Preservation of fertility in teenagers and young adults treated for haematological malignancies

Title page

Fertility preservation options in teenage and young adults treated for haematological malignancies

Summary

Background: Haematological malignancies account for 28% of new cancer diagnosis in the TYA group with lymphoma (Hodgkin's and non-Hodgkin) making up 19% and leukaemia (acute myeloid and acute lymphoblastic leukaemia) 9%. Malignant disease occurring in the TYA age group represents a unique challenge; it is a period of significant growth and change from a physiological, medical, social, and psychological perspective as well as a time of change from dependence on parent/guardians to independence.

Aim: The aim of the review is to present current thinking and evidence base for fertility preservation options for males and females in haematological malignancies

Main issues covered: The gonadotoxicity of different treatment modalities is discussed. Fertility preservation options have been presented with the evidence of their efficacy and safety. Some of the options such as egg and sperm freezing are established in practice whilst other options such as ovarian tissue freezing is evolving. Testicular tissue freezing in the pre-pubertal age group is experimental.

Authors

Miss Ephia Yasmin (MD), University College London Hospital
Prof. Rod T Mitchell (PhD), MRC Centre for Reproductive Health
Dr. Sheila Lane (PhD), Children's Hospital Oxford

Corresponding author: E Yasmin: ephia.yasmin@nhs.net

Introduction

Over 70% of young adults diagnosed with cancer will be cured of their disease but due to the intensity of treatment many will be left with long term health issues. Loss of fertility and problems with sexual health are among the most distressing long-term outcomes of treatment and are strongly linked with reduced quality of life and mental health problems (Logan et al., 2019, Thouvenin-Doulet et al., 2018[2] Bober et al., 2013[3]).

The term 'teenage and young adult' has no internationally recognised definition. In the UK, the National Cancer Registration and Analysis Service (NCRAS) in collaboration with the Teenage Cancer Trust (TCT), defines the TYA population as aged between 14-24 years and reports data and statistics on incidence and mortality to this convention. This is the definition used in this paper but the salient points are relevant to a broader definition as used in other countries.

Cancer in the TYA group is rare, with about 2-2,500 new diagnosis a year in the UK (Cancer Research UK[4]). A population-based study carried out to quantify the burden of young adult

cancers worldwide identified haematological cancers and brain tumours as the two most common malignancies in this age group (Fidler et al 2012[5]).

Haematological malignancies account for 28% of new cancer diagnosis in the TYA group with lymphoma (Hodgkin's and non-Hodgkin) making up 19% and leukaemia (acute myeloid and acute lymphoblastic leukaemia) 9% (ncin.org.uk[6]). Myelodysplastic syndrome, though rare should also be included amongst haematological malignancies diagnosed in the TYA cohort. Other haematological malignancies such as chronic lymphocytic leukaemia, myeloma and chronic myeloid leukaemia occur in the older age cohort..

Malignant disease occurring in the TYA age group represents a unique challenge; it is a period of significant growth and change from a physiological, medical, social, and psychological perspective as well as a time of change from dependence on parent/guardians to independence. These factors impact on treatment planning, decision making and issues of consent. Haematological malignancies, by their nature are particularly challenging as patients are often very unwell at the time of presentation, with a high burden of circulating disease and the need to start treatment urgently. Whilst discussion of the effect of planned cancer treatment on fertility is standard of care, knowledge of potential fertility treatment options and when they should be offered in haematological malignancies is not always so clear.

The aim of the review is to evaluate the gonadotoxicity of treatments of prevalent haematological malignancies in teenagers and young adults and present fertility preservation (FP) options available to the TYA group. This provides an evidence-based framework to help with fertility discussion and management at the time of diagnosis, relapse/resistant disease and in long-term follow up settings.

