gonadal suppression with gonadotropin-releasing hormone analogues and sex hormones are unlikely to be of benefit for male patients, although they may be of some help for female patients.
- With regards to the strategy of fertility preservation, the technologies of embryo and sperm cryopreservation are well established, with satisfactory data on their success and long-term safety. On the other hand, the practice of cryopreservation of oocytes, ovarian and testicular tissues is still experimental and should be offered to patients within institutional review board-approved clinical protocols.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Mitwally MFM. Effect of cancer and cancer treatment on human reproduction. *Expert Rev. Anticancer Ther.* 7(6) 811–822 (2007).

•• **First of a three-article series reviewing the effect of cancer and its treatment on human reproduction. This review outlines how cancer and its treatment can negatively affect reproduction both in males and females and the various mechanisms involved in such a negative impact.**

2 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2006. *CA Cancer J. Clin.* 56, 106–130 (2006).

•• **Updated information on the statistics and epidemiology of different cancer diagnoses.**

3 Kim SS, Soules MR, Battaglia DE. Follicular development, ovulation, and corpus luteum formation in cryopreserved human ovarian tissue after xenotransplantation. *Fertil. Steril.* 78, 77–82 (2002).

4 Blatt J. Pregnancy outcome in long-term survivors of childhood cancer. *Med. Pediatr. Oncol.* 33, 29–33 (1999).

5 Mattle V, Behringer K, Engert A, Wildt L. Female fertility after cytotoxic therapy – protection of ovarian function during chemotherapy of malignant and non-malignant diseases. *Eur. J. Haematol.* 75, 77–82 (2005).

• **Focuses on the fertility problems in women undergoing cytotoxic therapy for both cancer and noncancer indications, providing available options to minimize the negative effects of chemotherapy on fertility.**

6 Donnez J, Bassil S. Indications for cryopreservation of ovarian tissue. *Hum. Reprod. Update* 4, 248–259 (1998).

• **Comprehensive review of the various indications (malignancy and nonmalignancy) for ovarian tissue cryopreservation. The list of indications remains valid and up to date.**

7 Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Ovarian tissue and oocyte cryopreservation. *Fertil. Steril.* 86(5 Suppl.), S142–S147 (2006).

•• **Most recent recommendations of the American Society for Reproductive Medicine regarding the practice of fertility preservation in women undergoing cancer treatment, as well as other potential applications. The society stresses the still experimental nature of the practice of oocyte and ovarian tissue cryopreservation.**

8 Lee SJ, Schover LR, Partridge AH *et al.* American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J. Clin. Oncol.* 24(18), 2917–2931 (2006).

•• **Provides accurate and updated information of major help in counseling cancer patients on their options regarding fertility preservation. Also discusses the known demands and unmet needs in this area.**

9 Dargent D, Brun JL, Remy I. Pregnancies following radical trachelectomy for invasive cervical cancer. *Gynecol. Oncol.* 52,

105–108 (1994).

•• **First report in the literature on conservative surgery for preserving the uterine body for victims of early-stage cervical cancer interested in preserving fertility.**

10 Grant P. Radical trachelectomy. *Aust. N. Z. J. Obstet. Gynaecol.* 46(5), 372–374 (2006).

11 Gershenson DM. Fertility-sparing surgery for malignancies in women. *J. Natl Cancer Inst. Monogr.* 34, 43–47 (2005).

• **Comprehensive review of the careful selection of cancer patients suitable for conservative surgery to preserve their fertility.**

12 Kay TA, Renninson JN, Shepherd JH, Taylor MJ. Successful pregnancy following radical trachelectomy and *in vitro* fertilisation with ovum donation. *Br. J. Obstet. Gynecol.* 113, 965–966 (2006).

13 Hertel H, Kohler C, Grund D *et al.* German Association of Gynecologic Oncologists (AGO). Radical vaginal trachelectomy (RVT) combined with laparoscopic pelvic lymphadenectomy: prospective multicenter study of 100 patients with early cervical cancer. *Gynecol. Oncol.* 103, 506–511 (2006).

14 Plante M, Lau S, Brydon L, Swenerton K, LeBlanc R, Roy M. Neoadjuvant chemotherapy followed by vaginal radical trachelectomy in bulky stage IB1 cervical cancer: case report. *Gynecol. Oncol.* 101, 367–370 (2006).

15 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.* 75, 590–600 (1958).

