Sperm cryopreservation in cancer patients: a review

Cryopreservação de espermatozóides em pacientes com câncer: revisão da literatura

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ABSTRACT
Due to remarkable advances in the treatment of childhood cancer, we have seen great improvements in life expectancy, with up to 80% of children surviving their disease, resulting in a growing population of adult long-term survivors of childhood malignancies. Although oncological treatments are highly effective, a major concern is their adverse impact on fertility. In this review we will summarize the literature regarding sperm cryopreservation in cancer patients.

Keywords: cancer, spermatogenesis, cryopreservation, fertility.

INTRODUCTION
Cancer is the leading cause of death in the world, accounting for 7.6 million mortality cases in 2005. Cancer treatments (surgery, radiotherapy and chemotherapy) are undertaken to remove malignancies, prolong the patient’s life and improve their quality of life. Due to remarkable advances in the treatment of childhood cancer, we have seen great improvements in life expectancy, with up to 80% of children surviving their disease, resulting in a growing population of adult long-term survivors of childhood malignancies (Brenner et al. 2007). It is therefore estimated that, by the end of 2012, 1 in 250 young adults aged 20–29 years will be a cancer survivor. Although oncological treatments are highly effective, a major concern is their adverse impact on fertility. Currently, available drugs to prevent testicular damage from cytotoxic therapy have not proved helpful in humans so far. However, improved therapeutic regimens using less gonadotoxic protocols could enable spontaneous recovery of spermatogenesis, but their use is not always possible without compromising patient survival. In fact, since rapidly dividing cells are the target of chemotherapy and radiotherapy, these treatments act not only on cancer cells, but also on germ cells. Damage to reproductive function is a very frequent and well documented side effect associated with the treatment of malignant tumors. The first work describing chemotherapy-induced azoospermia was published in 1948 (Spitz 1948) and it has been suggested that the effects might be transient or permanent depending upon the individual variability in the sensitivity to reproductive damage. The severity of damage is dependent on the type of chemotherapy or radiotherapy, the treatment protocol and the age and gender of the patients.

The increasing success of cancer treatment and determined efforts to improve the quality of life after successful treatment has turned attention to the preservation of reproductive function, especially in young patients. Concern for future fertility is high among individuals newly diagnosed as having cancer. Approximately three-quarters of men and women younger than 35 years who are childless at the time of cancer diagnosis desire children in the future (Schover et al. 1999).

Fertility preservation is an emerging field that encompasses a variety of fertility therapies for patients anticipating medical treatment that could affect future reproductive outcomes. Although most frequently associated with cancer treatment, fertility preservation has also been used for medical conditions like lupus, glomerulonephritis, and myelodysplasia, as well as in adolescent females with conditions known to be associated with premature ovarian failure, such as Turner mosaicism. Current available strategies to preserve fertility are limited and vary by age and sex. Prepuberal males options include: (i) minimizing the testicular damage from cancer treatment or protecting spermatogonial stem cells in vivo; and (ii) cryopreserving testicular tissue prior to gonadotoxic treatment in the form of either cell suspension, tissue fragments or whole organ. For postpuberal males, sperm and embryo cryopreservation are well-established and effective methods of fertility preservation.

In this review we will summarize the literature regarding sperm cryopreservation in cancer patients.

FERTILITY PRESERVATION
As discussed above, cancer therapy can result in subfertility or sterility due to gonad removal or permanent damage to germ cells from adjuvant therapy. Loss of fertility in adult life is a major psychologically traumatic consequence of cancer treatment. Indeed, in a quality-of-life analysis of former oncological patients, about 80% viewed themselves as potential parents, and the vast majority of younger cancer survivors saw their cancer experience as pivotal in preparing them to be better parents (Schover et al. 1999). Therefore, since post-therapy recovery of spermatogenesis remains unpredictable, it is important to inform patients facing infer-
ility as a side effect of their treatment of all the options available to preserve their fertility.

