Review Article

Oncofertility: technical challenges in immature testicular tissue banking

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Abstract

The gonadotoxicity of cancer therapy ranges from severe to mild effects on spermatogenesis, structural, functional, and genetic integrity of spermatozoa. However, these effects are even more pronounced in prepubertal males due to the susceptibility of the prepubertal testis to cytotoxic therapy. The proliferating germ cells in the prepubertal testis are mitotic in nature, thereby becoming a target for anticancer agents. Immature testicular tissue (ITT) cryopreservation is offered as the only fertility preservation (FP) strategy for prepubertal males facing gonadotoxic risk, though it is still experimental in nature. Multiple cryopreservation protocols have been developed to ensure safe and efficacious storage of the tissue and to enable maximum recovery of germ cells during thawing. The thawed tissue can then be used for fertility restoration by either in vitro spermatogenesis or transplantation to reinitiate spermatogenesis and thereby result in the production of mature spermatozoa. However, there are many challenges to overcome to successfully offer this procedure as an established one. This mini-review gives an overview of the progress in ITT cryopreservation and fertility restoration procedures, along with the other challenges.

Keywords: Challenges, fertility preservation, fertility restoration, immature testicular tissue banking, prepubertal boys

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INTRODUCTION

Improvement in cancer cure rates has led to change in focus from survival rates to quality of life or survivor experience from cancer treatment. Cancer survivors are faced with short- to long-term adverse effects of cancer treatment, the prominent among them being transient or permanent infertility.[1] In prepubertal males, the cytotoxic effects are even more pronounced, due to high susceptibility of the immature testis containing spermatogonial stem and germ cells, that are mitotic in nature. The potential for sperm production in adulthood in these cancer survivors is dependent on the survival, proliferation, and differentiation of these cells into spermatids.[2] Male childhood cancer survivors show a significant reduction in fecundity and are less likely to sire a pregnancy compared to the sibling control subjects.[3] A high prevalence of infertility (46%) has been seen in this patient group when compared to their siblings,[4] especially after treatment with alkylating medications.[5] It is difficult to predict the severity of damage to an individual as the variables for gonadotoxicity due to chemotherapy include the type of drug(s) administered,

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cumulative dose, treatment duration, and age of the patient.[6]

Immature testicular tissue (ITT) banking

In prepubertal boys, ITT is cryopreserved with the intent for future use for fertility restoration through autologous transplantation of tissue or spermatogonial cells or by generation of spermatozoa through in vitro culture of the tissue.[7] This procedure is offered only in an experimental setting with use of an Institutional Review Board (IRB) (The Institute for Research in Biomedicine Barcelona)-approved protocol,[8] for prepubertal males fulfilling the Edinburgh selection criteria.[7] Valli-Pulaski *et al.* have reported that testicular tissue biopsy or cryopreservation can be offered as a feasible method of fertility preservation (FP) in male patients ranging from 5 months to 34 years.[9] Braye *et al.* summarize their experience of ITT banking during 2002–2018, highlighting the importance of the procedure to safeguard the fertility potential of boys with a high risk of germ cell loss and also stating the important challenges to setting up FP for prepubertal boys.[10]

Several international societies have recommended guidelines for ITT banking, with the common indication being malignant disease in childhood.[11,12] The cancers associated with impaired gonadal function even before commencement of treatment are Hodgkin's and non-Hodgkin's lymphoma, testicular cancer, extragonadal germ cell tumors, and other solid tumors.[13,14] Cancer pathology can affect reproductive health through several mechanisms, such as disruption of the hypothalamus-pituitary gonadal axis and consequently the production of reproductive hormones, or by directly affecting the testicular environment by invasion of cancerous cells or through the pro-inflammatory response resulting from the cancer.[14]

While cancer pathology can play a role in gonadal impairment, cancer treatment can result in direct damage to the gonadal cells, causing fertility decline. Cancer treatments such as total body irradiation, chemotherapy with alkylating agents, or conditioning before bone marrow transplantation are known to result in gonadal impairment.[15]

ITT banking can be invasive as well as experimental; hence consideration for FP in prepubertal boys should be based on the extent of risk to gonadal function.[7] Pre-pubertal boys facing high risk of fertility due to treatment can be taken before the initiation of therapy, or in some cases, patients who have already undergone one round of chemotherapy are also recruited to prevent further gonadal damage.[13]

Prepubertal patients with non-malignant disorders requiring hematopoietic stem cell transplantation (HSCT), such as sickle cell disease, bone marrow failure, thallasemia, etc., are also at risk of impaired fertility due to the conditioning with chemotherapy before HSCT. Hence, such patients can also be included in ITT banking.[14] Patients with testicular or sex chromosome disorders such as Klinefelter's syndrome, and cryptorchidism can also be potential candidates for ITT banking.[16] However, in certain conditions, such as Klinefelter's syndrome, clinical results so far are not in favor of ITT banking due to the suboptimal quality of gonadal tissues available during banking.[17]