• **First report on surgical mobilization of the ovaries away from the field of radiation to reduce the damaging effects of radiation on their function.**

16 Morice P, Thiam-Ba R, Castaigne D *et al.* Fertility results after ovarian transposition for pelvic malignancies treated by external irradiation or brachytherapy. *Hum. Reprod.* 13, 660–663 (1998).

17 Bisharah M, Tulandi T. Laparoscopic preservation of ovarian function: an underused procedure. *Am. J. Obstet. Gynecol.* 188, 367–370 (2003).

18 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.* 14(Suppl.), 77–80 (1993).

19 Scott SM, Schlaff W. Laparoscopic medial oophoropexy prior to radiation therapy in an adolescent with Hodgkin's disease. *J. Pediatr. Adolesc. Gynecol.* 8(5), 355–357 (2005).

20 Martin JR, Kodaman P, Oktay K, Taylor HS. Ovarian cryopreservation with transposition of a contralateral ovary: a combined approach for fertility preservation in women receiving pelvic radiation. *Fertil. Steril.* 87(1), 189.e5–e7 (2007).

21 Morice P, Castaigne D, Haie-Meder C *et al.* Laparoscopic ovarian transposition for pelvic malignancies: indications and functional outcomes. *Fertil. Steril.* 70, 956–960 (1998).

22 Sonmezer M, Oktay K. Fertility preservation in female patients. *Hum. Reprod. Update.* 10, 251–266 (2004).

23 Morice P, Juncker L, Rey A *et al.* Ovarian transposition for patients with cervical carcinoma treated by radiosurgical combination, *Fertil. Steril.* 74, 743–748 (2000).

24 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.* 187, 93–105 (2002).

25 Pereyra Pacheco B, Mendez Ribas JM, Milone G *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.* 81, 391–397 (2001).

26 Waxman JH, Ahmed R, Smith D *et al.* Failure to preserve fertility in patients with Hodgkin's disease. *Cancer Chemother. Pharmacol.* 19, 159–162 (1987).

27 Emons G, Grundker C, Gunthert AR *et al.* GnRH antagonist in the treatment of gynecological and breast cancers. *Endocr. Relat. Cancer* 10, 291–299 (2003).

28 Mullen P, Scott WN, Miller WR. Growth inhibition observed following administration of an LHRH agonist to a clonal variant of the MCF-7 breast cancer cell line is accompanied by an accumulation of cells in the G0/G1 phase of the cell cycle. *Br. J. Cancer* 63, 930–932 (1991).

29 Jonat W. The role of LHRH analogs in premenopausal breast cancer. In: *GnRH Analogues: the State of the Art 2001. A Summary of the 6th International Symposium on GnRH Analogues in Cancer and Human Reproduction.* Lunenfeld B (Ed.). Parthenon Publishing Group, NY, USA (2002).

30 Behringer K, Breuer K, Reineke T *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.* 23, 7555–7564 (2005).

31 Castelo-Branco C, Nomdedeu B, Camus A, Mercadal S, Martinez de Osaba MJ, Balasch J. Use of gonadotropin-releasing hormone agonists in patients with Hodgkin's disease for preservation of ovarian function and reduction of gonadotoxicity related to chemotherapy. *Fertil. Steril.* 87(3), 702–705 (2007).

32 Meirow D, Assad G, Dor J, Rabinovici J. The GnRH antagonist cetrorelix reduces cyclophosphamide-induced ovarian follicular destruction in mice. *Hum. Reprod.* 19(6), 1294–1299 (2004).

33 Danforth DR, Arbogast LK, Friedman CI. Acute depletion of murine primordial follicle reserve by gonadotropin-releasing hormone antagonists. *Fertil. Steril.* 83(5), 1333–1338 (2005).

34 Chapman RM, Sutcliffe SB. Protection of ovarian function by oral contraceptives in women receiving chemotherapy for Hodgkin's disease. *Blood* 58, 849–851 (1981).

35 Whitehead E, Shalet SM, Blackledge G *et al.* The effect of combination chemotherapy on ovarian function in women treated for Hodgkin's disease. *Cancer* 52, 988–993 (1983).

36 Montz FJ, Wolff AJ, Gambone JC. Gonadal protection and fecundity rates in cyclophosphamide-treated rats. *Cancer Res.* 51, 2124–2126 (1991).

37 Familiari G, Caggiati A, Nottola SA. Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. *Hum. Reprod.* 8, 2080–2087 (1993).