In order to reduce the deleterious effects of gonadotoxic therapies, different strategies have been tested, such as testicular preservation and the use of cytotoxic drugs. Limiting radiation exposure by shields or removing the testes from the radiation field should be implemented whenever possible. Gonadal protection through hormonal suppression is based on the principle that disruption of gametogenesis renders the gonads less sensitive to the effects of cytotoxic drugs or irradiation. Promising results were obtained in rodents (Shetty and Meistrich 2005), but not in non-human primates (Boekelheide et al. 2005) or humans (Brennemann et al. 1994; Blake et al. 2007), except in one clinical trial (Masala et al. 1997) where only moderate stem cell death was induced by chemotherapy. Anti-apoptotic agents (Otal et al. 2004; Carmely et al. 2009) and other cytoprotective substances (Lirdi et al. 2008; Okada et al. 2009) have also been used with partial success in rodents but not in humans. In conclusion, no effective gonadoprotective drugs are so far available for use in humans and studies aiming to identify factors regulating spermatogonial proliferation are therefore required to find novel targets for in vivo spermatogonial stem cells protection. Another potential alternative for preserving fertility in prepubertal boys involves storage of testicular tissue, in the hope that future technologies will allow its safe utilization. As prepubertal testicular tissue contains spermatogonial stem cells from which haploid spermatozoa are ultimately derived, these cells can either be cryopreserved as a cell suspension or in the form of tissue. However, although cryopreservation of testicular tissue is offered in some centers, it is still considered experimental; potential future uses include in vitro maturation of spermatagonia into spermatocytes or germ-cell transplant into native testicular tissue. Patients must be counseled that this technology is still being developed, and potential use of specimens is unlikely for several more years. Cell suspensions have been developed with a view to facilitating cryopreservation, as cell heterogeneity in tissue pieces renders tissue freezing more challenging. Preparation of cell suspensions requires mechanical and/or enzymatic digestion of tissue, compromising cell survival and cell-to-cell interactions necessary for cell proliferation and differentiation. For human testicular cell suspensions, post-thaw viability of up to 60% was achieved (Brook et al. 2001; Hovatta 2001). However, whether it is better to produce cell suspensions before or after cryopreservation is still unclear.

Cryopreservation of testicular tissue pieces may be considered as an alternative method capable of maintaining cell-to-cell contacts between Sertoli and germinal stem cells, thereby preserving the stem cell niche necessary for their survival and subsequent maturation. Other advantages of this method may be preservation of the Sertoli cells, since there is evidence of their reversion to a dedifferentiated state as a consequence of chemotherapy, and Leydig cells, whose preservation may be useful to alleviate the hormonal imbalance caused by cytotoxic therapy. Because of the complexity of the tissue architecture, cryopreservation protocols must strike a balance between optimal conditions for each cellular type, depending on the water content, size and shape of cells, and the water permeability coefficient of their cytoplasmic membrane. In addition, problems can arise when extracellular ice forms, as it can cleave tissues into fragments. None of the above mentioned approaches have proved efficient and safe in humans. The only established method to secure fertility in male cancer patients before gonadotoxic therapy is semen cryopreservation, which in time can be used for assisted reproduction technologies (ART).

SPERM CRYOPRESERVATION

Sperm cryopreservation provides a useful and effective method in infertility management for many men. In fact, cancer patients can use cryopreservation to preserve their fertility prior to treatments such as radiation and chemotherapy. The processes of freezing human semen and achieving successful fertilization via intrauterine insemination were established many decades ago (Bunge and Sherman 1953). At that time a successful, practical technique for cryopreservation of human spermatozoa was introduced by the demonstration that the sperm, after being frozen and stored in dry ice (~78°C), were capable of fertilization and the subsequent induced development of normal progeny. The introduction in 1963 of a method for freezing human semen in liquid nitrogen vapor and its storage at ~196°C was followed by reports of normal births with its use. The basic principles of technique refined since 1953 have proved suitable for the establishment of clinical cryobanks, which have resulted in normal, healthy offspring in various parts of the world.