Current approaches to ITT freezing protocols

Earlier, the purpose of testicular tissue cryopreservation was to effectively cryopreserve the spermiogenic cells, or spermatozoa, from the testis of azoospermic men undergoing infertility treatment; hence, glycerol was used as a cryoprotectant. However, with ITT as a FP strategy in cancer-affected prepubertal boys starting gaining significance, glycerol was no longer found to be optimal for ITT freezing.[18] The rationale for ITT banking was effective preservation and recovery of spermatogonial stem cells (SSC) by minimal cryoinjury to the tissue.[19] ITT cryobanking has been optimized through several approaches since the first trial by Bahadur *et al.*, who used different cryoprotectants such as glycerol and propane-1,2-diol on prepubertal and pubertal human testicular tissue to test the efficacy of cryopreservation by the slow freezing method.[20] Over the years, dimethyl sulfoxide (DMSO) was found to be a more appropriate cryoprotect for ITT, as first revealed by the findings from Keros *et al.*,[21] which was also later confirmed by other groups.[22-24] Wyns *et al.* established a protocol for prepubertal testicular tissue using 0.7 M DMSO and controlled rate freezing (CRF) for efficient freezing of the tissue.[25] A survey of European centers offering FP reported that most centers preferred DMSO and CRF for freezing of ITT.[26]

Vitrification was also tested as a cryopreservation method for ITT, as this method had shown promising results in gamete and embryo freezing. Curaba *et al.* compared conventional slow freezing with vitrification of ITT using 2.8 M DMSO as a cryoprotectant and showed similar tissue morphology post-thaw between the two freezing methods. The group recommended vitrification

as a more cost-effective method of freezing compared to CRF.[27] Poels *et al.* also compared vitrification versus slow freezing but found that the number of spermatogonial cells per tubule was reduced significantly in all groups – fresh, CRF, and vitrified human tissues that were thawed and xenografted into castrated nude mice.[28]

Several studies have also compared the freezing of isolated testicular cell suspensions with testicular tissue and proposed better outcomes with the former method. Brook *et al.*, in 2001, assessed the cryosusceptibility of cell suspensions isolated from testicular tissue of five adult patients, which was the first trial to establish freezing protocol for testicular cell suspensions (TCS).[29] Wyns *et al.* also proposed that TCS freezing might be a better alternative to ITT freezing, which would allow them to bypass the challenges of heat exchange in tissue freezing, thereby facilitating better spermatogonial stem cell survival.[30] Several other studies have confirmed similar findings.[24,31,32] Despite all the improvements made in freezing protocols, there is still no consensus on the optimal method. Further studies are awaited to establish the ideal freezing protocol.

Fertility restoration after ITT

Fertility restoration strategies from cryopreserved ITT are still under development across the world, with a focus on auto-transplantation of testicular tissue or propagated SSCs, and in vitro spermatogenesis.[13] The former method was shown to be successful in animal models where spermatogenesis could be reinstated after infusion of SSCs into the rete testis of mouse.[33,34] Healthy offspring were obtained after SSC transplantation in mice model.[35] Studies have demonstrated that the rete testis is an ideal site for injection of propagated SSC in bovine and primate models as well as in humans.[36,37] Using human SSCs obtained from adult and prepubertal testicular tissue, in vitro propagation and infusion into seminiferous tubules through xenotransplantation have been successfully demonstrated.[38]

Grafting of testicular tissue instead of propagated SSC is another alternative method of fertility restoration.[13] This method allows for the interaction of SSCs with the somatic cells, allowing for spermatogenesis within the natural niche. Xenografting of testicular tissue to ectopic sites has not been successful in animal models in establishing full spermatogenesis, which could be due to a temperature difference between the scrotum and other body sites. Xenotransplantation of human ITT to homotopic sites such as the scrotum or testis of mice has allowed for SSC proliferation and differentiation up to the spermatid stage, as well as better graft survival.[22,39] In non-human primates, healthy monkeys were born from sperm derived from ectopic xenografts through ICSI.[40] Another recent study was able to obtain healthy offspring after ICSI of sperm derived after ectopic transplantation of macaque testicular tissue to castrated immature macaques.[41]

