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Review

Impact of cancer and cancer treatment on male fertility

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ABSTRACT

While cancer, and especially testicular cancer and Hodgkin's disease, affects male fertility in many ways, the current increase of survival of male cancer patients of reproductive age or earlier has emerged as a new challenge to their subsequent ability to father children. Cancer treatments, including surgery, radiotherapy and chemotherapy, can have a transitory as well as a permanent detrimental impact on male fertility. Gonadotoxic effects and the length of time for sperm recovery after radiotherapy depends not only on initial semen quality, but also on gonadal dosage and the delivery method after chemotherapy, on the type of regimens and dosages and on the spermatogenesis phase that each drug impacts. Combination treatment with radiotherapy and chemotherapy will induce more gonadotoxicity than either modality alone. Although efforts to prevent gonadal toxicity in cancer treatment are routinely applied, sperm cryopreservation remains the gold standard to maintain male fertility after cancer survival. Fertility preservation for prepubertal boys presents the greatest problem due to the absence of mature sperm in their gonads. In this area, research efforts are concentrated on cryopreservation of immature gametes and, in particular, techniques for their maturation and proliferation after thawing.

Key words: Cancer, Cancer treatment, Male infertility, Sperm cryopreservation

INTRODUCTION

Advances in oncologic therapy have over the last several years led to improved survival outcomes for patients suffering from cancer. For men of reproductive age or younger, one of the key concerns encountered in the consideration of life after cancer treatment is their ability to father children. Studies have demonstrated that more than 50% of these young male survivors will desire paternity after treatment, including 75% of those who were childless at the time of diagnosis.[1] Therapeutic modalities such as chemotherapy and radiation therapy are highly effective in treating cancer, but their gonadotoxic side effects can severely impair fertility in an agent- and dose-dependent way.[2]

The importance of consistently addressing the issue of fertility preservation in the course of cancer diagnosis and therapy has been stressed, with organizations such as the American Society for Reproductive Medicine (ASRM) and the American Society of Clinical Oncol-

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ogy (ASCO) having issued formal recommendations urging clinicians to inform their patients about the potential impact of cancer treatments on fertility and to offer solutions for fertility preservation, including sperm cryopreservation when necessary.[3,4] Although sperm banking before the onset of cancer treatment is frequently advocated, circumstances that preclude the successful cryopreservation of sperm may exist in some cases.

The resumption of spermatogenesis after different kinds of therapy tends to be unpredictable, and studies of spermatogenesis in long-term cancer survivors have demonstrated evidence of persistent azoospermia or severe oligozoospermia in up to 24% of patients.[5] On the other hand, the eventual return of sperm production in many post-treatment cancer patients prompts the question of whether post-therapy sperm is an option for conception, either naturally or via assisted reproductive technologies.

The aims of this review are to discuss the pathophysiology of male infertility caused by cancer, and especially by cancer treatment, to present the means of prevention and maintenance of fertility, mainly by sperm cryopreservation, and to discuss the issue of cryopreserving and maturing thawed immature gametes collected from prepubertal testis of boys before cancer treatment.

PATHOPHYSIOLOGY

Testicular cancer

Testicular cancer represents 1% to 1.5% of male neoplasms and, in general, 5% of urological tumors, with 3-10 new cases occurring per 100,000 males every year in Western societies. An increase in the incidence of testicular cancer was detected during the 1970s and 1980s, especially in Northern Europe, and there has been a clear trend towards an increase in testicular cancer incidence in the last 30 years in the majority of the industrialized countries of North America, Europe and Oceania, though surprising differences in incidence rates between neighboring countries have been observed. Data from the Surveillance Epidemiology and End Results (SEER) Program during the years 1973 to 1998 show a continuing increased risk among Caucasian men in the USA only for seminoma. However, contemporary treatments have dramatically reduced the risk of dying from this disease to about 1 in 5000 patients.[6]