Search Methods

A literature search was conducted using PubMed, Embase and national library of medicine (NLM). Articles published in English language between 1Jan,1990 – 30June 2020 were identified. Fertility preservation guidelines: ASCO, ASRM, ESMO, BFS and CCLG guidance were also included. Reference lists of relevant publications identified during the literature search were also scrutinised and citation searches were performed. Search terms used were fertility preservation, haematological malignancy, childhood cancers, teenage and young adult cancers, egg cryopreservation, ovarian tissue freezing, sperm cryopreservation, testicular tissue freezing,

Effect of Haematological Malignancy and Treatment on Fertility

The well established long -term data sets for childhood cancer survivors which report fertility outcomes probably underestimate the situation in the TYA cohort as in childhood cancer a third of cases will be leukaemia which has both a high first line cure rate and relatively low gonadotoxic treatment. In the TYA cohort leukaemia accounts for less than 10% cases with lymphomas, brain tumours and carcinomas, where treatment tends to be more gonadotoxic, predominating. Long-term data sets specific to the TYA cancer group do not exist as they tend to be merged at one end with childhood cancer outcomes and at the other with those specific to adult cancers.

The childhood data sets such as the St Jude's Childhood Cancer Survivors Study, even if slightly underestimating fertility risks for the TYA group do give some indication of the scale of the problem. Analysis of this data reveal that there is about a 48% increased risk of

infertility in survivors compared to their siblings (relative risk [RR] 1·48 [95% CI 1·23-1·78]; p<0·0001), with pregnancy rates being 20% lower than in siblings and male cancer survivors only half as likely to father children.. In survivors <24 years of age at time of treatment, infertility was nearly 3-fold more common (RR 2·92 [95% CI 1·18-7·20], p=0·020) (Barton et al., 2013[9] , Chow et al 2016[10], Green et al 2010[11]). Alkyating chemotherapy agents and radiotherapy including conditioning treatment for stem cell transplants are the main threats to fertility in the treatment of haematological malignancies (Behringer et al., 2013[20].van der Kaaij et al., 2012[21].)

To appreciate the effect of cancer treatment on fertility and plan fertility preservation treatment , it is important to understand normal physiological development of the gonads..

In boys, normal testicular function is dependent on a complex interplay between several cell types including the germ cells and somatic Sertoli and Leydig cells. These cells are present within the testis at birth although they do not reach full maturation to support spermatogenesis until puberty. At puberty, under the influence of the pituitary hormones, the Leydig cells produce testosterone which leads to the development of secondary sexual characteristics and also, in combination with pituitary hormone stimulation of Sertoli cells, initiates and maintains spermatogenesis. (Anderson et al 2015[12]).

In the testes, chemotherapy damages germ cells including the spermatagonial stem cell population, leading to azoospermia. As the testosterone-producing Leydig cells are more resilient to the effects of chemotherapy it is possible to have normal pubertal development post-chemotherapy but still have associated azoospermia (Mitchell et al 2009[15]). It is known that in some cases spermatagonial stem cells can repopulate the seminiferous tubules after chemotherapy-induced damage and recovery of spermatogenesis has been reported in up to 25% patients after stem cell transplant, but this is dependent on factors such as age and myeloablative protocol. (Rovo et al 2006[16]).

In contrast, in girls, the total complement of ovarian primordial follicles (immature eggs) develops during fetal life, reaching a peak of 3 – 7 million at 5 – 6 months in-utero. This pool of non-growing follicle (NGF) is located in the cortex of the ovaries and declines throughout life, such that at birth there are between 1-3 million NGFs,declining by 50% by puberty with menopause occurring when there are less than 1000 NGF left.. The initial stages of development of the primordial follicles are independent of pituitary hormones with follicles that enter the development pathway becoming atretic before maturation. At puberty under the influence of the pituitary hormones a cohort of developing follicles progress through maturation with one or two mature eggs per month surviving to be released. The developing follicles produce anti-Müllerian hormone which can in some circumstances be used as an indicator of ovarian reserve (Wallace and Kelsey 2010[13]).

In the ovary, chemotherapy has both direct and indirect toxic effects on the ovarian reserve. There are several ways in which these gonadotoxic effects may be exerted. The hypothesis is that chemotherapy targets actively dividing cells thereby affecting all growing follicles within the ovary. The loss of the growing follicles may in turn lead to acceleration of recruitment of NGF from the resting pool to replace those lost. This is thought to cause further depletion of the ovarian reserve (Roness et al 2013[14]).

Radiotherapy causes ionizing damage to cells destroying NGF in the ovary and spermatagonia in the testis. A radiation dose of just 2Gy is known to deplete the NGF pool by 50% and 16Gy causes almost immediate infertility in a teenage girl (Wallace and Kelsey 2010[13]) In male patients as little as 0·1–1·2 Gy can impair spermatogenesis with doses 2–6Gy leading to permanent sterility (De Felice et al 2018[17]).

