Fertility preservation strategies for male patients with cancer

Darren J. Katz, Thomas F. Kolon, Darren R. Feldman and John P. Mulhall

Abstract | With the increasing number of patients surviving cancer, there is increasing interest in long-term quality of life, especially with respect to cancer-related infertility. Although infertility most commonly occurs as the result of treatment with gonadotoxic agents, it can also manifest before treatment has commenced. Current fertility preservation strategies for the postpubertal male patient with cancer focus on sperm cryopreservation before therapy. Sperm acquisition techniques should be discussed with the patient as early as possible, by either an oncologist or a specialist in male reproduction. For patients rendered infertile by cancer treatment who did not cryopreserve sperm beforehand, there are no techniques currently available to restore fertility. For the prepubertal male patient, cryopreservation of sperm is impossible. However, emerging research—primarily in animal models—into promising fertility preservation and restoration strategies might provide a clinical solution in the future. Advances in the protection and cryopreservation of spermatogonial stem cells (SSCs) might translate into clinical options for fertility preservation before treatment. Restoring fertility after treatment might also be possible via SSC autotransplantation or *in vitro* maturation of SSCs. Before any of these techniques become clinically viable, a number of scientific, logistical and ethical issues will need to be resolved.

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Introduction

Fertility preservation is an essential consideration of cancer management. In 2004, a report of the President's Cancer Panel recommended that all patients of reproductive age who are diagnosed with cancer be informed of the possibility of treatment-related infertility and the options for preserving fertility.[1] These sentiments were echoed by the American Society of Clinical Oncology, which recommended that consideration of fertility preservation be included as early as possible during treatment planning.[2] These statements focus on fertility preservation strategies for adults, with less emphasis on adolescents, young adults and children; however, younger patients represent an ever-increasing cohort of cancer survivors, and the potential consequences of cancer treatment on their fertility merits attention.

Approximately 13 million people worldwide are diagnosed each year with cancer.[3] In the USA, approximately 1.7 million people are expected to be diagnosed with cancer in 2013.[4] When all cancers, across patients of all ages, are considered, the 5-year relative survival rate has improved over the past few decades. For patients diagnosed with cancer between 1975 and 1977, the 5-year relative survival rate was 49%, whereas for those diagnosed in 2001–2007, it had increased markedly to 67%. Paediatric patients have experienced the most significant gains in 5-year relative survival, which increased from 58% in 1975–1977 to 83% in 2001–2007. However, there has been no significant improvement in 5-year survival rates since 1975 for the adolescent and young adult age group (15–29 years old).[5] Approximately 1 in 168 young adults in the USA will develop invasive cancer, and there were nearly 12 million cancer survivors in the USA in 2012.[5,6] For this substantial proportion of the population, there has been increasing focus on long-term quality-of-life issues, including fertility.

Cancer treatment that results in infertility is psychologically distressing for many patients[7–9] and those who have undergone fertility preservation might cope better with their cancer management.[10,11] Although infertile cancer survivors can become parents through adoption or gamete donation, most declare a preference for fathering a biological child.[8,12] A study examining young men who were recently diagnosed with cancer found that 51% would like to have children in the future, and this rate increased to 77% for those who were childless at diagnosis.[8] Another study reported that 70% of men with cancer wanted to father a child after chemotherapy.[10] For some patients, maintaining fertility is of such importance that they might choose a less efficacious treatment, as has been documented for some women with breast cancer.[13]

Despite the great importance of fertility preservation to patients with cancer, it is still not routinely discussed in many oncology practices.[8,14] In a survey of 200 young male cancer survivors, most of whom were treated at a dedicated cancer centre, only 51% recalled being offered sperm cryopreservation prior to their cancer treatment.[8] The optimal time for fertility preservation

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Key points

- Infertility related to cancer is a major issue for many cancer survivors and should be discussed as early as possible during treatment planning
- Impairment of fertility related to cancer can manifest before, during or after treatment
- Existing fertility preservation strategies for men focus on acquiring sperm for cryopreservation before therapy; patients rendered infertile by cancer treatments who did not cryopreserve sperm beforehand are unable to father a biological child
- Promising advances in spermatogonial stem cell research might lead to future fertility preservation and restoration options for male patients with cancer
- A number of scientific, logistical and ethical barriers might need to be overcome before investigational fertility preservation strategies can be used clinically, especially for prepubertal patients
- Development of, and adherence to, clinical care pathways, education of oncological health-care providers and involvement of male reproductive specialists should be included in the management of infertility in male patients with cancer

is before the initiation of any oncological therapy that can affect spermatogenesis, so it is essential that fertility management is discussed with all patients with cancer before treatment commences. Practitioners who deliver cancer care should be aware of the relationship between cancer treatment and infertility. Moreover, they need to be cognizant of the options for fertility preservation or at least be able to counsel patients regarding where to access information about fertility preservation.