In vitro spermatogenesis, or in vitro differentiation of testicular germ cells, is an alternative method of fertility restoration, especially in patients with malignant hematological diseases who face the risk of contamination with malignant cells during transplantation. Tesarik *et al.*, was the first study to show potential to generate sperm in vitro from the adult testicular tissue of azoospermic patients. Haploid spermatids were derived in vitro, which resulted in healthy babies after a round spermatid injection.[42] Similar findings were observed in another study that attempted to obtain sperm from adult patients with cryptorchidism.[43] However, the outcomes from prepubertal testicular tissue culture have been quite different. Organotypic culture systems using semi-permeable membranes have been used to culture human prepubertal testicular tissue and found that while the system demonstrated survival of spermatogonia and maintenance of seminiferous tubule architecture, complete spermatogenesis could not be re-instated.[44,45]

Complete spermatogenesis in vitro has been reported in animal models, with some studies showing the production of fertile offspring after ICSI with in vitro-derived sperm using an organ culture system.[46] Different culture systems have been developed over time to improve the outcome of in vitro spermatogenesis (IVS) in mice,[47,48] and rat models.[49] In humans, one study attempted to culture tissue derived from adult males with gender dysphoria using chitosan and reported haploid spermatids in the seminiferous tubules in 34 days of culture instead of 72 days.[50] While there have been no studies so far demonstrating sperm production in vitro from human prepubertal testicular tissue, a few studies were able to derive meiotic and post-meiotic germ cells from prepubertal testicular tissue using organotypic culture conditions or 3D culture systems,[51,52] but the functionality of these cells remains to be established.

The lack of further progress in this direction of fertility restoration from ITT emphasizes the need for further research to establish protocols for successful FP and restoration.

Challenges in ITT

The main hurdle of ITT banking is that it is not only invasive but also experimental in nature, owing to the lack of establishment of successful protocols in FP and restoration. This prevents the option from offered to other patients with low or medium risk of infertility. However, treatment strategies can vary based on individual responses to the treatment, allowing for subsequent treatments to involve high-risk gonadotoxic drugs. Also, the literature on the gonadotoxicity of chemotherapy drugs has mostly been extrapolated from animal studies, making it difficult to confirm the risk classification of gondotoxicity.[14,16] Hence optimization of patient selection criteria for ITT is required taking into consideration the above aspects.

In prepubertal boys with hematological diseases, testicular tissue retrieval can be considered invasive as it may have additional complications such as infection or bleeding. Also, the risk of reintroducing the malignant cells during transplantation of tissue or SSCs is also considered to be high during fertility restoration in these patients.[16] Detection of malignant cells before transplantation can be done through methods such as minimal residual disease PCR to detect leukemic cells in an in vitro propagated human SSC population.[53]

The freeze-thaw process during banking and fertility restoration is also expected to affect the germ cells, leading to their depletion or compromise in quality.[19] There is also the concern that genetic and epigenetic stability is likely to be compromised due to the cryopreservation, thawing, transplantation, or in vitro culture. Animal studies have demonstrated the maintenance of genomic stability of germ cells after freeze-thawing[35,54] and even up to second-generation offsprings.[55] However, there have been no studies on prepubertal human testicular tissue to assess the extent of damage caused to the genome and epigenome due to manipulations involved in the FP and restoration processes. This brings about a need for optimization of freeze-thaw protocols to minimise germ cell loss, maintain genetic integrity, and their functionality.

Fertility preservation referrals

While ITT banking is still experimental in nature, it is the currently available technique for prepubertal boys facing the risk of fertility loss recommended by the American Society of Clinical Oncology (ASCO).[56] However, the number of patients availing of this FP option is very low, either due to poor referrals, or a lack of awareness, or a lack of time.[57,58] It has been shown that the majority of healthcare providers involved in oncofertility programmes are unaware of the FP guidelines.[59,60] Also, healthcare providers perceive patient survival and cure from cancer as the primary goals. Therefore, it is felt that FP could interfere with or delay the cancer treatment.[61]

Oncologists and gynecologists or fertility specialists play an important role in a successful oncofertility program; hence, their knowledge of the available FP options is likely to correlate with the frequency of patients opting for FP services.[62] Another major hurdle is the economic burden on patients due to a lack of insurance coverage or funding in developing countries that needs to be addressed.[63] Similar challenges have been reported in developed countries as well, along with other religious or cultural restrictions and legal barriers.[64]

CONCLUSION

ITT banking is the only FP option that is presently offered to prepubertal boys facing a significant risk of gonadal impairment due to cytotoxic therapy. Though there have been major advances in the protocols of ITT cryopreservation as well as in restoration, the technique is purely experimental in nature as of now. Due to societal, emotional, and financial factors associated, the referrals for this approach remain low. There are several challenges that still need to be overcome for the ITT cryopreservation to become a standard FP option.

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Conflicts of interest

There are no conflicts of interest.

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