Fertility preservation 1

Fertility preservation in men with cancer

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During the past decade, advances in cancer treatment have increased survival rates of both boys and men. However, cancer treatment itself can compromise fertility, especially exposure to alkylating agents and whole body irradiation, which cause substantial germ cell loss. Children and adolescents with testicular cancer, leukaemia, and Ewing sarcomas are at the highest risk of developing permanent sterility from cancer treatment. Consequently, various strategies to preserve fertility are necessary. Sperm cryopreservation is an effective but underused method to safeguard spermatozoa. In the past few years, large advances have been made in prepubertal germ cell storage aimed at subsequent transplantation of testicular tissue and associated stem cells. Although still experimental, these approaches offer hope to many men in whom germ cell loss is associated with sterility. The derivation of male gametes from stem cells also holds much promise; however, data are only available in animals, and the use of this method in human beings is probably many years away.

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This is the first in a **Series** of three papers about fertility preservation

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Introduction

From puberty onwards, testicular stem cells proliferate into differentiated spermatogonia, enter meiosis, and eventually become spermatozoa in a process called spermatogenesis. Because of the stem-cell properties of the testicular germ cells (ie, spermatogonia type A), spermatogenesis is maintained throughout a man's life. Although ageing is associated with a gradual depletion of testicular germ cells, an important natural loss happens in association with specific genetic disorders. However, the main cause of germ-cell loss is iatrogenic. Exposure to gonadotoxins (eg, alkylating agents), might cause massive germ-cell loss, often with long-term sterility as a result. Although advances in cancer treatment are associated with improved survival rates, the treatment itself can compromise fertility and prevent people who have had cancer from starting their own family.

Oncofertility is a novel interdisciplinary approach aimed at maximising the reproductive future of people who have had cancer by offering various fertility preservation techniques. This approach is being introduced in more clinics worldwide. In men, sperm cryopreservation is a well established, although underused strategy to circumvent sterility after germ-cell loss. In the past few years, new fertility preservation strategies have been developed that might drastically change the reproductive options for both men and boys facing germ-cell loss and hence sterility. We discuss the causes of testicular germ-cell loss and fertility preservation options.

Epidemiology and pathophysiology of germ cell loss

Naturally occurring depletion of the germ-cell line in the ageing testes is a poorly understood phenomenon. Ageing is associated with a regression of the germinal epithelium[1] and a gradual decrease in sperm output.[2] Findings of germ-cell transplantation experiments[3] in mice have shown that this regression results from a deterioration of the stem cell niche rather than from an intrinsic defect of the germ cell itself.

Loss of germ cells has been reported in association with specific genetic disorders—ie, the presence of Yq-microdeletions[4] and 47,XXY Klinefelter's syndrome.[5] For patients with Klinefelter's syndrome, the present hypothesis is that germ cells carrying an extra X chromosome are predestined to go into apoptosis.[5] However, even with the loss of germ cells and the high prevalence of azoospermia, testicular spermatozoa can be obtained after testicular biopsy in about 50% of adult patients with Klinefelter's syndrome.[6]

By contrast with naturally occurring germ cell loss, the mechanisms of drug-induced germ cell loss (eg, caused by gonadotoxic treatments) are well understood. Cancer treatment protocols have long-term health consequences, including gonadal toxic effects. However, other factors affect gonadal function in men with cancer. Testicular cancer has a more negative effect on semen quality than do other cancers.[7] As well as the type of cancer, downregulation of reproductive hormones by some tumours and the metabolic condition of the patient before initiation of treatment can interfere with spermatogenesis. Malnutrition with deficiencies of elements essential for germ-cell maintenance and spermatogenesis can arise in patients with cancer.[8,9]

Search strategy and selection criteria

We searched PubMed between July 1, 2013, and Jan 31, 2014, for publications with the following keywords and their combinations: "fertility", "spermatogenesis", "sperm", "testis", "stem cell", "germ cell", "cancer", "chemotherapy", "azoospermia", "sperm DNA damage", "semen parameters" "radiotherapy", "fertility preservation" and "male infertility", "radiation therapy", and "assisted reproduction". We aimed to select publications in the past 5 years, without excluding relevant older publications.