Testicular tumors impair fertility by disturbing spermatogenesis by destruction of surrounding tissue, local secretion of human chorionic gonadotropin and other paracrine factors, intrascrotal temperature elevation and alterations in local blood flow. New vessels can be generated by means of angiogenesis, this having been observed to occur during tumor vascularization. In addition, another process known as tumor-derived vasculogenesis, in which malignant cells give rise to endothelial cells, has also been reported to occur in a number of tumor types. These effects also concern the healthy testis.[7,8] Testicular tissue adjacent to the tumor will also undergo disruption in spermatogenesis, this explaining how testicular tumors cause even more impairment to spermatogenesis than Hodgkin's disease (HD).[9] Congenital cryptorchidism hinders the normal transformation of neonatal gonocytes into type A spermatogonia, which are the precursors for normal spermatogenesis. Abnormal development of gonocytes is followed by the development of carcinoma in situ, which carries a potential risk for growth of testicular cancer. Moreover, the testicular dysgenesis syndrome theory links testicular tumors and infertility by proposing that in utero exposure to stressors and hormonal disrupters during testicular embryologic development alters the normal development and function of the primordial germ cells and results in infertility or cancer, or both.[7,19,20]

Apart from the primary effect of testicular cancer on fecundity, a large multicenter study suggested that fertility decline is mainly due to the cancer therapy rather than to the tumor itself. Of 451 patients with testicular cancer who tried to start a pregnancy, 208 of 228 were able to do so before initiating therapy compared with 110 of 164 after therapy.[10]

Other cancers

Malignancy unfavorably affects fertility through a large number of mechanisms via endocrine and nutritional alterations as well as induction of a hypermetabolic state whereby tumor cells, due to their rapid proliferation and apoptosis, produce large quantities of spermatotoxic metabolites. The stress following cancer or its treatment can result in hormonal alterations that have deleterious effects on sperm production

and function. Moreover, malignancy that involves deficiencies in vitamins, minerals and trace elements, which play a critical role in the maintenance of a male's reproductive capacity, can contribute to male infertility, affecting aspects that range from physical testicular development to spermatogenetic quality.[11]

Tumors may promote an autoimmune response by producing antisperm antibodies, preventing sperm motility, or by releasing cytokines, leading to germ cell and Leydig cell injury.[11] Fever usually associated with Hodgkin's disease (HD) negatively affects semen parameters. Low-grade fevers were associated with asthenozoospermia or oligoasthenozoospermia, while even azoospermia was noted at greater body temperatures.[12,13] Besides the global effects of malignancy on reproductive health, certain malignancies such as HD and germ cell tumors produce direct gonadotoxic effects. However, the effects on semen parameters of other types of cancers of young males such as leukemia and sarcoma remain debatable, with early studies revealing inconsiderable pretreatment semen abnormalities[10] and others suggesting significant differences in these patients compared with healthy donors.[12,14]

GONADOTOXIC EFFECTS OF CANCER TREATMENT

The three main options for cancer treatment are surgery, radiotherapy and chemotherapy. A complete knowledge of these treatment modalities is crucial to enable the selection of the most effective treatment with the greatest cure rate and least detrimental side effects. Preserving the patient's quality of life in the "post-cancer period" remains a paramount target of physicians dealing with cancer.

Surgery

Radical unilateral orchiectomy remains the standard treatment for testicular tumors. After orchiectomy, a 50% decrease in sperm concentration occurs during the first few months and 10% of patients with preoperative normal sperm counts will become azoospermic.[15]

Partial orchiectomy has become a favored option as a method to preserve hormonal and sperm cell production in carefully selected patients. Although primarily used with perfect tumor control results for prepubertal benign teratomas,[16] Heidenreich et al. adopted this technique for adult testicular cancers as well. The guidelines established by the German Testicular Cancer Study Group suggest parenchymal sparing for patients with bilateral testicular tumors or a unilateral tumor in a solitary testis. In these cases strict instructions should be followed to avoid tumor relapse or metastasis. These include patients with organ-confined tumors and with a tumor diameter up to 2 centimeters, who present multiple negative biopsies from the tumor bed and are eligible for close follow-up with high compliance. These patients should undergo adjuvant local radiotherapy postoperatively to avoid local recurrence, especially in the presence of carcinoma in situ. In these cases, a 98.6% disease-free survival rate at a minimal follow-up period of 7 years has been reported.[17]