In this Review we will outline the issues related to infertility for both prepubertal and postpubertal male patients with cancer. The currently available fertility preservation approaches will be reviewed with a subsequent discussion of experimental strategies that are under investigation for fertility preservation both before and after cancer treatment.

Cancer-related male infertility

Evidence suggests that male survivors of cancer are more likely to be infertile than unaffected men. According to a study of over 6,000 men who were diagnosed with cancer before the age of 21 years and had survived for >5 years following diagnosis, patients were found to be 44% less likely to procreate than their healthy siblings.[15] Another study found that only 33% of male survivors of childhood cancer had normal semen parameters.[16] However, this relationship between cancer and infertility is multifactorial. Even before treatment has been received, cancer can affect a patient's fertility potential.

Pretreatment infertility

Spermatogenesis might be impaired in male patients with cancer before oncological treatment has begun.[17,18] A study underway at the Children's Hospital of Philadelphia has revealed that, when adjusted for age, 40.7% of boys diagnosed with non-testis cancer had neither type A dark spermatogonia nor primary spermatocytes in the testis before receiving treatment, which is suggestive of abnormal maturation of germ cells.[19] Moreover, an epidemiological study reported pretreatment oligozoospermia in 28% of men with testicular cancer, 25% of men with Hodgkin lymphoma and 57% of men with leukaemia.[18]

Multiple aetiologies of impaired spermatogenesis can exist for a specific cancer; for example, testicular cancer can affect spermatogenesis not only via testicular dysgenesis, but also through local effects of the tumour, autoimmune phenomena, endocrine alterations and other systemic mechanisms.[20,21] Common to all types of cancer is the potential psychological effect of diagnosis; stress is known to have a deleterious effect on spermatogenesis.[22]

Post-treatment infertility

Effect of chemotherapy on fertility

Chemotherapeutic agents are cytotoxic to cells that have a high mitotic rate. These drugs have the ability to penetrate the blood–testis barrier and can affect the germinal epithelium.[23] Spermatogonia differentiate rapidly and are, therefore, very sensitive to chemotherapy; also at risk, although less mitotically active, are spermatogonial stem cells (SSCs).[24] The extent of germinal damage depends on the patient's age, type of drug and cumulative dose. Importantly, synergism between individual agents administered concurrently might lower the threshold doses needed to affect fertility.[25]

Alkylating agents, such as cyclophosphamide, chlorambucil and procarbazine, can have potent and prolonged anti-spermatogenesis effects (Box 1). For example, studies of gonadal function after chemotherapy for Hodgkin lymphoma show that alkylating regimens can impair fertility in 89–100% of male patients.[26,27] Dosage is also an important factor; comparative studies of patients with testicular cancer found that total cumulative doses >400–600 μg/m^2 of cisplatin altered spermatogenesis and gonadal function, whereas lower doses did not.[28,29] These studies included both prepubertal and postpubertal male patients, demonstrating that patients of all ages are at risk of testicular failure from gonadotoxic chemotherapy.

Effect of radiation therapy on fertility

The testis is one of the most radiosensitive organs in the body. Direct doses of radiation as small as 0.1 Gy can cause damage to the most vulnerable of germinal cells (the spermatogonia) and result in transient oligozoospermia for up to 18 months.[30] As the dose increases, injury to different types of germ cells results, leading to more profound oligozoospermia or azoospermia and an increase to the delay in recovery (if recovery occurs at all). At a radiation dose of 2 Gy, spermatocytes are affected, causing azoospermia to develop within about 2 months and recovery potentially taking 30 months. At 4–6 Gy, radiation damages the spermatids, causing a rapid onset of sustained azoospermia that can last 3–5 years and in some cases might be permanent.[30] Although not true for other tissues, fractionation—where the total radiation dose is spread out over multiple treatments—seems to exacerbate the damage to germ cells.[31,32] Fractionated doses of >1.2 Gy are associated with permanent sterility.[31,32]