Cytotoxic treatment impairs spermatogenesis in most patients, at least temporarily, and occasionally results in sterility. The differentiating spermatogonia are most sensitive to chemotherapy, causing a maturation depletion with progressive loss of spermatozoa in the months after the start of chemotherapy.[10] The effect of chemotherapy on spermatogenesis varies substantially depending on the combination of drugs used, and on the cumulative dose given.[11] Nitrogen mustard derivatives, alkylating drugs, and cisplatin seem to have the most detrimental effect on germ cell proliferation (panel). Other agents are not likely to cause permanent sterility, and recovery to normal sperm counts can be expected. Alkylating agents are often used in combination with other chemotherapeutic drugs, substantially increasing their germ cell toxic effects.[12] Combinations of cytotoxic drugs (eg, the nitrogen-mustard, oncovin [vincristine], procarbazine, prednisone [MOPP] regimen) used in patients with Hodgkin's disease, have a high risk for permanent sterility.[13,14] By contrast, the other cytotoxic regimen given to patients with Hodgkin's disease—adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine (ABVD)—is associated with a much lower risk of future infertility. In testicular cancer chemotherapy, the combination of bleomycin, etoposide, and cisplatin has an intermediated risk of sterility of 20%.[15,16] Boys with metastatic soft tissue sarcomas and Ewing sarcomas have a high chance of becoming sterile from cancer treatment. Chemotherapy, in combination with whole-body irradiation for leukaemia is also detrimental for testicular function.[14] The effect of most chemotherapy regimens on spermatogenesis is well described and was recently reviewed.[13] Severe oligo-zoospermia and azoospermia often arise in the first 6 months after the start of chemotherapy; recovery depends on the amount of stem-cell loss and their regeneration and can take up to 5 years. Even in men with prolonged azoospermia, spermatozoa can be found in 30–50% of testicular sperm extractions.[17] Chemotherapy protocols are changed regularly, and continued follow-up of patients during adolescence is needed to establish the potential negative effects on future fertility.

The production of spermatozoa can return after chemotherapy depending on the cytotoxic drug used and on the cumulative dose given. For example, cyclophosphamide, often used in children with leukaemia, has a dose-dependent effect on spermatogenesis (a dose >10 g/m² is associated with increased risk of sterility).[18] Age of administration is not prognostic for future fertility. Prepubertal age is believed to be a quiescent period in gonadal development with the absence of spermatogenesis, but chemotherapy has an equal negative effect on germ cells irrespective of whether given during childhood or adolescence.[19]

Chemotherapy and radiation therapy can result in DNA abnormalities of germ cells and potentially increase the risk of disturbed growth, childhood diseases, congenital abnormalities, and cancer in the children of individuals who have had cancer. Although only a few long-term follow-up studies have been done, initial data suggest no increase in abnormalities in children of men who have had chemotherapy or radiotherapy treatment for cancer.[20,21] However, researchers of a more recent large study[22] concluded that children of men with a history of cancer have a small but significant increase in the risk of major congenital abnormalities; therefore, further detailed studies of offspring from patients with cancer are necessary.

***Panel:* Risk of chemotherapy for impairment of spermatogenesis**

High risk
- Cyclophosphamide
- Ifosfamide
- Chlormethine
- Busulfan
- Melphalan
- Procarbazine
- Dacarbazine
- Chlorambucil
- Nitrogen-mustard, oncovin (vincristine), procarbazine, prednisone (MOPP)

Medium risk
- Cisplatin
- Carboplatin
- Doxorubicin
- Bleomycin, etoposide, cisplatin (BEP)
- Adriamycin, bleomycin, vinblastine, dacarbazine (ABVD)

Low risk
- Vincristine
- Methotrexate
- Dactinomycine
- Bleomycin
- Mercaptopurine
- Vinblastine

Data taken from Wallace and colleagues.[14]