Total body irradiation (TBI) and pelvic radiotherapy in females will also have an effect on the uterus. In pre-pubertal girls this will lead to a very small uterus that may not be able to support a pregnancy (Larsen et a., 2003[18], Gerstl et al., 2018[19]). In post-pubertal girls whilst the size of the uterus may not be affected, the damage to uterine vasculature is likely to lead to an increased risk of miscarriage.

In females, the number of years of fertility post treatment 'the fertility window' will be affected by the size of the reserve at the time of gonadotoxic treatment. In a study of Hodgkin Lymphoma patients receiving alkylating agents those below 30 years at the age of treatment had a 45% risk of immediate post treatment premature ovarian insufficiency (POI) versus 82% in the over 30 age cohort (Behringer et al., 2013[20]). The concept of 'fertility window' is particularly important when considering fertility preservation options in girls and whether fertility preservation should be offered pre or post cancer treatment.

Type of treatment protocol will influence risk. Cumulative risk of POI after alkylating chemotherapy being 60% (95% CI, 41% to 79%) and only 3% (95% CI, 1% to 7%) after non-alkylating chemotherapy (van der Kaaij et al., 2012[21]). The 'cyclophosphamide equivalent dose' (CED) concept based on data from the St Jude's life time cohort study (Green et al 2014[22]) allows comparison of gonadotoxic risk across different treatment regimens. A CED above 5g/m^2 is associated with a significant lifetime risk of infertility in both boys and girls. However, there is considerable variation in individual susceptibility to chemotherapy and therefore additional factors such as age, diagnosis and ovarian gonadal reserve also play a role in determining gonadotoxicity (Anderson et al, 2015[12]). Spermatogonial stem cells on the other hand are susceptible across all ages from pre-puberty to adulthood.

Table 1 summarises the gonadotoxic risk rating of the standard UK treatment regimens commonly used in haematology malignancies

Fertility Preservation Treatment

Cancer and fertility guidelines across the world are aligned in recommending that the gonadotoxicity and risk of infertility of proposed cancer treatment should be discussed with all patients at diagnosis and at times of change of treatment protocol. Referrals for discussion of fertility risk and fertility preservation treatment should be made as early as possible. Whilst the awareness about the need to discuss fertility preservation is increasing it is still not entrenched in practice. A survey amongst stem cell transplant specialists in Europe demonstrated that 87% of the professionals were aware of and informed patients of potential risks to fertility, however, in practice, they referred only 56% of their male and 36% of female patients for consultation about fertility preservation (Alexandroni et al., 2019[23]). Evidence suggests that informing, counselling and addressing fertility concerns are best done at or around the time of diagnosis (Logan et al., 2019[24]). In the TYA population there will be a very diverse mindset about fertility with some younger patients having never considered such issues and being heavily dependent on parents/guardians for decision-making and consent. For others fertility may be considered as overwhelmingly important and they may be completely independent in their decision making about fertility options. Therefore, counselling and consent for fertility preservation treatment must take account of physical and psychological differences that are present across the TYA age-range. This requires sensitive discussions and close collaboration between all specialists involved in the care. Barriers in availing fertility preservation consultation before oncologic treatments include urgent need to commence treatment, lack of medical fitness, lack of training and

knowledge, inadequate (or complete lack of) referral pathways, reluctance from clinicians to prioritise fertility issues and the cost of fertility preservation treatment if public funding does not exist. Where public funding does not exist, patient or parents may not be able to afford fertility treatment which can add extra stress at a time when major life changing decisions have to be made at speed. Good information counselling and active management of fertility throughout the cancer treatment pathway and after care is essential. (Young et al., 2019[25]).