During the last 30 years several improvements in sperm cryobanking have occurred. Storage in liquid nitrogen has become the standard and extenders containing cryoprotectants have been added to the medium. These extenders have several functions, including: (i) optimizing osmotic pressure and pH, (ii) providing an energy source to prevent undesirable use of intracellular sperm phospholipid, (iii) preventing bacterial contamination by including an antibiotic and (iv) allowing for semen dilution while offsetting the deleterious effect on survival produced by high dilution. Before the in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) era, cryopreserved semen was used only for intrauterine inseminations. However, because of the deleterious effects of the freezing and thawing of the semen, the post-thaw quality was often not good enough for intrauterine inseminations. The introduction of new, sophisticated ART during the last decades such as IVF and ICSI overcame these severe sperm concentration and motility problems. These techniques provided patients, who banked semen and became infertile after treatment, with a reasonable chance of conception.

Although complex techniques have improved fertilization potential of the thawed sperm, the freezing and thawing methods expose spermatozoa to much physical and chemical damage, and several improvements have been made to the process of cryopreservation and thawing.

SPERM CRYOPRESERVATION TECHNIQUE

The most commonly reported detrimental effect of cryopreservation on human spermatozoa is a marked decrease in motility. It occurs despite many advances in cryopreservation methodology. The primary cause of cellular damage during cryopreservation is that the formation of intracellular ice. However, cell survival depends on the nature of the suspending medium, and understanding the profound protective effect of this medium has led to the development of numerous protective agents. Cryoprotectants are low-molecular-weight and highly permeable chemicals that serve to protect spermatozoa from freeze damage. There are four main known cryoprotectants: glycerol, ethylene glycol, dimethyl sulphoxide and 1,2-propanediol. Cryoprotectants act by decreasing the freezing point of a substance, reducing the amount of salts and solutes present in the liquid phase of the sample and by decreasing ice formation within the spermatozoa.

Before cryopreservation semen samples are collected by masturbation after 2 or 3 days of abstinence and liquefied at room temperature. Usually, semen analysis is performed prior to processing for cryopreservation. There are two main freezing techniques used in sperm cryopreservation: slow freezing and rapid freezing (vitrification).

The slow freezing method may be manual or automated involving a semi-programmable freezer. It is performed by simultaneously decreasing the temperature of the semen while adding cryoprotectant in a stepwise manner and eventually plunging the samples into liquid nitrogen.

In particular, control of the cooling rate is often primitive. Although sperm have been frozen successfully with a number of manual techniques, including the dropping of semen on to dry ice to form pellets and the holding of semen in the vapour phase of liquid nitrogen, significant differences in cooling rate between different samples can be...
be a problem. The resulting straw-to-straw variation and loss of viability may not be important where sperm counts are normal, but in the case of oligozoospermic or asthenozoospermic samples these losses may be highly significant. Therefore, programmable freezers have proven valuable in reducing variability between freezes, despite the increased cost associated with the purchase of equipment.

Improvements in cryopreservation of human spermatozoa have been attempted in the past by the use of different cryoprotectants and extenders, and in particular, by altering the cooling rate, usually a linear reduction in temperature with time.

The cooling rate dependency of cell recovery of many cell types may be predicted from computer models of cell osmotic behavior during freezing. However, the predicted results with spermatozoa have not been in agreement with experimental observations. For example, although conventional models have suggested that human sperm cells are normal, but in the case of oligozoospermic or asthenozoospermic samples these losses may be highly significant. Therefore, programmable freezers have proven valuable in reducing variability between freezes, despite the increased cost associated with the purchase of equipment. Improvements in cryopreservation of human spermatozoa have been attempted in the past by the use of different cryoprotectants and extenders, and in particular, by altering the cooling rate, usually a linear reduction in temperature with time.

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changes in temperature, volume changes associated with the movement of water, cryoprotectants and osmotic stress due to increased salt concentration.