Prevention of germ cell loss in men

Spermatogenesis is highly sensitive to the effects of chemotherapy and irradiation; therefore, the main strategy to minimise germ cell loss is to choose treatment and combinations that have a less severe effect on spermatogenesis. Gonadotropin-releasing hormone analogues or antagonists that temporarily suppress the production of gonadotropins have been used to preserve spermatogenesis, and although some promising data exist in non-human primates,[23] this treatment is not recommended by the American Society of Clinical Oncology.[13]

Cryopreservation and the subsequent storage of semen samples is the main option for fertility preservation in men (and boys producing sperm in the ejaculate) who are

undergoing chemotherapy or radiotherapy regimes that might affect gonadal function. Semen was first stored in 1953,[24] and it can be successfully used for conceptions after several decades in storage.[25] Well developed national guidelines recommending sperm storage exist,[13,26,27] and state that patients should be fully informed about the risks of infertility before potential gonadotoxic treatment, and that sperm banking should be considered and available for all patients when future fertility is an issue. Additionally, a coordinated approach between the health-care professional in charge of cancer treatment and the specialists in reproductive medicine is recommended. Ideally, several semen samples should be collected 48 h apart,[27] but because time is always a concern, patients should provide samples without delay, which can mean obtaining more than one sample in a day. The objective is to collect enough sperm for subsequent infertility treatment without delaying chemotherapy or radiotherapy.

With the widespread availability of guidelines, one would assume that sperm storage is universally offered. However, this is not the case.[28,29] Poor adherence to recommendations by health-care professionals and the paucity of robust comprehensive systems need urgent attention.[30] Additionally, not all men who are offered sperm storage actually bank sperm. Some patients have difficulty producing semen because of stress, severe illness, and sexual inexperience. For these individuals, electro-ejaculation or testicular sperm extraction might be appropriate.[31,32]

Although research into this specialty is preliminary, the decision to bank sperm depends on many factors such as educational qualification, previous children, and the advice given by the treating oncologist.[33] More study of these areas is warranted.

Despite recent research, it remains difficult to predict, on an individual basis, whether permanent sterility will ensue from the disease, and treatment and generalisation are difficult to achieve with a high degree of accuracy. A key question remains: what is the association between cancer type and fertility prospects (ie, semen characteristics)? Controversy exists about the effect of cancer on semen parameters before treatment. Van Casteren and colleagues[9] noted reduced semen quality in 64% of men referred for sperm banking; in 12% of these men, azoospermia or only non-motile spermatozoa were present and cryopreservation could not be done. Poor semen quality was commonly recorded before the start of chemotherapy in men with testicular tumours,[7,9] especially in men with a non-seminoma testis.[9]

Most data for semen characteristics of patients relate to men with testicular cancer and Hodgkin's lymphoma. Previous data suggested that semen characteristics were minimally affected in patients with early stage Hodgkin's disease, but can be reduced in those with advanced stage diagnosis.[34] Recently, several large studies have added more confidence to these conclusions. For example, Hotaling and colleagues[35] addressed semen characteristics before and after cryopreservation in 373 men diagnosed with cancer. The total number of motile sperm in semen was significantly lower in men with testicular cancer, myeloid leukaemia, and lymphoid leukaemia than in semen from controls (ie, men without cancer). Patients with Hodgkin's lymphomas, prostate cancer, brain cancer, sarcoma, and lymphocytic cancer were not significantly different (ie, had similar number of motile sperm). Additionally, men with testicular cancer, myeloid leukaemia, and lymphoid leukaemia had a significantly lower percentage survival of motility after cryopreservation than did men with other cancers, suggesting that more samples should be stored in these patients. Rives and colleagues[36] studied the history, histological diagnosis, stage, and effect of orchiectomy in 1158 men (aged >12 years) with testicular cancer. Investigators noted that seminomas changed sperm production more than did non-seminoma tumours and seemed to preferentially impair spermatogenesis in the tumour-bearing testes. Semen quality was decreased in patients with advanced testicular tumours and in men with a history of cryptorchidism. The main conclusions were that sperm banking should be done before orchiectomy, and that testicular sperm extraction should be offered during scrotal surgery in cases of azoospermia. Cryopreservation adversely affects the quality of samples of all men except some groups, and some groups are more susceptible—eg, those with testicular cancer.[35]