Fertility Preservation Treatment Algorithms in Haematological Malignancy

The fertility preservation options available to patients will also vary depending on level of physical and emotional maturity across the TYA spectrum. Puberty is a complex process and occurs in a continuum spanning several years. In females, the onset of menarche is often used to mark puberty and therefore anyone who has had a period is considered post-pubertal. However, onset of menarche is a late phenomenon in the pubertal process. The hypothalamo-pItuitary ovarian axis takes time to settle and menstrual irregularity is very common in the first 2 years after menarche. It is important to appreciate that even if post-menarchal, processes involved in procuring eggs (ovarian stimulation, monitoring and egg retrieval) may not be easy. Similarly, for pubertal and young adult males, obtaining sperm by masturbation can be challenging. The reproductive medicine specialists and andrologists will be able to make an assessment of the pubertal status with the help of physical examination and endocrine profile. Help from a counselor or psychologist trained in dealing with paediatric and TYA population and fertility are crucial in assisting this population and their parents in the decision making process.

Fertility preservation treatment algorithms for haematological malignancies, including issues that are specific to the TYA population, are presented in algorithms 1 – 3. (Figures 1-3) They synthesise the balance of risks and benefits at each treatment decision point. These are guidelines and every case will need careful consideration and should involve a multidisciplinary team approach of haematologists, oncologists, reproductive medicine specialists, psychologists, patients and parents/guardians.

Fertility Preservation Treatment Options

Female Treatment Options

A) Treatment options prior to cancer treatment

1. *Egg and embryo freezing*

Embryo or oocyte (egg) cryopreservation are established techniques for fertility preservation in adults. However, their use in the TYA population is highly dependant on the physical and emotional maturity of the individual. They require ovarian stimulation in the form of daily self-administered hormone injections followed by collection and freezing of oocytes by vitrification which has been shown to be superior to slow freezing (Martinez et al., 2014[26]). As a result, these options are not available to pre-pubertal patients. A period of about 2-3 weeks is required for egg/embryo freezing. Ovarian stimulation can start at any time point in the menstrual cycle with equivalence in terms of number of oocytes retrieved to the conventional early follicular phase start. (ESHRE guideline, 2019[27]). Although embryo freezing is more established in practice, oocyte freezing will be more appropriate for the

post-pubertal TYA group as this negates the need for a partner to produce sperm for fertilisation of the oocyte at the time of collection and who at a future time whatever the status of the relationship would have to provide consent to use of the embryos. Oocyte survival after vitrification and warming is very high (>97%) (Cobo and Diaz, 2011[28], Cobo et al., 2016[29]). Although monitoring of stimulation and oocyte retrieval are usually performed vaginally, these can also be performed abdominally in patients where transvaginal monitoring is inappropriate and where patients decline transvaginal oocyte retrieval. The supra-physiological levels of oestrogen resulting from ovarian stimulation is generally not a problem in haematological malignancies; however, there is an increased risk for venous thrombosis and a risk assessment must to be done prior to stimulation. Patients who are panctyopenic at presentation or have significant mediastinal disease that makes deep sedation unwise may not be able to have egg freezing.

In healthy women, the live birth rate per frozen oocyte is around 6% and continues to improve steadily due to advances in vitrification protocols (Cobo et al., 2011[30]). Probability of live birth depends on the age of the patient and the number of oocytes frozen. In practical terms, for a patient to have a 60% chance of a live birth they will need to store at least 10 eggs. Younger ages and higher egg numbers are associated with higher probability of live birth (Doyle et al., 2016[31]). In The UK, data on success rates are reported on the Human Fertilisation and Embryology (HFEA) reporting platform. Live birth per embryo transfer cycle from stored eggs is between 35 – 40% but this will vary with womens age when eggs were stored.

Ovarian stimulation and oocyte cryopreservation is not recommended once chemotherapy has commenced until approximately 6 months after the end of treatment, due to the risk of DNA damage in the oocytes (Arnon et al 2001[32]).

2. *Ovarian tissue freezing*

Ovarian tissue cryopreservation is a fertility preservation option for girls who are not mature enough or do not have time available for egg collection. For TYA patients that are still prepubertal this is the only option available for storing material that might be used to restore fertility after treatment has been completed. This treatment requires a laparoscopic procedure to either remove an ovary or excise ovarian cortex. After either of these procedures, the ovarian cortex is cryopreserved (frozen at very low temperatures – less than -170C). When the young woman wishes to start a family, if her remaining ovary has ceased functioning, the stored tissue can be defrosted and auto-transplanted onto the remaining ovary and ovarian fossa (orthotopic) to allow natural fertility to occur. If this is not possible due to concerns about pelvis vasculature following intense radiotherapy, heterotrophic sites such as arm or abdomen can be chosen for subsequent ovarian stimulation, oocyte retrieval, and *in-vitro* fertilization (IVF). Use of heterotopic sites appears to be less successful than orthotopic transplants. (Demestere et al 2009[33],)