Moreover, in all cases of nonseminomatous germ cell testicular cancer in patients with preoperative azoospermia, sperm dissection should be done after removal of the testis. In these patients, failure to extract sperm from the excised testis could necessitate a contralateral testicular biopsy, especially in men with second or greater stage of nonseminomatous germ cell testicular cancer. The latter patients have a higher risk of post-chemotherapy germ cell aplasia.[18] Finally, 5% of testicular cancer patients will develop metachronous cancer in the contralateral testis, this supporting the parenchymal-sparing approach of the initial tumor.[19]

In patients undergoing unilateral orchiectomy for treatment of testis cancer serum testosterone is not affected in the follow-up period. However, these patients present higher age-adjusted LH levels than controls, indicating that they may be at risk of developing prematurely reduced Leydig cell function and hypogonadism as well as androgen deficiency syndrome of aging males (ADAM).[20,21]

Retroperitoneal lymph node dissection together with radical orchiectomy is an additional mechanism potentially inducing fertility damage due to the injury of the adjacent sympathetic ganglia, which are responsible for emission and ejaculation. In almost all patients, classic retroperitoneal lymph node dissection involving excision of all retroperitoneal lymph nodes, extending from the T12 to the L3 nerve level

on both sides, ultimately results in anejaculation.[15] In a high percentage of patients with low-stage disease and in selected patients with more advanced disease, modifications in the surgical template and nerve sparing techniques have markedly diminished the frequency of these complications, preserving ejaculation without harmful effects on relapse rates.[22] In these patients rendered anejaculatory after surgery, adrenergic treatment or electroejaculation to stimulate antegrade ejaculation might be a less invasive option for sperm retrieval before proceeding to surgical sperm harvesting for assisted reproduction.

Radiotherapy

Radiotherapy remains a treatment mainstay for many malignancies in men of reproductive age. The gonadotoxic effect of radiotherapy depends on the gonadal dosage and the delivery method. Radiation doses begin to adversely affect spermatogenesis at 0.1-1.2 Gy, with irreversible damage at a 4 Gy dose.[23] Leydig cells are more resistant to radiation-induced injury, withstanding up to 30 Gy.[24] Although recovery of spermatogenesis may start as much as 9 years after treatment,[25] better radiation techniques with more accurate dose delivery and protection of the gonads have allowed complete spermatogenesis recovery in 9-18 months post therapy with doses up to 1 Gy, 30 months with doses up to 2-3 Gy and 5 years with doses up to 4 Gy. While the highest dose limits at which permanent azoospermia becomes inevitable remains uncertain, doses of over 1.2 Gy will definitely increase the time of spermatogenesis recovery if it eventually occurs.[26] Long-term follow-up of patients with Stages I and IIA seminoma 8 years after radiotherapy revealed a 64% natural pregnancy rate, and 50% of those patients had complete recovery of spermatogenesis with a mean sperm concentration of 24 million/ml. None had long-term azoospermia. No correlation was found between either the radiation dose or the interval from radiotherapy and the sperm count.[27]

Combination treatment with radiotherapy and chemotherapy will induce more gonadotoxicity than either modality alone.[19] Additionally to the effects on sperm concentration, irradiation increases sperm DNA fragmentation and that may continue for up to 2 years after treatment. Therefore, it affects fertilization rates even after spermatogenesis recovery.[23] Damage could be due to direct irradiation or, frequently, to scattering radiation during treatment of adjacent tissues. Fractionated radiation dosing, though the most common form of radiotherapy, is more harmful for sperm than bioequivalent single-dose radiotherapy.[28] During radiotherapy for HD or retroperitoneal lymph node metastasis of testicular cancer, the testes receive an incremental dose of scattered radiation, resulting in greater levels of spermatocytic damage. Even though shielding the gonads will decrease the radiation exposure, scattered radiation may still cause significant damage. Bieri et al demonstrated that the gonads typically receive 2-3 Gy with an inverted Y field used for the treatment of HD.[29] Proper adaptation of the irradiation field, careful coning to avoid radiation scatter as well as shielding of the gonads are compulsory cautions. The modern "clamshell" gonadal protection system restricts external radiation scatter more than the older system. As a result, there is a reduction of the overall testicular dose.[27] Moreover, diagnostic imaging using ionizing radiation should be used sparingly in reproductive age men. Post-orchiectomy surveillance by chest x-ray and CT should be limited to well established surveillance protocols.