National guidelines suggest that sperm storage should be done before chemotherapy and radiotherapy, and if this is not possible, men should be warned of a potentially increased risk of genetic damage in sperm collected after initiation of chemotherapy or radiotherapy.[13] Whether sperm DNA damage is increased in men with cancer is controversial.[37,38] Chemotherapy might not have a negative effect on sperm DNA integrity;[39] however, radiotherapy is associated with a permanent increase in sperm DNA damage compared with the DNA fragmentation of patients who have received only chemotherapy.[38] Whether cryopreservation itself induces DNA damage remains controversial.[40]

Treatment options with cryopreserved samples

Assisted reproductive treatment is often needed after cancer treatment in men with azoospermia, when the only semen available has been cryopreserved before cytotoxic treatment.[21,41] Results are generally good in these men.[42] Intracytoplasmic sperm injection has the advantage of allowing assisted reproduction even if only one semen sample of poor quality has been cryopreserved. In a retrospective study[43] in which three methods of assisted reproduction were compared, intracytoplasmic sperm injection was most successful; women needed a median of three cycles to get pregnant, whereas women who had conventional in-vitro fertilisation needed six cycles, and those who had intrauterine insemination needed eight. A

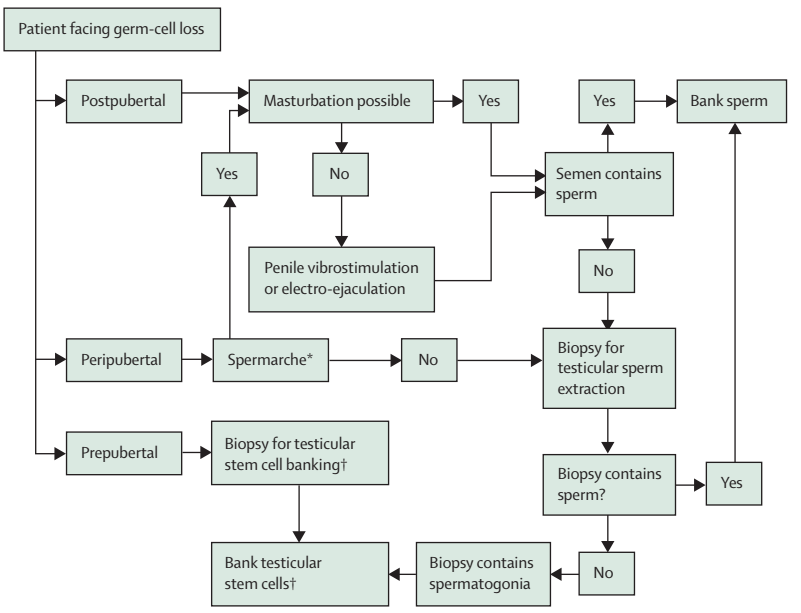

Figure: **Present options for patients with cancer undergoing gonadotoxic treatments with a high risk of sterility**
*When physical examination and endocrine parameters suggest onset of spermarche. †Part of experimental protocol aiming at transplantation or in-vitro culture of testicular stem cells.

more recent report on the use of cryopreserved samples from 57 patients (127 cycles) corroborated these findings; a pregnancy rate of 12% for intrauterine insemination, 28% for in-vitro fertilisation, and 32% for intracytoplasmic sperm injection, with no obvious difference in success between cancer diagnosis.[44] If spermatogenesis recovers after cancer treatment, fresh semen is usually used for assisted reproductive treatment if no spontaneous pregnancy has happened. Although the use of cryopreserved samples for fertility treatment is successful, few patients use their samples (often <10%).[45]

Up to now, follow-up information applies to children born after spontaneous conception, and might be different for children born after artificial reproductive techniques. Unfortunately, follow-up data for large cohorts of children born after assisted reproductive treatment by use of frozen-thawed sperm from men with cancer are currently not available in the scientific literature.