Worldwide about 360 auto-transplantations have taken place (Gellert et al 2018[35]) Reports from different groups reveal over 130 live births in over 21 countries with a pregnancy rate between 30 – 50% (Hoekman et al., 2020[34]), without increased risk of miscarriage or congenital abnormality (Gellert et al 2018[35]). These figures however, need to be treated with caution as there is no established worldwide database and negative outcomes are frequently under-reported in medicine.

In haematological malignancies, especially leukaemia, there is significant concern about presence of malignant cells/ minimal residual disease (MRD) in the ovarian tissue especially at diagnosis when there will be circulating malignant cells. A review of the available literature by Dolmans in 2018 concluded that auto-transplantation of frozen-thawed ovarian tissue in leukaemic patients carried a significant risk of the tissue containing residual disease (Dolmans and Masciangelo, 2018[36]). Despite this, there have been several case reports of live births from auto-transplanted tissue in leukaemia survivors (Shapiro et al., 2018[37],) without evidence of subsequent disease relapse. However, at this time, most centers storing ovarian tissue from patients with leukaemia, do so with the intention that the tissue will only be used when technology which does not require tissue auto-transplantation, such as in-vitro maturation of primordial follicles becomes available (Dolmans and Masciangelo 2018[36]). The systematic review conducted by Bastings and colleagues (2012)[38] evaluated the safety of autotransplantation of ovarian tissue obtained in different malignancies. The risk of malignant contamination was found to be highest in Leukaemia and of least concern in Lymphoma. A recent review of worldwide experience of auto-transplantation of ovarian tissue (Gellert et al 2018[35]), reported Lymphoma to be the diagnosis in 20% cases (n=53) with no reports of disease relapse post tissue transplantation.

In contrast to eggs, it is possible to collect ovarian tissue after the start of chemotherapy as the NGFs are dormant and less susceptible to damage from the mutagenic effects of the chemotherapy (Meirow et al 2007[39]). If tissue is to be collected after chemotherapy has commenced it is important to identify a window between courses of treatment when the patient has marrow recovery making the procedure safe and demonstrating clearance of the chemotherapy. In haematological malignancies, collection of tissue after a patient has shown response to treatment and disease burden/MRD is significantly reduced, minimises potential malignant contamination of the ovarian tissue (Shapira et al 2020[40]). In a recent series published by Poirot, 22 out of 31 patients having auto-transplant of previously cryopreserved ovarian tissue had prior chemotherapy exposure before the tissue was collected. The cumulative incidence of pregnancy (Kaplan-Meier) at 3 years after ovarian tissue auto-transplantation was 36%, with no difference related to previous chemotherapy exposure (Poirot et al., 2019[41])

3. *Ovarian transposition*

If pelvic radiotherapy is required, as can be the case with lymphoma, the ovaries can be surgically transposed away from the field of irradiation. In case of midline radiotherapy, ovaries can be transposed laterally toward the pelvic wall and in case of lateral radiotherapy field, the ovaries can be moved medially behind the uterus (Irtan et al., 2013[42]). Ovarian transposition can be performed laparoscopically including robotics. The patient has to be fit for laparoscopic surgery and success depends on the dose of radiotherapy scatter, initial ovarian reserve, maintenance of blood supply to the ovary and absence of concomitant gonadotoxic chemotherapy. After completion of cancer treatment, natural conception may be possible but if there has been significant radiation damage to the uterus a surrogate pregnancy may be necessary.

4. GnRH agonist suppression

The role of GnRH analogs in protecting ovaries before and during chemotherapy is widely debated, particularly for patients with hematological malignancies (Chen et al 2019[43], Lambertini et al 2019[44]). The exact mechanism of how GnRH agonist may protect ovarian reserve is unclear. The initial activation of primordial follicles is GnRH independent as

demonstrated by this activity being present from before birth in a phase when pituitary hormones play no part in follicle growth. Although there is evidence of benefit in some groups of breast cancer patients (Lambertini et al 2018[45]), there is no conclusive evidence of a protective role in haematological cancers. However, some guidelines do include them as a potential adjuvant treatment especially as they have the benefit of reducing blood loss due to the menstrual cycle. The menopausal side effects are poorly tolerated by some patients which can limit use.