The effect of prostate radiotherapy on reproductive function has recently been studied. Given that the dose received by the testes from I^{125} brachytherapy of the prostate is close to 0.18 Gy, recent evidence shows that prostate brachytherapy has a minimum or even no effect on spermatogenesis. However, due to the prolonged half-life of the isotopes used, the recommendations are to delay attempts at conception for up to 3-12 months after treatment.[30,31]

Chemotherapy

Gonadotoxicity caused by chemotherapy is due to the fact that it targets rapidly proliferating cells. The deleterious effect of chemotherapeutic drugs on spermatogenesis depends on various parameters such as type, dosage, the initial semen quality and the location of the toxicity in the spermatogenetic cycle.[32] Mutations occurring early in stem cell spermatogonia cause permanent damage in spermatogenesis in comparison to mutations in later stage spermatogonia, which lead to transient spermatogenic disruption.[33] Table 1 details the effects of some of the most frequently used chemotherapeutic drugs.[32,34]

Many chemotherapeutic drugs cross the blood-testis barrier and harm germ cells directly or by causing hyalinization and fibrosis of testicular interstitial tissue.[32]

Combination drug treatments consisting of alkylating agents such as mustine, vincristine, procarbazine and prednisolone constituted the main treatment for HD until the early 1990s. Thus, permanent germ cell depletion was produced, resulting in Sertoli cell-only testicular histological features, even with limited treatment cycles.[26] Other alkylating agents such as cyclophosphamide and isophosphamide caused per-

Table 1. Examples of different cytotoxic agents

Group	Agent	Mode of Action	Degree of Gonadotoxicity
Alkylating agents		Addition of alkyl groups to DNA, altering DNA structure and function	
	Chlorambucil		Prolonged azoospermia
	Cyclophosphamide		
	Procarbazine		
	Melphalan		
	Carmustine		Azoospermia in adulthood after treatment before puberty
	Lomustine		
	Busulfan		Azoospermia likely, but always given with other highly sterilizing agents
	Ifosfamide		
Platinum analogs		Formation of DNA adducts, DNA interstrand cross-links	
	Cisplatin		Prolonged azoospermia
	Carboplatin		Prolonged azoospermia not often observed at indicated doses
Antibiotics	Actinomycin-D	Binding to DNA inhibiting RNA synthesis	Azoospermia likely, but always given with other highly sterilizing agents
	Doxorubicin	Triggering of topoisomerase II-dependent DNA	Can be additive to above agents in causing prolonged azoospermia, but causes only temporary reductions in sperm count when used solo
	Bleomycin	Single- and double-strand breaks in DNA	Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible
Antimetabolites	Fluorouracil	Pyrimidine analog	Only temporary reductions in Sperm count at doses used in conventional regimens, but additive effects are possible
	6-Mecaptopurine	Purine analog	Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible
	Thioguanine	Purine analog	Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible
	Methotrexate	Antifolate	Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible
Plant derivatives	Vincristine Vinblastine	Inhibition of formation of microtubules	Can be additive to above agents in causing prolonged azoospermia, but causes only temporary reductions in sperm count when used solo
	Etoposide	Inhibition of topoisomerase II activity	Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects possible
Miscellaneous	Prednisone	Inhibition of RNA synthesis	Unlikely to affect spermatogenesis
	Interferon	Stimulation of macrophages and natural killer cells	No effects on spermatogenesis