Peripubertal options for prevention of germ cell loss

The onset of production of spermatozoa (spermarche) starts at puberty, but it is not exactly known in what stage of pubertal development spermatozoa are produced.[46] Some data from urine examination and electro-ejaculation done in pubertal boys suggest that clinical parameters of puberty (eg, testicular size), Tanner stage, and a rise in reproductive hormones do not always coincide with spermatogenesis.[31,47] Spermatozoa can even be found in the urine of boys who have no clinical signs of puberty.[48] In a cohort of 80 pubertal boys, van Casteren and colleagues[9] could not do semen cryopreservation in 14 patients because of azoospermia or the absence of motile spermatozoa. Pretreatment levels of reproductive hormones could not predict the outcome of electro-ejaculation in this group of pubertal boys.

In boys and adolescents, production of semen through masturbation is not always possible because of psychosexual reasons. In boys that are unable to produce sperm, electro-ejaculation can be offered. However, this method of sperm harvesting is not widely used in children because it requires general anaesthesia. Findings of Hovav and colleagues'[31] study of electro-ejaculation in six boys (aged 15–18 years) showed that the procedure was successful in all cases. If no spermatozoa can be found in semen or urine, a testicular sperm extraction seems the only option to collect spermatozoa, especially in boys that are at high risk of becoming azoospermic after cancer treatment. Results of a recent, larger study of 30 adolescents treated with electro-ejaculation showed a sperm recovery rate of 60%.[49] This figure rose to 70% when testicular sperm extraction was done in men who could not undergo electro-ejaculation.[49] The figure shows the options for peripubertal boys together with those of adult men and prepubertal boys.

Preservation of stem cells in prepubertal boys

Before puberty, spermatogenesis is absent and cryopreservation of spermatozoa is not an option. In prepubertal boys facing drug-induced germ-cell loss, some oncofertility programmes collect and freeze testicular stem cells to preserve fertility of survivors of childhood cancer.[50] This approach is based on the early work by Brinster and colleagues in the mouse,[51] which showed that testicular stem cells can be transplanted into seminiferous tubules devoid of germ cells and reinitiate spermatogenesis. Recently, spermatozoa able to fertilise oocytes were obtained by this procedure in both prepubertal and adult recipient macaques pretreated with chemotherapy.[52] Thus, as a preventive strategy, a prepubertal boy facing sterilising chemotherapy, can opt to undergo a testicular biopsy to cryopreserve testicular stem cells. After being cured from cancer, the frozen-thawed stem cells can be reintroduced into the seminiferous tissue in the hope that sperm production will restart.

Data from studies of mice[53,54] and human beings[55] suggest that cryopreservation of testicular tissue containing spermatogonial stem cells can be done with dimethylsulphoxide and sucrose as cryoprotectants either in an uncontrolled slow freezing procedure[53,54] or in an ultrarapid vitrification protocol.[53–55] These simple protocols do not need expensive biofreezers, which allows the collection and cryopreservation of testicular tissue at a procurement site distant from the banking and transplantation site.[53]

Freezing tissue allows subsequent transplantation either by infusion of a testicular cell suspension into the seminiferous tubules[56] or intratesticular grafting of tissue.[54] Orthotopic intratesticular grafting has the

advantage of conserving the stem cell niche, but is inappropriate whenever a risk of testicular malignant contamination exists (eg, in patients with leukaemia).[57] Whenever such a risk does exist, infusion of decontaminated cell suspensions is a possible solution, although present decontamination procedures are insufficient to avoid cotransplantation of cancer cells.[58]

Up to now, even in animal models, the efficacy of this procedure is low, but in-vitro expansion of the stem cells either before freezing or after thawing might improve the success of the procedure in human beings.[59] Therefore, testicular germ cell transplantation might be restricted to children that have a high risk (>80%) of becoming sterile because a testicular biopsy in a young child with small testes might diminish future spermatogenesis after recovery from cancer treatment.[14] Because both testicular stem-cell cryopreservation and transplantation are still under investigation, this preventive strategy remains controversial.[60] However, parents of boys facing childhood cancer already accept this strategy as shown by a recent survey.[61]

Because of the invasive nature of procuring testicular tissue and the present experimental character of testicular stem cell cryopreservation for later transplantation, consent from the parents, and whenever possible from the prepubertal child, is mandatory before testicular tissue can be taken and stored.[62]

Derivation of male gametes from stem cells

As well as testicular stem cell transplantation, in-vitro culture of testicular stem cells is being studied in the mouse for its potential to generate post-meiotic male gametes.[63,64] In-vitro spermatogenesis would circumvent the risk of cotransplanting cancer cells and allow the production of male gametes in boys or men who had bilateral orchiectomy.