The most common cause of gonadal impairment after radiotherapy is scatter from radiation directed at

adjacent tissues. Notably, the testicles can receive up to 1–2% of radiotherapy directed at the abdominopelvic region during treatment for malignancies of the prostate, bladder and rectum.[33] Although necessary, radiation exposure from diagnostic and follow-up CT for cancer can also be of concern, given that the average abdominal CT scan uses 25 mGy of radiation.[34]

Fertility preservation strategies

Sperm cryopreservation

Sperm cryopreservation is the most reliable method of preserving male fertility prior to cancer therapy. Owing to advances in *in vitro* fertilization (IVF) techniques, even patients with severe oligozoospermia are candidates for sperm cryopreservation. Sperm can be cryopreserved for several decades; according to one report, sperm frozen for 28 years was used successfully for IVF that resulted in a live birth.[35] Overall, IVF success rates for cryopreserved sperm from patients with a previous malignancy compare favourably to patients who have cryopreserved sperm for other male-factor-related reasons.[36]

Attempts at fertility preservation should be performed prior to commencement of cancer therapy owing to the vulnerability of the germinal epithelium to gonadotoxic therapies. Sperm analysis has shown that the integrity of sperm DNA might be affected after just one treatment session.[18] However, if a patient decides to attempt sperm cryopreservation after cancer treatment, experts recommend waiting at least 12 months after the last treatment session (whether chemotherapy or radiation therapy).[37]

Ejaculation through masturbation is commonly used to collect semen for sperm cryopreservation. This method is relatively simple and can be performed at home if necessary. Depending on semen quality, more than one sample might need to be procured. We suggest separating the sample into multiple vials for freezing, and if the sample is of good quality, one vial usually equates to one attempt at IVF. The number of sperm required per vial is small given that <10 oocytes are typically retrieved during an IVF cycle.[38] However, it is worth noting that more than one-third of viable sperm might be lost upon thawing of a cryopreserved specimen,[39] and more than one cryopreserved vial might be needed per attempt at IVF in some circumstances.

Some patients might be unable to ejaculate on demand for a variety of reasons, including sickness, age, pain, psychological reasons, cultural factors or religious barriers.[40] For some of these patients, outpatient penile vibratory stimulation can be used.[41,42] We suggest that this technique not be used for boys who have never masturbated owing to potential psychological ramifications. For patients unable to procure a masturbatory or penile vibratory stimulation semen specimen, electroejaculation can be used.[41,42] Electroejaculation might also be preferable for patients under time constraints; it is not unusual for a patient to be told they have <24 h to procure a semen specimen before chemotherapy commences. However, electroejaculation requires general anaesthesia, with its attendant risks.[42] For this reason, at our institutions almost every electroejaculation procedure is combined with another procedure that necessitates general anaesthesia (such as port placement or bone marrow aspiration), especially for paediatric patients.

In some circumstances, sperm might be collected internally. For men who experience retrograde ejaculation, sperm can be collected from the urinary bladder after orgasm. For men with documented obstructive azoospermia, or when electroejaculation is unsuccessful, sperm can be extracted from the testes via testicular sperm extraction (TESE) or from the epididymis using microsurgical or percutaneous epididymal sperm aspiration.[42] Patients with normal spermatogenesis can undergo percutaneous sample collection, but most men with cancer and nonobstructive azoospermia require an open approach. For these patients, microsurgery (micro-TESE) has been shown to have a higher sperm retrieval rate than standard open biopsy (63% versus 45%),[43] but it is more technically complex. For patients with cancer and nonobstructive azoospermia who have undergone chemotherapy, micro-TESE yields the best results compared with nonmicrosurgical approaches.[44] Data assessing the utility of testicular biopsy before chemotherapy are sparse. One study found that sperm was successfully retrieved using open biopsy in approximately 45% of men with cancer and nonobstructive azoospermia before initiation of chemotherapy.[45]

TESE can also be safely used to extract sperm from teenagers with cancer who are unable to bank sperm through conventional methods before they receive treatment.[46] If necessary—for example, in the case of testicular cancer—testicular sperm can be retrieved *ex vivo* from a testis at the time of orchiectomy.[47] Encouragingly, several studies suggest that cryopreserved testicular sperm yields pregnancy rates that are not significantly different from those achieved with fresh sperm.[48–50]

