MADRID
22ND CONGRESS
JUNE 22 - 25 | 2017
European Hematology Association

Fertility preservation in patients with hematological malignancies - Section 3

Fertility preservation in pre-pubertal and adult males

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Take-home messages

- Semen cryopreservation for future use in ART is an effective means of preserving fertility in post-pubertal men at risk of infertility.
- Fertility preservation in prepubertal males remains experimental and currently there are no established clinical options to preserve fertility in this patient group.
- Current research is focused on developing *in vitro* and *in vivo* strategies to preserve fertility for childhood cancer survivors.

Introduction

Childhood cancer rates have increased dramatically (29% since the 1970s) over recent decades and currently 1 in 500 children in the UK will develop cancer.[1] Improved long-term survival (>80% 5 year survival) has resulted in a dramatic increase in the number of young adults experiencing late effects of treatment.[2] One of the most frequent effects is infertility which occurs in the majority of males receiving high-dose alkylating agents, commonly used in childhood cancer.[3] Unlike the situation in females and for adult men, there is currently no prospect of preserving fertility in prepubertal males at risk of infertility because mature gametes are not present until puberty (Table 1).[3] This short review will summarise the current state of the art for fertility preservation in males at risk of infertility as a result of cancer treatment and highlight progress towards potential future clinical approaches for prepubertal boys.

Current state of the art

For fertility preservation in adolescent and adult males at risk of infertility due to cancer treatment there is the established option of semen cryopreservation for future use for insemination, IVF and ICSI.[4] This approach is widely used, albeit with inter-center variation in provision. Despite the fact that in many cancer patients there is a decline in semen parameters, the success rates with *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are similar to those of standard IVF for infertile couples.[4] However, for many patients it is not possible to obtain a semen sample for a variety of reasons. These include religious, cultural and psychosocial factors and many adolescents and young adults face difficulties producing a semen sample as a result of emotional immaturity.[3] For these patients, it may be possible to perform surgical testicular sperm extraction (TESE) for cryopreservation.[4]

For prepubertal patients that have not yet achieved spermatogenesis, obtaining sperm for storage is not possible.[3] Currently, there are no established clinical options to preserve fertility in these patients. Potential approaches might involve protecting the gonad in-situ either by modifying treatment regimens[5] or alternatively by co-administering treatments that may prevent the damage.[6] A number of promising rodent studies have shown that hormonal (e.g. GnRH antagonists, sex steroids) manipulation can protect or restore of fertility; however, the limited evidence in humans does not support this approach for clinical applications.[5] Recent studies involving administration of granulocyte colony-stimulating factor (G-CSF) have demonstrated protection of fertility in animal models, including Rhesus monkeys; however, this approach has not yet been translated into humans.[6]

Over the last decade a number of centers have begun to cryopreserve testicular tissue from prepubertal boys prior to gonadotoxic cancer treatments.[4] This approach remains experimental in the absence of any clinical applications for the cryopreserved tissue.[7] Testicular tissue cryopreservation requires a surgical biopsy, ideally to coincide with a planned theatre procedure. Tissue may be stored according to a number of freezing protocols that have been described for prepubertal testis tissue which demonstrate viability of the spermatogonial stem cells (SSC) after thawing.[8-10] However, in the absence of a proven strategy to restore fertility using this cryopreserved tissue, the true functional capacity of the SSC within the

stored tissue is unknown.

Several approaches have been proposed for subsequent use of cryopreserved prepubertal testicular tissue to generate mature gametes and/or restore fertility.[5] This includes *in vitro* culture of testicular tissue and (xeno) transplantation of testicular tissue or SSCs. *In vitro* culture of neonatal mouse testis using a soft agar system has been shown to result in the generation of functional sperm from the spermatogonia, resulting in the generation of progeny following ICSI.[11] *In vitro* generation of sperm from human prepubertal testicular tissue has not been reported. The safety of *in vitro* generated spermatozoa (e.g. epigenetic stability) is an important consideration for *ex vivo* strategies and therefore transplantation methods may offer an alternative approach. Transplantation of SSC from neonatal mouse testis directly into the seminiferous tubules of a germ-cell ablated mouse has been successful in generating functional gametes that can produce progeny.[12] SSC transplantation has also been successful in Rhesus monkeys for generating sperm that are capable of fertilization using ICSI.[13] Similar to SSC transplantation, testicular tissue (xeno) grafting has also resulted in the generation of functional gametes in rodents[14] and monkeys;[15] however, for prepubertal human tissue xenografts, germ cell differentiation did not proceed beyond spermatocytes.[16] Both SSC transplants and testis tissue transplants have the potential to re-introduce malignancy, which may be a particular issue for hematological malignancies.[17] Therefore, development of these strategies for future clinical use would require robust systems to ensure this cannot occur.

Future perspectives

Over recent decades, significant progress has been made towards developing strategies to preserve fertility in young people treated for cancer. For prepubertal boys facing gonadotoxic treatments there are no clinical options to preserve fertility. The focus for future research should be on developing strategies to protect the gonad in-situ and also on techniques to generate viable gametes from cryopreserved testicular tissue. Generation of functional germ cells from pluripotent stem cells has recently been described[18] although this approach remains in its infancy. For any future strategy that is developed for clinical use, particular attention must be paid to avoidance of re-introduction of malignancy, ensuring the genetic and epigenetic stability of the germ cells, and careful follow-up of offspring.

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Table 1. Options for fertility preservation in males with cancer.

		Prepubertal	Adolescent	Adults
Established	Semen cryopreservation	×	✓*	✓
	Testicular sperm extraction	×	✓*	✓
Experimental	Protection of the *in situ* gonad	✓	✓	✓
	SSC transplantation	✓	✓	–
	Testicular tissue transplantation	✓	✓	–
	In vitro maturation	✓	✓	–
	Pluripotent stem cells	✓	✓	–

**Option for pubertal individuals.*

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