38 Tilly JL. Molecular and genetic basis of normal and toxicant-induced apoptosis in female germ cells. *Toxicol. Lett.* 102, 497–501 (1998).

39 Tilly JL. Apoptosis and ovarian function. *Rev. Reprod.* 1, 162–172 (1996).

40 Morita Y, Tilly JL. Oocyte apoptosis: like sand through an hourglass. *Dev. Biol.* 213, 1–17 (1999).

41 Johnson DH, Linde R, Hainsworth JD *et al.* Effect of a luteinizing hormone releasing hormone agonist given during combination chemotherapy on posttherapy fertility in male patients with lymphoma: preliminary observations. *Blood* 65, 832–836 (1985).

42 Brennemann W, Brensing KA, Leipner N *et al.* Attempted protection of

spermatogenesis from irradiation in patients with seminoma by D-tryptophan-6 luteinizing hormone releasing hormone. *Clin. Investig.* 72, 838–842 (1994).

43 Kreuser ED, Hetzel WD, Hautmann R *et al.* Reproductive toxicity with and without LHRHA administration during adjuvant chemotherapy in patients with germ cell tumors. *Horm. Metab. Res.* 22, 494–498 (1990).

44 Dafopoulos K, Griesinger G, Schultze-Mosgau A *et al.* Cumulative pregnancy rate after ICSI with cryopreserved testicular tissue in non-obstructive azoospermia. *Reprod. Biomed. Online* 10, 461–466 (2005).

45 Brook PF, Radford JA, Shalet SM *et al.* Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. *Fertil. Steril.* 75, 269–274 (2001).

46 Geary S, Moon YS. The human embryo *in vitro*: recent progress. *J. Reprod. Med.* 51(4), 293–302 (2006).

47 Michelmann HW, Nayudu P. Cryopreservation of human embryos. *Cell Tissue Bank.* 7(2), 135–141 (2006).

48 Mitwally MFM, Casper RF. Aromatase inhibition: a novel method of ovulation induction in women with polycystic ovarian syndrome. *Reprod. Technol.* 10, 244–247 (2000).

49 Mitwally MF, Casper RF. Aromatase inhibition reduces gonadotrophin dose required for controlled ovarian stimulation in women with unexplained infertility. *Hum. Reprod.* 188, 1588–1597 (2003).

50 Mitwally MF, Casper RF. Aromatase inhibition reduces the dose of gonadotropin required for controlled ovarian hyperstimulation. *J. Soc. Gynecol. Investig.* 11(6), 406–415 (2004).

51 Tulandi T, Martin J, Al-Fadhli R *et al.* Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil. Steril.* 85, 1761–1765 (2006).

52 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.* 23, 4347–4353 (2005).

53 Oktay K, Hourvitz A, Sahin G *et al.* Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *J. Clin. Endocrinol. Metab.* 91, 3885–3890 (2006).

54 Levi Setti PE, Albani E, Novara PV, Cesana A, Morreale G. Cryopreservation of supernumerary oocytes in IVF/ICSI cycles. *Hum. Reprod.* 21, 370–375 (2006).

55 Borini A, Sciajno R, Bianchi V, Sereni E, Flamigni C, Coticchio G. Clinical outcome of oocyte cryopreservation after slow cooling with a protocol utilizing a high sucrose concentration. *Hum. Reprod.* 21, 512–517 (2006).

56 Shaw JM, Oranratnachai A, Trounson AO. Fundamental cryobiology of mammalian oocytes and ovarian tissue. *Theriogenology* 53, 59–72 (2000).

57 Baka SG, Toth TL, Veeck LL, Jones HW Jr, Muasher SJ, Lanzendorf SE. Evaluation of the spindle apparatus of *in-vitro* matured human oocytes following cryopreservation. *Hum. Reprod.* 10, 1816–1820 (1995).

58 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.* 54, 102–108 (1990).

59 Matson PL, Graefling J, Junk SM, Yovich JL, Edirisinghe WR. Cryopreservation of oocytes and embryos: use of a mouse model to investigate effects upon zona hardness and formulate treatment strategies in an *in-vitro* fertilization programme. *Hum. Reprod.* 12, 1550–1553 (1997).

60 Falcone T, Bedaiwy MA. Fertility preservation and pregnancy outcome after malignancy. *Curr. Opin. Obstet. Gynecol.* 17(1), 21–26 (2005).