Data from Giwercman et al and Lee et al.[29,31]

manent azoospermia in 80-90% of cases.[35] In order to decrease these side effects, other combinations were used including mustine, vinblastine, procarbazine and prednisolone to produce the same curative result with less toxicity. Unfortunately, the fact is that the majority of the aforementioned combinations still include the alkylating agent mustine, which causes azoospermia in 90% of patients.[36] The first nonalkylating agent combination, adriamycin, bleomycin, vinblastine and dacarbazine that have the same therapeutic efficacy, is regarded as an innovational chemotherapy regimen for the treatment of HD with a post-treatment sperm recovery rate of 90% within 1-5 years after treatment.[37] The 5-year failure-free survival rate for the latest combination was 61% compared with 50% for those receiving the more toxic combination.[38] In a recent study, the follicle-stimulating hormone (FSH) level was measured as a marker of testicular dysfunction in 355 patients treated for HD using three different treatment modalities. FSH levels increased in 3% of patients receiving radiotherapy alone, 8% of patients receiving nonalkylating agents chemotherapy and 60% of those receiving alkylating agents chemotherapy.[39] Modern treatment regimens including bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone for advanced stage HD presented high overall response rates, but 90% of patients developed azoospermia.[40] The disease stage defines the appropriate chemotherapeutic regimens for germ cell tumors. The most common chemotherapeutic regimens for metastatic germ cell tumors are bleomycin, etoposide and cisplatin. Although platinum-based regimens may result in impermanent azoospermia, spermatogenic recovery may begin between the second and fourth year after therapy is completed.[22] Fifty percent of patients recover spermatogenesis within 2 years and 80% within 8 years.[26]

Dose reduction and alternative regimens are investigated in clinical trials in order to reduce the drug-related toxicity without compromising the cure rate. The pretreatment clinical features of prognostic value, including tumor pathologic features, primary site, metastatic sites and serum tumor marker levels, have been used for stratification of various chemotherapeutic regimens. Approximately 90% of patients with good prognosis achieve complete remission with either four cycles of etoposide and cisplatin or three cycles of cisplatin, etoposide, and bleomycin. Conversely, for patients with intermediate- and poor-risk germ cell tumors, four cycles of bleomycin, etoposide and cisplatin are still the gold standard.[41] New chemotherapy combinations, including gemcitabine and paclitaxel, oxaliplatin and paclitaxel, gemcitabine and cisplatin, are producing significant response rates in metastatic germ cell tumors refractory to cisplatin-based chemotherapy. However, these drugs obviously increase toxicity in heavily pretreated patients, while our knowledge about their toxic effects on the reproductive system is still limited.[42] A study conducted in 22 patients with advanced-stage testicular cancer who had received a 5-day treatment combination of bleomycin, etoposide and cisplatin revealed a high percentage of DNA fragmentation in comparison to pretreatment levels.[43] Other recent developments such as epidermal growth factor receptor or vascular endothelial growth factor inhibitors also need more studies on their reproductive side effects.

Dose-dependent Leydig cell dysfunction after chemotherapy is proven, although these cells are more resistant to chemotherapy and radiotherapy damage due to their lower turnover rate. Of the patients treated with a high dose of cisplatin 600 mg/m^2, 45% had Leydig cell dysfunction compared with 27% of patients treated with lower doses. The serum testosterone levels remained unaffected after bleomycin, etoposide and cisplatin regimens.[22] Advances in chemotherapy regimens will hopefully allow targeted therapy with the minimal appropriate dose and toxicity.

PREVENTION

The protective effects of gonadal shielding or removing testes from a radiation field are obvious. Thus, preservation of gonads function could be achieved if therapy was targeted only at malignant cells. Modern protocols are aimed in this direction.

Over the past few years research has been focused on cytoprotective strategies to minimize the side effects of cancer treatments on fertility. Strategies to optimize fertility can be used before, during or after treatment. Early efforts focused on prophylactic down-regulation of the pituitary gland by either gonadotropin-releasing hormone (GnRH) agonists or testosterone to induce a quiescent state in the

gonads, making them less susceptible to damage. It might be possible to decrease the sensitivity of the stem cells to chemical and radiation injury by stopping spermatogenesis, this being indicated by the fact that the prepubescent testis is less sensitive to gonadal toxicity than the active adult one.[44] The preservation of spermatogenesis can be supported by GnRH agonists, while, in addition, as has been shown in animal studies, GnRH analogs, provided these are administered immediately after initiation of specific cancer therapies and for the first 10 weeks, can also play a critical role in faster spermatogenesis recovery.