Box 1 | Effects of antitumour agents on sperm production*

Permanent or sustained oligozoospermia or azoospermia
- Total body irradiation (for bone marrow transplant, stem cell transplant)
- Testicular radiation of >2.5 Gy in men or >6 Gy in boys (testis cancer, acute lymphocytic leukaemia, non-Hodgkin lymphoma, Hodgkin lymphoma)
- Procarbazine (Hodgkin lymphoma)
- Cyclophosphamide (acute lymphocytic leukaemia, non-Hodgkin lymphoma, sarcoma)
- Cisplatin, carboplatin‡ (testis and other germ cell tumours, head and neck cancer, lung cancer, unknown primary tumours, lymphomas, breast cancer)

Temporary oligozoospermia or azoospermia
- Doxorubicin, bleomycin, vinblastine (testis cancer, non-Hodgkin lymphoma, Hodgkin lymphoma)
- Testicular radiation (scatter) <1 Gy
- No effect on spermatogenesis
- Interferon-α, prednisolone
- Radioactive iodine (thyroid cancer)

Unknown effect on spermatogenesis
- Tyrosine kinase inhibitors, such as sunitinib or imatinib (renal cancer, gastrointestinal stromal tumours, chronic myeloid leukaemia)
- Monoclonal antibodies, such as bevacizumab (colorectal, lung, renal cancers)

*Chemotherapy using a combination of these agents will alter the impact on fertility.
‡Permanent or sustained oligozoospermia or azoospermia is possible but unlikely when administered at standard dose.

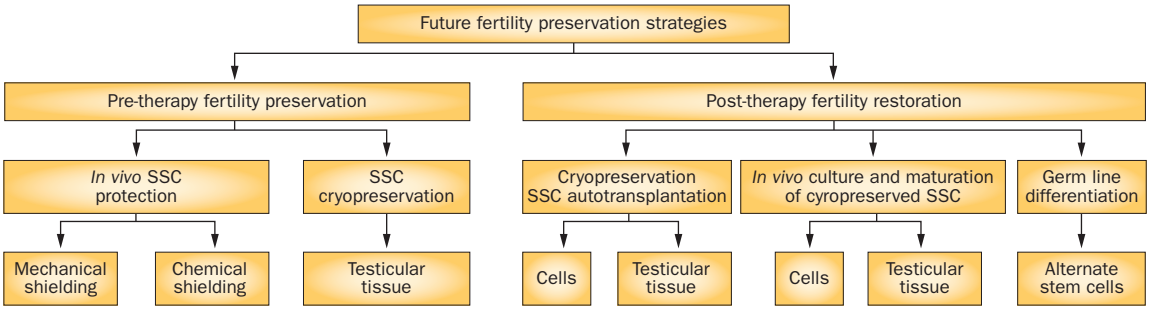

Figure 1 | Potential options for fertility preservation in the future. Strategies for pre-therapy fertility preservation are likely to focus on SSC protection, either mechanically or chemically, or cryopreservation. After therapy, fertility can be restored using autotransplantation techniques. Abbreviation: SSC, spermatogonial stem cell.

Other approaches for fertility preservation

Several methods have been suggested to limit testicular damage and preserve gonadal function during treatment that affects fertility. Physical gonadal shielding, using a lead shield, during abdominopelvic radiation is a well-established method for minimizing scatter radiation to the testicles in both adults and children.[51–53] Gonadal shields have also been demonstrated to protect testicular growth and function from inadvertent scatter radiation in children and adolescents.[54]

Less gonadotoxic alternatives to alkylating chemotherapy regimens can be employed for certain malignancies. Recent research suggests that for patients with Hodgkin lymphoma, treatment with doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) rather than with multiple or large doses of alkylating agents is associated with lower rates of fertility impairment.[26] Only one-third of patients treated with ABVD experienced azoospermia and most fully recovered.[26]

For amenable testicular tumours, organ-sparing surgical techniques can be used to preserve fertility with minimal compromise of oncological outcomes. Organ-sparing surgery can be used for tumours in a solitary testis, bilateral testicular tumours or metachronous contralateral tumours in postpubertal boys and for epidermoid cysts, teratoma, juvenile granulosa cell tumours and benign Leydig or Sertoli cell tumours in prepubertal boys.[55,56] Some surgeons advocate active surveillance of very small testicular lesions detected incidentally in subfertile men.[57]