As a preventive strategy, both testicular stem cell transplantation and in-vitro spermatogenesis require collection of testicular tissue containing spermatogonial stem cells before germ cell loss. For those patients having lost their germ cells and hence their reproductive potential, the generation of male gametes from stem cells would be a theoretical option. In 2004, Geijsen and colleagues[65] published landmark findings that showed haploid cells could be produced in-vitro from blastomeres isolated from mice embryos. However, the embryos obtained after injection of these haploid cells into oocytes were unable to produce offspring. In the past few years, primordial germ cell-like cells were produced from both embryonic stem cells and induced pluripotent stem cells in mice.[66,67] Once transplanted to the testis of an otherwise sterile recipient mouse, these in-vitro-derived cells developed into spermatozoa that could fertilise oocytes, and eventually produced fertile offspring. Although this research might be a last hope for men who do not have testicular stem cells or have lost them, any clinical application in human beings remains speculative, not just because of the need to extrapolate this research in human beings, but also because of uncertainties about genetic and epigenetic consequences for the offspring.

Conclusions

In men, spermatogenesis is driven by testicular spermatogonial stem cells that can self-renew, enter meiosis, and eventually differentiate into mature spermatozoa. Loss of these germ cells can be part of genetic disorders such as 47,XXY Klinefelter's syndrome. However, cytotoxic treatments and irradiation are the main causes of germ cell loss and can disrupt spermatogenesis temporarily but unfortunately also permanently in some patients. Therefore, patients with cancer in particular face long-term sterility because of germ-cell loss.

Because of the very high survival rates for cancers, such as leukaemia, Hodgkin's lymphoma, and testicular cancer, the treatment focus is increasingly shifting towards quality of life. For both young adult and survivors survivors of childhood cancer, preservation of reproductive potential is an important quality of life issue.[68] Oncofertility is a new emerging specialty in reproductive medicine that aims to preserve fertility in patients undergoing sterilising cancer treatments.

In adult men, fertility can be preserved by cryopreservation of mature spermatozoa. Thanks to advanced techniques of assisted reproduction (ie, intracytoplasmic sperm injection), only few spermatozoa are needed to fertilise the ovum. As a result, even storing one poor quality semen sample containing only a few spermatozoa is worthwhile when considering preservation of a man's fertility potential. Moreover, recent data suggest that cancer survivors have a slight increased risk for congenital malformations in their offspring; thus, banking semen before starting any gonadotoxic treatment has become a general guideline.

Nevertheless, sperm banking is still underused, partly because this recommendation is not always supported by the treating oncologist, and partly because patients do not always opt to bank their semen or fail to produce a sample for storage. The latter problem is more prevalent in adolescents and peripubertal boys.

Because active spermatogenesis only starts from puberty onwards, prepubertal boys cannot benefit from sperm banking. However, they harbour stem cells in their testes that can be cryopreserved for later transplantation back into their testes, analogous to transplantation already successfully done in rodents and primates. Although prepubertal testicular stem cell banking is being introduced into clinical practice, this approach should be regarded as experimental in view of the paucity of evidence of successful transplantation and the scarce safety data for this method. Creation of spermatozoa from stem cells other than spermatogonial stem cells is theoretically a reproductive option for men who have lost their testicular

germ cells. However, although this technology is under research in rodents, this is an even more demanding strategy to develop in human beings.

Contributors

HT designed the concept of the review. All authors were involved in both literature search and writing of the paper. CLRB did the final editing of the manuscript. All authors approved the final manuscript.

Declaration of interests

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