Specifically, using GnRH analogs in a rat model, germ cell repopulation occurred at a rate of 90% and 100% after 20 weeks of procarbazine treatment or 3.5Gy irradiation, respectively.[45] However, cytoprotective strategies are as yet unevidenced in humans.

RECOVERY OF HUMAN SPERMATOGENESIS AFTER CANCER THERAPY

The survival and ability of mitotically quiescent spermatogonia (type Adark) to resume mitotic activity and to produce differentiating spermatogonia (type Apale) affect spermatogenesis recovery after a cytotoxic treatment. In men, recolonization of surviving spermatogonia can first be detected 6 months after a dose of 0.2 Gy, 9-18 months after a dose of 1 Gy and more than 4 years after a dose of 10 Gy.[46,47,65] The complete wastage of spermatogonial stem cells after radiotherapy is thought to be the main cause of permanent infertility. Observations in adult monkeys revealed that testicular irradiation with doses of 0.5-4 Gy leads to an immediate decline of Apale spermatogonia, while the number of Adark spermatogonia remains initially unchanged.[48,49] Following irradiation, the quiescent Adark spermatogonia start to proliferate, this, at higher doses of irradiation, being followed by decrease in number of both Apale and Adark spermatogonia.

The type of therapy affects spermatogenesis after allogenic Hematopoietic Stem Cell Transplantation (HSCT). One-third of adult HSCT patients receiving high-dose cyclophosphamide treatment had sperm in the ejaculate after a recovery period of 1 year and 80% in 7 years after treatment. These patients given cyclophosphamide combined with busulphan or thiotepa presented initial sperm recovery after 3 years and 50% of them had sperm in the ejaculate after 7 years. Therefore, recovery after HSCT may be underestimated if sperm samples are evaluated too early.[47]

TREATMENT

Sperm cryopreservation

Sperm cryopreservation (sperm banking) is an established fertility preservation method that should always be the first-line option for men of reproductive age.[50] Assisted reproduction techniques enable conception in men with severe oligospermia. Intracytoplasmic sperm injection (ICSI) with cryopreserved sperm succeeds in up to 50% of cancer patients.[51] The use of cryopreserved sperm for in vitro fertilization (IVF) and ICSI produces results equal to fresh sperm.[52] It is the physician's role to inform the patient about this hopeful option.

It is notable that in 20% of Tanner II boys spermiogenesis has already started allowing cryopreservation of sperm from that age.[53] Thus, pubertal boys with testis volumes over 10-12 ml should give semen samples before cancer therapy.[54,72] It is safer to obtain 2-3 ejaculates per patient because quality of samples in cancer patients is usually lower. Moreover, sperm should be collected prior to cancer therapy initiation, since sperm quality after treatment is always worse.[55] Electro-ejaculation, penile vibratory stimulation and search for spermatozoa in urine samples or testicular sperm extraction from testis biopsy are useful alternatives for spermatozoa retrieval for boys unable to ejaculate.[56] A post-masturbation urine sample is easy to collect as well as being less invasive.[54,57]

In many patients, the sperm banking option is not considered for a number of reasons, including urgency to initiate treatment, cost, lack of adequate facilities and poor prognosis of the underlying condition.[58] Low sperm quality in pre-treated cancer patients has been a poor prognostic factor for sperm cryopreservation but advances in assisted reproduction techniques (ICSI) have revised criteria for this option.[56,58] Testicular extracted spermatozoa have equal value in ICSI attempts to those from ejaculated sperm.[59]

Cryopreservation of testicular tissue

In prepubertal boys there is the potential option

of cryopreservation of testicular tissue or isolated germ cells being used for fertility preservation. In one study, sperm were recovered in 14 of 31 azoospermic patients with malignant lymphoma or testicular germ cell cancer.[60] Recent studies have shown that immature testicular tissue preserves its function after cryopreservation. Cryopreservation using dimethyl sulfoxide as a cryoprotectant was found to be able to maintain spermatogonia, Sertoli cells and stromal compartments. Cryopreservation of spermatogonia of adolescent and adult men, who may already be azoospermic at the moment of cancer diagnosis, can be achieved if there is proper storage of gonadal tissue and an optimal amount of collected material.[61]