Investigational strategies

If the fertility preservation strategies mentioned above have failed, the only options left to men rendered infertile by gonadotoxic cancer therapy who wish to become fathers are adoption or the use of donor sperm. No methods currently exist that can re-establish fertility, and the techniques discussed in this section that are aimed at fertility preservation before treatment or fertility restoration after treatment are still in the research phase and remain experimental (Figure 1).[58] Prepubertal male patients with cancer might seek parenthood several decades after their treatment, and it is hoped that these advances will translate into viable reproductive options by the time contemporary prepubertal patients are ready to start a family.

Fertility preservation before treatment

In vivo spermatogonial stem cell protection

In addition to the established method of mechanical gonadal shielding, chemical gonadal shielding has also been proposed to protect the testes from gonadotoxic agents.[59] The testes might be less susceptible to damage if the hypothalamic–pituitary–gonadal axis is suppressed by hormonal therapy. Thus, gonadotropin-releasing hormone (GnRH) agonists have been used, occasionally with other hormones such as testosterone, to provide gonadal protection in rat models,[60–63] although human trials have not demonstrated a consistent benefit (Table 1).[64–68] The variation in signalling pathways between different species might account for this lack of clinical success. Notably, in prepubertal nonhuman primates, germ cell proliferation has been demonstrated to be gonadotropin independent.[69] Hopefully, future studies will identify other hormonal regulatory mechanisms that are active in the prepubertal human testis and might become the target of future suppressive therapies. Indeed, GnRH analogues might be more effective in other areas of fertility preservation, such as enhancing the success of SSC transplantation.[70]

Cytoprotective agents have also been investigated as a potential means of chemically shielding SSCs *in vivo*. The antioxidant carnitine has been shown to be gonadoprotective against chemotherapy and radiotherapy in rats.[71,72] Other antiapoptotic agents have shown success in animal models,[73,74] but no successful trials of cytoprotective agents in humans have been conducted and currently there are no clinical options available.

Spermatogonial stem cell cryopreservation

The most promising experimental approach for fertility preservation prior to gonadotoxic therapy is the cryopreservation of SSCs. These immature diploid germ cells can be cryopreserved in testicular tissue or in a cell suspension.[75,76] Although this approach is still in the investigative phase, clinical protocols for the prepubertal male are currently in place at several institutions.[77] By the time these children wish to consider parenthood, it is hoped that advances in reproductive technology will enable haploid spermatozoa to be produced from these cryopreserved SSCs. The latest development in this context is the maturation of murine SCCs into

spermatids *in vitro*.[78] Encouragingly, these spermatids were functional and produced healthy and reproductively competent progeny via microinsemination.

To date, testicular tissue cryopreservation has been more successful for the storage of SSCs than cell suspension. Human testicular tissue has a post-thaw viability of up to 95% compared with only 66% for post-thaw cell suspension.[75,79] Freezing testicular tissue enables preservation of not only germ cells but also supporting Sertoli cells, which might maintain crucial cell-to-cell interactions and account for the improved SSC survival in cryopreserved tissue compared to cell suspension.[80] The method of choice for cryopreservation of immature testicular tissue is currently under investigation; preliminary data from studies of vitrification of human and primate immature testicular tissue are promising.[81,82]

Table 1 | Hormonal gonadal protection before treatment

Study	*n*	Cancer	Hormonal suppression	Spermatogenesis recovery rate	
				Treatment	Controls
Johnson *et al.* (1985)[66]	5	Hodgkin lymphoma	GnRH agonist	20%	No controls
Waxman *et al.* (1987)[68]	30	Hodgkin lymphoma	GnRH agonist and testosterone	Nil	Nil
Redman and Bajorunas (1987)[67]	45	Hodgkin lymphoma	Testosterone	70%	68%
Fossa *et al.* (1988)[65]	15	Testis	Medroxyprogesterone acetate	30%	66%
Kreuser *et al.* (1990)[136]	14	Testis	LHRH analogue	100%	67%
Brennermann *et al.* (1994)[64]	20	Testis	GnRH agonist and antiandrogen	100%	100%

Abbreviations: GnRH, gonadotropin-releasing hormone; LHRH, luteinizing hormone-releasing hormone. Permission obtained from Cambridge University Press © Mulhall, J. P. *et al. Fertility preservation in male cancer patients* (Cambridge University Press, 2013).