Table 2 summarises efficacy of fertility preservation options in females

B) **Treatment options post cancer treatment**

Counselling in the after care setting should include active fertility management so that patients get appropriate advice according to need including advice on contraception. If patients have not been able to store eggs/embryos or tissue prior to cancer treatment there may be a window when menstrual cycles return and if there is sufficient ovarian reserve this could be the time when eggs or tissue could be collected. It often takes 6 – 9 months for the ovarian function to recover and menses to restart. Regular review including monitoring of pubertal development, endocrine function, and ovarian reserve is advised in the after care setting.

Future prospects pertaining to fertility preservation in haematological malignancies

1. Artificial ovary

The development of artificial ovaries is a novel experimental technology that aims to produce mature oocytes ready for in-vitro fertilization through an ex-vivo multistep strategy including sequential in-vitro cultures of ovarian tissue, follicles, and oocytes. A novel 3D printed artificial ovary impregnated with mice primordial follicles has recently been shown to be successful in mice with production of healthy pups (Laronda et al., 2017[46], Salama and Woodruff I.2019[47]). Further research and studies are needed to adapt this technique to produce artificial human ovaries and establish this in clinical practice (Laronda et al., 2017[46]). This technology would potentially allow ovarian follicles in stored tissue to be used to mature oocytes without the need for auto-transplantation.

2. In vitro follicular growth systems

Ability to grow primordial follicles in-vitro is another new technology that removes the risk of auto-transplantation of ovarian tissue with malignant potential. Telfer and Mclaughlin in Edinburgh have managed to grow and mature human primordial follicles to the pre-antral and antral follicle stages (Telfer and Mclaughlin, 2012[48], Mclaughlin et al., 2018[49]). More work is required before in-vitro culture can be clinically applied and offered.

Male Treatment Options

Overt testicular involvement occurs in about 2% of boys at the time of diagnosis of childhood acute lymphoblastic leukemia (ALL) (Hijiya et al., 2005[50]). However, sub-clinical infiltration of leukaemic cells into the testes occurs more frequently. In one published series, testicular biopsies taken at diagnosis in children with acute lymphoblastic leukaemia contained leukaemic cells in up to 25% cases (Akhtar et al., 1991[51]). In adult testicular autopsy samples from patients who have died of leukaemia, but had no clinical evidence of testicular

involvement prior to death, leukaemic infiltrates in the testis were found in 40–60% of patients (Richie, 1998[52]).

Malignant infiltrates or inflammation within the testis may disrupt the spermatogonial stem cell (SSC) niche leading to reduction in sperm quality and quantity. This may be one reason why it is not always possible for post-pubertal boys to produce sperm in an ejaculated semen sample at diagnosis of haematological malignancy.

Post-pubertal boys who can produce a semen sample prior to the start of cancer treatment should be strongly encouraged to do so even if the proposed first-line treatment has low gonadotoxicity because should they relapse on treatment or require treatment escalation they will not then be able to store sperm during subsequent chemotherapy exposure due to the risks of DNA damage to developing sperm (Rives et al, 2017[53]).

A. Treatment options prior to cancer treatment

1. Collection of spermatozoa

a) Ejaculated semen sample

The potential for sperm collection from the TYA population is dependent on the physical and emotional maturity of the individual. In adolescent males who are post-pubertal, the most established approach to preserving fertility is the cryopreservation of ejaculated mature sperm. This is dependant on the ability of the individual to produce a semen sample through masturbation. In most post-pubertal males, the process of providing sperm for cryopreservation is effective, inexpensive, and non-invasive.

b) Electro-ejaculation

Electro-ejaculation under general anesthesia (Stahl et al 2012[56]) is an alternative technique that can be used to obtain mature sperm in pubertal boys who are unable to produce a semen sample through masturbation prior to commencement of chemotherapy. This technique should not be used in patients with hematological malignancy who are pancytopenic as it may lead to a significant risk of sepsis from bacteria in the gut.