Appropriate freezing protocols are necessary to guarantee the survival of the spermatogonial cells. Testicular tissue can be cryopreserved as a cell suspension or in the form of tissue. Cell suspensions are thought to be easier to cryopreserve, but preparation of cell suspensions requires mechanical or enzymatic digestion of tissue, which can compromise cell survival.[62] For human testicular cell suspensions, a post-thaw viability of 60% was achieved, regardless of the cryoprotective agent.[62,63] Cryopreserved and thawed testicular tissue pieces can be used in testicular xeno and autografting. Cryopreservation of testicular tissue preserves cell-to-cell contacts between germ cells and maintains the stem cell niche necessary for their survival. Cryoprotection with ethylene glycol and dimethyl sulfoxide (DMSO) and slow-programmed freezing have been used for cryopreservation.[64,65] DMSO has proved to preserve better structures between germ cells[66,67] and has most effectively maintained tissue capacity to start spermatogenesis in non-human tissue.[68] Moreover, slow-programmed freezing seems to better maintain morphology.[69] Further modification of this protocol with addition of sucrose managed to reduce the spermatogonial and Sertoli cell loss during freezing-thawing. In addition, spermatogonia increased after orthotopic xenografting.[70]

A recent study investigated testicular biopsy side effects from boys with solid tumors at high risk for treatment-related gonadal damage.[36] No adverse short-term[58] or long-term effects[49,74] were evident on biopsy. However, other complications may follow gonadal biopsies among leukemic patients. It is known that traumatic lumbar puncture may cause leukemia relapse in the central nervous system.[71,72] Therefore, the testicular biopsy involves potential risk at the time of the onset of leukemia.

Another question is the timing of biopsy. A recent study searched the effects of lymphoblastic leukemia and its treatment on spermatogonia in 28 prepubertal testicular biopsies and testicular function after sexual maturation.[49] Therapy without cyclophosphamide had no significant effects on spermatogonial numbers. Surviving germ cells have been a base for recovery of spermatogenesis in non-irradiated boys.

Only the necessary amount of testicular tissue has to be removed for cryopreservation. According to morphological studies, it is estimated that one testis of a 10-year old prepubertal boy contains approximately $83x10^6$ germ cells.[73] In the testis of the juvenile rhesus monkey (16-19 months of age) there are approximately $14x10^6$ Adark spermatogonia, all of which are putative spermatogonial stem cells. A mean biopsy size of 0.34 mm^3, used in the study of Kvist et al, which is 10% of the testicular volume of the juvenile rhesus monkey, would maximally result in a $14x10^5$ Adark spermatogonia collection.[74]

Due to the fact that many men with cancer and/or undergoing cancer treatment may become permanently infertile, these patients need to be informed not only about fertility preservation options but also as to other alternatives, such as adoption or sperm donation.

CONCLUSION

Cancer, and especially its cytotoxic treatments, may impair male fertility in numerous ways, thus excluding these men from the opportunity to father children. Improvements in cancer treatment toxicity and selective deliveries of the therapeutic agents yield better outcomes, reduce sperm damage and ensure faster recovery of spermatogenesis. Nevertheless, a great deal of research work still needs to be done in this area.

Meanwhile however, all male patients who are keen to become fathers after treatment should be counselled properly by a fertility specialist regarding the potential risks of their disease and the side effects of its therapy, along with the potential to

overcome all these hurdles. To date, sperm banking has been the only clinically available method: this can certainly preserve mature spermatozoa from men of reproductive age to be used for IVF. With regard to the latter, numerous investigations are ongoing to improve safety and efficacy.

Fortunately, in many boys who are in early puberty spermiogennesis has been started, allowing cryopreservation of sperm. In younger boys, fertility maintenance is still a problem and efforts for improving techniques for testicular tissue or spermatogonial cryopreservation and transplantation and testis xenografting are being carried out. However, at the moment they are not routinely applied for clinical reasons.

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