Fertility restoration after treatment

Spermatogonial stem cell autotransplantation

SSC autotransplantation is based on the hypothesis that previously cryopreserved SSCs can restore fertility when reimplanted into the same patient.[83] Given that germ cells undergo a theoretically indefinite cycle of self-renewal, they might, therefore, be able to restore long-term fertility.[58,83] SSC autotransplantation was first described in 1994 in a mouse model and has since been attempted in several other species, including goat and domestic chicken, with successful fertility restoration outcomes.[84–86] However, there has been only limited success in primates.[86,87,88] Attempts to autotransplant SSCs have been undertaken in humans but differentiation of transplanted SSCs from native SSCs has not been successful and, therefore, no results have been published.[89]

The steps underlining successful SSC autotransplantation are becoming delineated. Germ line stem cells have the ability to colonize the seminiferous tubule and differentiate through the process of spermatogenesis.[90] This process is facilitated by the unique milieu of the testicular microenvironment.[91] Donor cells can be injected into the efferent ductules, the rete testis or directly into the seminiferous tubules.[75,92] Some studies have demonstrated that these injection techniques are possible in humans.[75,93]

Another method of SSC autotransplantation is to graft previously cryopreserved testicular tissue back into the patient. The potential advantage of grafting testicular tissue is that SSCs are returned to the patient with the surrounding architectural and pericellular support network. The aim is for revascularization of the graft, which will generate spermatogenesis. Sperm can then be harvested from the graft and used for assisted reproduction techniques, such as direct injection into an egg via intracytoplasmic sperm injection (ICSI). Such tissue grafting was first attempted in animal models a decade ago and generation of progeny by ICSI has been achieved in mice and rabbits.[94–96]

Several studies have revealed the capacity of human prepubertal SSCs to survive in mouse seminiferous tubules and for human prepubertal testicular tissue to survive xenographically in mice.[97–99] Prepubertal human SSCs were transplanted into mouse testicles and migrated to recipient mouse seminiferous tubules. These human SSCs were found in small colonies but with a greater succss rate than similar procedures using somatic cells. These colonies of human germ cells resembled the initial germ cell expansion of mouse gonocytes transplanted to mouse recipients after approximately 2 weeks.[98] Likewise, Sertoli cells and spermatogonia survived for 4–9 months after xenotransplanation of human prepurbertal testicular tissue to mice, suggesting the possibility of using xenografts to preserve human SSCs before autotransplantation.[98,99]

In vitro culture of spermatogonial stem cells

The goal of *in vitro* culture of SSCs is to enable maturation into haploid gametes that can be used for ICSI. SSCs from prepubertal animals (mice and cattle) have been propagated *in vitro*, and completion of spermatogenesis from spermatogonia has been achieved in mice.[78,100,101] Sato and colleagues[78] demonstrated for the first time that neonatal mouse spermatogonia can be propagated *in vitro* into functional spermatids that can produce offspring via microinsemination. Research into culture methods that can facilitate human *in vitro* spermatogenesis have also demonstrated propagation of spermatogonia, albeit only partially; primary spermatocytes have been successfully matured into round spermatids and then into normal late spermatids in culture.[102,103] Although late spermatids might be able to fertilize an oocyte, no human pregnancies have been reported as a result of such fertilization.

Similar to autotransplantation of SSCs, it has been postulated that *in vitro* maturation of human testicular tissue might be more successful than maturation of cells as it retains the pericellular support network and niche, including Sertoli cells.[104,105] Several investigators have attempted *in vitro* maturation of human testicular tissue but with only limited propagation of spermatogenesis.[104,105] Thus, it has been suggested that Sertoli cells are only necessary for the early stages of spermatogonial differentiation.[106] From these experiments, it is clear that each stage of spermatogenesis requires complex cellular

and hormonal interactions, many of which are not yet fully understood. Ongoing research into the testicular microenvironment will facilitate complete spermatogenesis from SSCs and hopefully lead to successful ICSI, embryo development and, ultimately, pregnancy.