c) Testicular Extraction of Sperm (TESE)

TESE under general anesthesia, (Schrader et al. 2003[55]), is a technique that can be considered for post-pubertal boys at high risk of infertility due to cancer treatment who cannot produce sperm in an ejaculated semen sample. They must be fit for a general anaesthetic and preferably not pancytopenic. This technique may also be helpful in boys in very early puberty where a proportion of seminiferous tubules within the testis may contain sperm even if it is absent in ejaculate. An early morning testosterone, LH and FSH level within the pubertal range and a testicular volume indicating pubertal development can be helpful in predicting the likelihood of sperm being present and therefore whether TESE might be appropriate.

Cryopreserved sperm can be used later in life for intrauterine insemination or *in vitro* fertilization, with or without an intracytoplasmic sperm injection if azoospermia should occur post treatment. Sperm collected prior to cancer treatment will be free from any risk of micro-metastatic contamination. Collection of sperm must take place prior to the start of chemotherapy to prevent mutagenic changes in the developing sperm. It takes 60 -90 days for mature sperm to develop and so sperm retrieved after the start of chemotherapy may have acquired DNA damage. These sperm may be at significantly increased risk of

aneuploidy (Rives et al., 2017[53]) and DNA damage has even been identified in sperm up to 2 years post-treatment (Beaud et al., 2019[54]). Therefore, if semen cryopreservation is being considered it should be performed before treatment commences

2. Testicular Tissue Cryopreservation

Testicular tissue cryopreservation to preserve spermatagonial stem cells is the only fertility preservation treatment option available to pre-pubertal boys. It may also be considered in boys who either could not produce a semen sample containing sperm or who did not collect sperm prior to the start of their chemotherapy (Picton et al 2015[57]; Goossens et al 2020[5]). This treatment involves surgical removal of testicular tissue under a general anaesthetic. The treatment is relatively new and is still regarded as experimental. To date there have been no reports of human babies being born using stored human testicular tissue, in part due to the age of the boys storing tissue and the fact that most will not have requested restoration of fertility. There is however, good evidence from work in animals that the tissue remains viable during cryopreservation and auto-transplantation of testicular tissues has resulted in live births of healthy offspring in a several species including non-human primates (Fayomi et al 2019[59]).

Future use of testicular tissues stored from patients with leukaemia and other haematological malignancies will require assessment of leukaemic infiltration and this may restrict use of tissue to in-vitro methods for sperm maturation and ICSI/IVF (Jahnukainen et al 2015[60]). If testicular tissue storage is considered for patients they must be fully aware of the experimental nature of the technology and limitations of tissue auto-transplantation.

Table 3 summarises success rates of sperm retrieval in different options.

B. **Treatment options post cancer treatment**

On completion of treatment it can take some time for sperm function to completely recover and it is advised that patients delay testing for sperm quality and quantity for at least 2 years following cessation of treatment (Beaud et al., 2019[54]). At 5 years post cancer treatment, when most patients are considered to be cured of their cancer, a semen analysis can be undertaken to look at sperm quality and quality. If this is shown to be within normal parameters any stored sperm/tissue could be discarded.

Post high dose treatment such as stem cell transplant, if there has been no testicular radiation, there can be very slow recovery of sperm production over a number of years as surviving spermatogonial stem cells repopulate the seminiferous tubules (Rovo et al,2006[16]).

Counselling in the after-care setting should include active fertility management so that patients get appropriate advice according to need including advice on contraception.

Conclusion

In summary, fertility preservation in teenage and young adults with haematological malignancies is complex. Central to the management of fertility in this population is an assessment of physical and emotional maturity for each individual which will guide counselling and determine the available fertility preservation options. The risk of fertility damage and the possible fertility preservation options must be discussed with all patients and their family before the start of cancer treatment. In each case the appropriate fertility

preservation advice will depend upon a complex interplay of factors weighing risk of future infertility against the risk of fertility preservation treatment. These considerations must be on a case-by-case basis and require a multidisciplinary team approach with input from experts in haematology/oncology, fertility, surgery and tissue/gamete storage and a well-developed patient pathway for fertility preservation treatment. Above all fertility issues must be taken seriously and actively managed in a coordinated and compassionate patient centered manner.

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