61 Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonckt A, Demylle D, Dolmans MM. Ovarian tissue cryopreservation and transplantation: a review. *Hum. Reprod. Update* 2(5), 519–535 (2006).

62 Jeremias E, Bedaiwy MA, Gurunluoglu R, Biscotti CV, Siemionow M, Falcone T. Heterotopic autotransplantation of the ovary with microvascular anastomosis: a novel surgical technique. *Fertil. Steril.* 77(6), 1278–1282 (2002).

63 Bedaiwy MA, Falcone T. Ovarian tissue banking for cancer patients: reduction of post-transplantation ischaemic injury: intact ovary freezing and transplantation. *Hum. Reprod.* 19(6), 1242–1244 (2004).

64 Bedaiwy MA, Hussein MR, Biscotti C, Falcone T. Cryopreservation of intact human ovary with its vascular pedicle. *Hum. Reprod.* 21(12), 3258–3269 (2006).

65 Oktay K, Newton H, Aubard Y, Salha O, Gosden RG. Cryopreservation of immature human oocytes and ovarian tissue: an emerging technology? *Fertil. Steril.* 69, 1–7 (1998).

66 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.* 32, 273–279 (2006).

67 Newton H, Aubard Y, Rutherford A, Sharma V, Gosden R. Low temperature storage and grafting of human ovarian tissue. *Hum. Reprod.* 11, 1487–1491 (1996).

68 Donnez J, Dolmans MM, Demylle D *et al.* Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 364, 1405–1410 (2004).

69 Meirow D, Levron J, Eldar-Geva T *et al.* Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N. Engl. J. Med.* 353, 318–321 (2005).

70 Oktay K. Spontaneous conceptions and live birth after heterotopic ovarian transplantation: is there a germline stem cell connection? *Hum. Reprod.* 21(6), 1345–1348 (2006).

71 Oktay K, Buyuk E, Veeck L *et al.* Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. *Lancet* 363, 837–840 (2004).

72 Eppig JJ, O'Brien MJ. Development *in vitro* of mouse oocytes from primordial follicles. *Biol. Reprod.* 54, 197–207 (1996).

73 Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. *Hum. Reprod.* 1, 81–87 (1986).

74 Weissman A, Gotlieb L, Colgan T, Jurisicova A, Greenblatt EM, Casper RF. Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. *Biol. Reprod.* 60(6), 1462–1467 (1999).

75 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* 20, 11–16 (2006).

76 Sonmezer M, Shamonki MI, Oktay K. Ovarian tissue cryopreservation: benefits and risks. *Cell Tissue Res.* 322, 125–132 (2005).

77 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.* 11, 1668–1673 (1996).

78 Seshadri T, Gook D, Lade S *et al.* Lack of evidence of disease contamination in ovarian tissue harvested for cryopreservation from patients with Hodgkin lymphoma and analysis of factors predictive of oocyte yield. *Br. J. Cancer* 10, 1007–1010 (2006).

79 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.* 25, 4107–4111 (2005).

80 Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? *Lancet Oncol.* 6, 209–218 (2005).

81 Chung K, Irani J, Knee G *et al.* Sperm cryopreservation for male patients with cancer: an epidemiological analysis at the University of Pennsylvania. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 113(Suppl. 1) S7–S11 (2004)

82 Lass A, Akagbosu F, Abusheikha N *et al.* A programme of semen cryopreservation for patients with malignant disease in a tertiary infertility centre: lessons from 8 years' experience. *Hum. Reprod.* 13, 3256–3261 (1998).

83 Foley SJ, De Winter P, McFarlane JP *et al.* Storage of sperm and embryos. Cryopreservation of sperm should be offered to men with testicular cancer. *Br. Med. J.* 313, 1078 (1996).

84 Schover LR, Brey K, Lichtin A *et al.* Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors. *J. Clin. Oncol.* 20, 1880–1889 (2002).

85 Schover LR, Rybicki LA, Martin BA *et al.* Having children after cancer: a pilot survey of survivors' attitudes and experiences. *Cancer* 86, 697–709 (1999).

86 Glaser AW, Phelan L, Crawshaw M *et al.* Fertility preservation in adolescent males with cancer in the United Kingdom: a survey of practice. *Arch. Dis. Child.* 89, 736–737 (2004).

87 Agarwal A, Ranganathan P, Kattal N *et al.* Fertility after cancer: a prospective review of assisted reproductive outcome with banked semen specimens. *Fertil. Steril.* 81, 342–348 (2004).