Another potential use of *in vitro* stem cell culture is to propagate SSCs to a sufficient number for autotransplantation. Calculations suggest that SSCs from a typical 0.2 ml biopsy sample of prepubertal testicular tissue must be propagated 1,300-fold for autotransplantation into a typical adult-sized testicle.[107] This degree of proliferation has been achieved using human adult spermatogonia, but given that SSC autotransplantation is still in the research phase this culture method has no current clinical utility.[107,108]

Induced pluripotent stem cells

The use of other stem cells that have the potential to differentiate into germ line cells might have a future role in patients rendered infertile by gonadotoxic treatments who did not cryopreserve sperm or SSCs prior to therapy. Induced pluripotent stem cells (iPSCs) from both mice and humans have been demonstrated to differentiate into primordial germ cells. Zhu *et al.*[109] investigated the potential of mouse iPSCs to differentiate into SSCs and late-stage male germ cells. iPSC-derived SSCs were able to differentiate into male germ cells ranging from spermatogonia to round spermatids, as demonstrated by *VASA* (also known as *DDX4*) and *SCP3* (also known as *CTDSPL*) expression. Furthermore, Yang *et al.*[110] showed that human iPSCs could differentiate into male germ cells *in vitro* and reconstituted seminiferous tubules could provide a functional niche for exogenous iPSC-derived male germ cells. The derivation of male germ cells from iPSCs has potential application for treating male infertility and provides an ideal platform for elucidating the molecular mechanisms of male germ cell development.

Genes that have been shown to promote iPSC formation have also been linked to cancer, as some are also known oncogenes (such as *TP53* and *MYC*).[111] Inactivation or deletion of the tumour suppressor *TP53* significantly increases reprogramming efficiency, but has the risk of increasing tumour formation.[112] Similarly, 20% of mice transplanted with *MYC*-induced iPSC developed lethal teratomas.[113] Omitting *MYC* allows for iPSC formation, although reprogramming efficiency might be significantly reduced. In order to avoid the problems of tumorigenesis and low throughput observed with these genetic methods, others have tried alternate nongenetic vectors, such as adenovirus, plasmids and naked DNA or protein compounds.[114–116] Further refinements to the methodology that yield higher efficiencies might lead to the production of safer iPSCs without oncological potential.

Potential concerns of experimental strategies

Although experimental protocols for testicular cryopreservation exist, concerns regarding the risks of testicular biopsy have been raised, especially in prepubertal patients. However, the available data on testicular biopsies in this age group—performed under trial protocols—suggest no short-term or long-term adverse effects.[77,117,118] These protocols mandate that testicular biopsies are performed only in conjunction with another necessary procedure (such as placement of a central venous catheter or medication port, or bone marrow aspiration), thereby limiting exposure to anaesthesia.

Another potential concern is the reintroduction of malignant cells to a patient who no longer has cancer. The testis is an immunoprivileged site and might harbour malignant cells that evade detection by currently available techniques.[119,120] Thus, research into the development of assays that can detect and remove malignant cells—especially those of haematological cancers—from testicular tissue are of vital importance. A number of techniques, such as magnet-activated or fluorescence-activated flow cytometry, are currently in development for this purpose.[121–123] The theoretical risk of malignant cell reimplantation might also be circumvented by the use of *in vitro* maturation of SSCs and subsequent microinsemination of mature spermatids.[124]

The potential use of xenograft material for fertility preservation or restoration might be associated with a risk of infection. However, assessing and quantifying the risk is not possible at this time.[125] Until potential infectious dangers, such as viruses, are eliminated, these techniques will remain experimental.

The development of IVF was associated with concerns about birth defects,[126] and similar concerns have been raised regarding progeny derived from SSCs. For example, it has been suggested that the accelerated *in vitro* maturation of SSCs might subvert the normal DNA control mechanisms of *in vivo* maturation.[58] The only available data on birth defects associated with SSCs are from experiments in animal models, but there are reports of genetic birth defects associated with germ cell transplantation and haploid gametes matured *in vitro*.[104,127] Even if fertility restoration methods prove to be viable, such safety issues will need to be addressed prior to any clinical application.