During cryopreservation, the initial cooling process causes extensive chemical and physical damage to sperm cell membranes due to changes in lipid-phase transition and/or increased lipid peroxidation. It is well established that the production of reactive oxygen species leads to an increase in lipid peroxidation after cryopreservation and that this event is associated with a loss of sperm motility. As previously suggested (Chatterjee and Gagnon 2001), the injury to human spermatozoa induced by conventional cryopreservation occurs mainly during thawing and has been related to diminishing sperm quality. The initial cellular damage during cooling and/or structural damage to the cytoskeleton and/or antioxidant enzymes during cryopreservation.

Cryopreservation of human spermatozoa is also known to have negative effects on sperm motility and velocity due to acrosomal leakage and degeneration. It has been reported that irregular interaction between DNA and nuclear proteins can lead to impaired motion parameters in spermatozoa. Normozoospermic semen samples appear to be more tolerable to damage induced by freezing and thawing compared with oligozoospermic samples. It has been reported that more spermatozoa could be recovered after five refreeze–thaw cycles in normozoospermic samples and after two refreeze–thaw cycles in oligozoospermic specimens samples (Verza Jr et al. 2009). It has also been reported that up to half of men presenting with testicular cancer or lymphoma have impaired semen quality (O’Connell et al. 2002) and some investigators have shown that damage to sperm cryopreserved from cancer patients is more severe than that from normal donors (Hallak et al. 2000). However, other researchers have demonstrated a similar decline in quality after cryopreservation in control and cancer groups (Agarwal 2000). Rofeim and Gilbert (2005) demonstrated that sperm integrity remains unchanged after the initial cellular damage induced by the freezing process. Sperm from men with lymphoma and testicular cancer also tolerated the cryopreservation well up to 5 years. In addition it was reported that refreezing of human semen by the technique of liquid nitrogen vapor allows the retrieval of viable spermatozoa after thawing in cancer patients (Verza and Esteves 2008). Advances in cryopreservation techniques and better initial semen quality in cancer patients, perhaps due to early referral and semen collection before the start of gonadotoxic therapy, may explain an increased tolerability of sperm to cryoinjury. Moreover, with advanced ARTs only few spermatozoa are needed to achieve fertilization. As Hallak et al. (1998) demonstrated, after a median storage time of 49 months for cancer patients, the pregnancy rate per cycle of ART was 4%. This finding is significant for cancer patients where infertility can last up to 5 years after radiotherapy or chemotherapy.

Cryopreservation Prior to Cancer Treatment

Cryopreservation is an integral component of fertility preservation management in cancer patients and much of its successful application will affect success rates of ARTs. As described above, freezing and thawing semen further reduces sperm count, motility and viability. Although, additional samples and longer abstinence periods may be used to achieve higher total sperm counts, the need to initiate lifestyle change before therapy urgently often becomes a barrier in the process.

Semen banking should ideally be done before the start of cancer treatment. Theoretically semen collection and storage is feasible after the initiation of chemotherapy and radiation therapy, at least until azoospermia ensues. However, it is advisable to wait for 12–18 months because of the time taken for the recovery of spermatogenesis and significant increase in the frequency of sperm aneuplody persisting for 18 months or more after initiation of anti-cancer treatment. Post-pubertal men are generally able to ejaculate, however, some young cancer patients may not be able to produce a sample by masturbation. A strong vibrator or a rectal electric probe can be used to stimulate ejaculation in these boys; however it should be used under anaesthesia to avoid pain. Another concern with electroejaculation is the possible reduction in sperm motility.

In 2006, the American Society of Clinical Oncology published guidelines recommending that oncologists discuss the possibility with reproductive-age cancer patients and offer referral for fertility preservation consultation and therapy (Lee et al. 2009).

Despite these measures, referral patterns are still inconsistent in many centers, even large multidisciplinary ones, and many reproductive-age patients still start treatment without discussion of or opportunity for fertility preservation. Nearly half (45%) of oncologists surveyed at one large university Medical Center about effective qualitative changes in this field center reported never referring patients to a reproductive endocrinologist for fertility consultation (Forman et al. 2009).