88 Audrins P, Holden CA, McLachlan RI *et al.* Semen storage for special purposes at Monash IVF from 1977 to 1997. *Fertil. Steril.* 72, 179–181 (1999).

89 Blackhall FH, Atkinson AD, Maaya MB *et al.* Semen cryopreservation, utilisation and reproductive outcome in men treated for Hodgkin's disease. *Br. J. Cancer* 87, 381–384 (2002).

90 Sanger WG, Olson JH, Sherman JK. Semen cryobanking for men with cancer – criteria change. *Fertil. Steril.* 58, 1024–1027 (1992).

91 Shin D, Lo KC, Lipshultz LI. Treatment options for the infertile male with cancer. *J. Natl Cancer Inst. Monogr.* 34, 48–50 (2005).

92 Bennett CJ, Seager SW, McGuire EJ. Electroejaculation for recovery of semen after retroperitoneal lymph node dissection: case report. *J. Urol.* 137, 513–515 (1987).

93 Hultling C, Rosenlund B, Tornblom M *et al.* Transrectal electroejaculation in combination with *in-vitro* fertilization: an effective treatment of anejaculatory infertility after testicular cancer. *Hum. Reprod.* 10, 847–850 (1995).

94 Meseguer M, Garrido N, Remohi J *et al.* Testicular sperm extraction (TESE) and ICSI in patients with permanent azoospermia after chemotherapy. *Hum. Reprod.* 18, 1281–1285 (2003).

95 Kliesch S, Behre HM, Jurgens H *et al.* Cryopreservation of semen from adolescent patients with malignancies. *Med. Pediatr. Oncol.* 26, 20–27 (1996).

96 Bahadur G, Ling KL, Hart R *et al.* Semen production in adolescent cancer patients. *Hum. Reprod.* 17, 2654–2656 (2002).

97 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.* 4, 9–15 (2002).

98 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.* 20, 429–430 (1998).

99 Hawkins MM, Draper GJ, Smith RA. Cancer among 1,348 offspring of survivors of childhood cancer. *Int. J. Cancer.* 43, 975–978 (1989).

100 O'Donovan M. An evaluation of chromatin condensation and DNA integrity in the spermatozoa of men with cancer before and after therapy. *Andrologia* 37, 83–90 (2005).

101 Arnon J, Meirow D, Lewis-Roness H *et al.* Genetic and teratogenic effects of cancer treatments on gametes and embryos. *Hum. Reprod. Update* 7, 394–403 (2001).

102 Thomson AB, Campbell AJ, Irvine DC *et al.* Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. *Lancet* 360, 361–367 (2002).

103 Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. *Proc. Natl Acad. Sci. USA* 91, 11298–11302 (1994).

104 Orwig KE, Schlatt S. Cryopreservation and transplantation of spermatogonia and testicular tissue for preservation of male fertility. *J. Natl Cancer Inst. Monogr.* 34, 51–56 (2005).

105 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.* 69, 412–420 (2003).

106 Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF, Nieschlag E. Germ cell transfer into rat, bovine, monkey and human testes. *Hum. Reprod.* 14, 144–150 (1999).

107 Jahnukainen K, Hou M, Petersen C, Setchell B, Soder O. Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia. *Cancer Res.* 61, 706–710 (2001).

108 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.* 19, 948–953 (2004).

109 Tesarik J, Bahceci M, Ozcan C, Greco E, Mendoza C. Restoration of fertility by *in vitro* spermatogenesis. *Lancet* 353, 555–556 (1999).

110 Lee DR, Kim KS, Yang YH *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.* 21, 471–476 (2006).

111 Kvist K, Thorup J, Byskov AG, Hoyer PE, Mollgard K, Yding Andersen C. Cryopreservation of intact testicular tissue from boys with cryptorchidism. *Hum. Reprod.* 21, 484–491 (2006).

112 Keros V, Hultenby K, Borgstrom B, Fridstrom M, Jahnukainen K, Hovatta O. Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. *Hum. Reprod.* 22, 1384–1395 (2007).

113 Wyns C, Curaba M, Martinez-Madrid B, Van Langendonckt A, Francois-Xavier W, Donnez J. Spermatogonial survival after cryopreservation and short-term orthotopic immature human cryptorchid testicular tissue grafting to immunodeficient mice. *Hum. Reprod.* 22, 1603–1611 (2007).

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