The ethical concerns surrounding fertility preservation strategies also require consideration. Because many of these techniques involve the use of tissue or cells from children who are too young to give consent, parental involvement is necessary. Before any samples are taken, it is imperative to have an open and candid discussion with parents to emphasize that all currently used fertility preservation methods are investigational and that there are no clinically validated techniques available that can restore fertility using prepubertal cells or tissue. Posthumous reproduction is possible for patients who had sperm cryopreserved before death; ethical dilemmas and discussion in this area become especially complicated when the potential for posthumous reproduction from cryopreserved sperm or germ cells of a child are considered.[128]

Developing a clinical care pathway

Physicians specializing in men's reproductive health must strive to disseminate information on all techniques that might assist male patients with cancer to become

biological fathers after completion of treatment. Several fertility preservation strategies are currently either underutilized or not employed.[129,130] In a survey of oncologists from two major US cancer centres, almost half of the physicians offered fertility preservation to <25% of eligible patients.[130] Indeed at our institution, a recent study demonstrated that only 26 male teenagers over the last 7 years were referred for tertiary fertility preservation.[46] Education-based strategies might have a meaningful effect on fertility preservation rates. For example, the age at which spermatozoa development commences, marking the potential ability to cryopreserve semen, is not well understood by clinicians and parents alike. According to one study, 20% of healthy boys aged 11.0–12.5 years old, as well as 20% of boys at Tanner stage II, had spermaturia,[131] although the ability to make sperm is different from the practical capability of procuring a semen specimen from a young adolescent. Thus, assessing whether or not a pubertal boy has started to produce spermatozoa can be a challenging situation for the clinician, parent and child.

Notably, the average age of onset of masturbation is 12 years old, and up to 80% of 13-year-olds masturbate, according to questionnaire data.[132,133] Several studies have reported the cryopreservation of viable sperm from 12-year-old boys obtained via masturbation, some with testicular volumes as low as 5 ml.[134,135] We believe that if more health-care professionals and parents were aware of the ability of young adolescents to produce a semen sample for cryopreservation, fertility preservation would be performed more often for children with cancer. Private discussion between a health-care professional and the patient in the absence of a parent or guardian has been identified as an important factor in facilitating a young person to donate a specimen by masturbation.[134] In this situation, the physician must be careful to avoid the appearance of coercion, especially in discussions with an adolescent.

For patients of any age who are unable to produce a suitable specimen via masturbation, the treating physician should be aware of other specialized approaches for obtaining sperm and refer onwards as required. As mentioned previously, electroejaculation and penile vibratory stimulation can be utilized in such situations, but for a postpubertal patient who is azoospermic, either before or after gonadotoxic therapy, TESE remains the best option for obtaining sperm for ICSI.[44]

Conclusions

As cancer survival rates continue to improve, more attention is being directed toward survivorship and enhancing long-term quality of life of patients with cancer. Maintenance of fertility is an important consideration for cancer survivors. In order to achieve the desired oncological outcomes, cancer specialists frequently have to administer gonadotoxic treatments to their patients. All modalities of oncological therapy, including chemotherapy, radiotherapy and some surgical procedures, carry some risk to fertility. Some treatments can be modified, or adjunctive protective measures employed, to limit the impact on fertility, but often there are few alternatives and infertility results. Many physicians fail to discuss the potential effect that such therapies have on fertility potential, and therefore the option of fertility preservation, with their patients. Numerous guidelines recommend that fertility preservation be discussed as early as possible during cancer management so that strategies can be employed to preserve the patient's fertility. One such simple measure is the cryopreservation of sperm after masturbation; more complex techniques such as electroejaculation and TESE are also available, and patients should be referred to a fertility specialist if necessary.

For those patients who did not cryopreserve sperm prior to gonadotoxic therapy and have been rendered infertile, there are currently no options for restoring fertility, though there is a wealth of research aimed at ultimately achieving that goal. Most of these studies involve research into SSCs and their ability to be cryopreserved, thawed and transplanted back into the patient after cancer treatment. Investigation into *in vitro* maturation of SSCs for future use, in ICSI or for autotransplantation, is also ongoing. However, there are still many processes involved in sperm maturation that need further delineation. Nevertheless, there have been some encouraging results from experiments in animal models. These promising findings laid the foundation for the development of research protocols for cryopreservation of SSC from prepubertal children, and studies using these protocols are currently being conducted at several institutions. Although these protocols are still considered investigational and might involve complex ethical considerations, it is hoped that with time they will lead to clinical application and be integrated into future fertility preservation strategies.

Review criteria

We searched for original articles focusing on fertility preservation in MEDLINE and PubMed published between 1950 and 2013. The search terms we used were "fertility preservation", "males" and "cancer". All papers identified were English-language full text papers. We also searched the reference lists of identified articles for further papers.

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