In fact, although it is well established that fertility in adult life may be severely impaired by gonadotoxic therapies, the awareness of most oncologists of this subject is still insufficient. Several studies have described the lack of knowledge on the part of oncologists about ART.

The success rates of IVF and ICSI treatments using cryopreserved semen are currently almost as high as those using fresh semen. However, it has been also reported that oncologists are not concerned with the impact of freezing and thawing semen on sperm and spermatozoa before chemotherapy (Arcad et al. 1999) specially because of the relatively small number of men making use of it following completion of treatment is less relatively low. In a previous report on the use and outcome of cryopreserved semen of cancer patients, Van Casteren ey et al. (2008) described an average success rate of achieving parenthood using cryopreserved semen of at least 54%. Other studies reported a success rate ranging from 33% to 73% (Revel et al. 2005).

In a recently published work (Bonetti et al. 2008), we retrospectively evaluated the semen characteristics and attitudes of men patients who had sperm banking before cancer treatment. For this, 98 male cancer patients were referred to our center for sperm banking before receiving potential gonadotoxic therapy, chemotherapy, and/or radiotherapy. Patients were asked to collect semen samples in a minimum of 30 days after testicular cancer patients whose sperm concentration had started chemotherapy immediately after enrolment into the sperm cryopreservation program; those in the latter category collected only one or two samples. Demographic parameters, semen characteristics, destination of sperm banked samples and questionnaires answered by the patients regarding cryopreservation time were evaluated. The cancer diagnoses were testicular (56.1%), prostate (15.3%), Hodgkin’s lymphomas (9.2%), non-Hodgkin’s lymphomas (7.1%), leukemia (3.1%) and other malignancies (9.2%). Patients with testicular cancer presented lower sperm concentration; however, there were no differences with the percentage of normozoospermic patients among cancer type groups. A shorter time between cancer diagnosis and sperm banking was observed for testicular and prostate cancer patients. Nevertheless, more than 80% of the sperm samples remain cryopreserved in our sperm bank.

In our study, testicular cancer patients more frequently requested sperm cryopreservation, followed by prostate cancer least. Also, we found that the mean time from diagnosis of cancer to the semen collection to cryopreservation was 4.5 months, but this period for testicular and prostate cancer patients was shorter. Reasons for this may be a higher level of awareness of the need for sperm banking by the medical team treating patients with cancer.
of the reproductive organs, or by the patient himself, who then influences the awareness level of the cancer site regarding fertility issues. However, despite of the fade of the cryopreserved samples, we recommend that all men who are about to receive cancer treatment that could impair fertility, should be advised that sperm cryopreservation is at this moment the only established and reliable method to preserve fertility. Moreover, semen can be stored for a reasonable long time without affecting pregnancy chance. Cancer patients also need to be made aware of the financial costs involved in the semen cryopreservation and ART as the insurance companies will not always cover these costs.

CONCLUSION
Postcancer quality of life studies revealed male factor infertility as one of the most devastating long-term side effects of anti-cancer therapy. As cancer survival has increased largely during the last decades, oncologists should now be more aware of the long-term quality of life. In fact, many young survivors of cancer want to have children in the future. They also often consider adopting a child rather than living without children. However, some patients are not informed of the deleterious effects of cancer chemotherapy on spermatogenesis, and many patients do not know about the availability of sperm cryopreservation. In recent years, new ART has substantially increased the chances of becoming a father using cryopreserved semen. Male cancer patients might have an impaired pretreatment semen quality, but this certainly should not rule out sperm banking, since with ICSI only a few motile spermatooza are needed. In conclusion, sperm cryopreservation, at this moment, is the only established and reliable method to preserve fertility in men. Cancer physicians should inform and strongly recommend sperm cryopreservation, to all men at risk of becoming infertile after receiving gonadotoxic.

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