Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults

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Abstract

Preservation of gonadal function,is an important priority for the long-term health of cancer survivors of both sexes and all ages at treatment.. The loss of an opportunity for fertility is a prime concern in both male and female cancer survivors, however the endocrine consequences of gonadal damage are also central to long-term health and wellbeing. Some fertility preservation techniques, such as semen and embryo cryopreservation for the adult man and woman respectively, are established and successful and the recent development of oocyte vitrification has greatly improved the potential to cryopreserve unfertilised oocytes from women. Despite being recommended for all pubertal males, sperm banking is not universally practised in Paediatric Oncology centres, and there are very few 'adolescent-friendly' facilities. All approaches to fertility preservation have particular challenges in children and teenagers, including ethical, practical and scientific issues. For the young female, cryopreservation of ovarian cortical tissue with later replacement has now resulted in at least 35 live births, but is still regarded as experimental in most countries. For pre-pubertal males, testicular biopsy cryopreservation is offered in some centres, but it is unclear how that tissue might be used in the future, and to date there is no evidence that fertility can be restored. For both sexes these approaches require an invasive procedure, and there is an uncertain risk of tissue contamination in haematological and other malignancies. Decision making for all these approaches requires an assessment of the individual's risk of loss of fertility, and is being made at a time of emotional distress. The development of this field requires better provision of information for patients and their medical teams as well as improvements in service provision, to match technical and scientific advances.

Search strategy and selection criteria

We searched Medline between Jan 1, 1990, and Sept 1, 2014, for reports published in English using the search terms "fertility preservation", "cancer", "childhood cancer", "gonadotoxic", and "cancer treatment" in several disjunctive and conjunctive combinations. We mainly selected publications in English from the past 5 years, but did not exclude older, significant publications. We also checked the reference lists of articles identified by this search strategy.

Introduction

Treatment for cancer may affect reproductive and endocrine function in both men and women, and loss of fertility remains a major concern of patients [1]. While survival rates in young people with

cancer were low in the 1960's, major advances in treatment, particularly the use of multi-agent chemotherapy, and in supportive care, have resulted in markedly improved rates of cure over recent decades. Cancer affects 1 in 800 children: current data suggest that around 80% will be alive five years from diagnosis and 70% will become long-term survivors. With increasing numbers of long-term survivors, gonadal function and fertility have become important concerns for these young men and women.

If the planned treatment is deemed to put gonadal function and future fertility at risk, fertility preservation options should be considered and discussed with the patient before treatment commences. This requires greater awareness, knowledge and willingness by oncologists to discuss fertility issues: there is evidence that this is increasing [2, 3] but many patients receive little information [4, 5]. Discussing fertility prognosis at the time of diagnosis puts an additional burden on the treating team but for the patient and their family can have a positive psychological effect and can be acceptable even if there are no realistic fertility preservation options available [6, 7]. Recent years have seen the development of new approaches for fertility preservation, with rapid translation of some into clinical practice. Highlighting which approaches remain experimental (which should therefore be offered only in the context of an approved clinical trial) is particularly important when counselling patients about to commence cancer treatment. In this review we discuss the assessment of risk to fertility, possible mechanisms of gonadal damage and propose a schema-based approach to counselling for individual patients.

Which patients are at risk?

Consideration of the degree of risk to gonadal function in both males and females is critical for provision of the most accurate information to the patients, and to allow examination of potential fertility preservation strategies, which may be time consuming, invasive, and in some cases experimental [8]. The risk of infertility for some young men and women will be low, whereas others will be facing a near certainty of loss of gonadal function. Consideration of this can be usefully structured into intrinsic and extrinsic factors (Table 1) [9]. Extrinsic factors centre on the proposed treatment which will reflect the diagnosis and stage of disease. Treatments known to have the most significant risk to gonadal function in both males and females include total body irradiation and chemotherapy conditioning before bone marrow transplantation, radiotherapy to a field that includes the gonads and some chemotherapy agents (e.g. alkylating agents) [10-14].

In the female, radiotherapy to a field that includes the ovaries will cause depletion of the remaining

non-growing follicle (NGF) pool in a dose dependent manner. The dose to deplete the NGF pool by 50% (LD50) has been estimated to be less than 2Gy [15]. Using our understanding of the normal decline in the NGF pool with increasing age in healthy females [16] we have calculated the effective sterilising dose for age at treatment. (Figure 1A). The older the patient the smaller their NGF pool and therefore the smaller the dose required to deplete the remaining NGF pool to 1000 NGF's or less and therefore cause immediate premature ovarian insufficiency (POI). At the age of 12 years 18.3 Gy to the ovary furthest away from the radiation field will cause immediate POI for most females, whereas for a 28 year old 14 Gy will be sterilising for most females. (Figure 1A). The combined effect of age at treatment and the patients' ovarian reserve (defined as NGF numbers in the ovary and displayed as 25th, 50th or 75th centile) is illustrated for a hypothetical patient receiving 5Gy to her ovary (Figure 1B). For a patient aged 6 years, depending on their ovarian reserve she will develop POI at between 24 and 32 years; if treated at age 22 years, POI is predicted at between 33 and 41 years. In Figure 1C we illustrate the same principle for a patient receiving TBI at a dose of 14.4 Gy. If treated at age 6 years she will develop POI either immediately or by 14 years, and if at 22 years, POI is predicted immediately or by 25 years if she is initially on the 75th centile for ovarian reserve.

For females, radiotherapy to other reproductive organs is also relevant, notably to the uterus which is associated with a range of adverse reproductive outcomes including miscarriage, premature delivery and stillbirth [17-19]. Radiotherapy may also have adverse affects on reproductive function through damage to the hypothalamus and pituitary [20]; this may manifest in relatively subtle ovulatory dysfunction developing with increasing time since treatment [21]. Likewise surgery may directly impact on the specific reproductive organs or may indirectly affect fertility, for example, through intra-abdominal adhesions impacting on ovarian and fallopian tube function. Consideration of these issues will allow classification of the patients as being at low, medium or high risk of gonadal dysfunction. However depending on the patient's response to treatment, the treatment plan may be required to change and a patient initially classified as low risk becomes high risk as e.g. radiation is required to a field that includes the pelvis. [11].

Extrinsic factors also include service provision related issues, i.e. what fertility preservation therapies are realistic and available to the patient, and the time-scales required to achieve them. Semen cryopreservation can be achieved with minimal delay; ovarian and testis tissue cryopreservation may also be rapidly achievable as no pre-treatment is required, but techniques involving ovarian stimulation require approximately 2 weeks. More than one option may be available and potentially appropriate, highlighting the need for rapid and clear communication between

oncology and reproductive medicine services. The rapidly evolving nature of this field further underlines the importance of seamless communication between specialties. Where fertility preservation strategies remain experimental there are further issues such as ethical approval, funding and staffing to consider.

Intrinsic considerations focus around the patient's individual susceptibility to reproductive damage from the proposed therapy, but also include psycho-social factors. These which will include consideration of familial and cultural/religious views and beliefs. The importance of age in women has long been recognised to be a very important determinant of the likelihood of ovarian failure after cancer therapy [22]

, [23]. This stratification can also be seen even in very young patients, thus adolescents were at approximately two to three-fold higher risk of acute ovarian failure than girls under the age of 12 when treated with radiotherapy [24]. Much of this effect of age is likely to reflect the progressive loss of follicles within the ovary, with depletion resulting in POI and the menopause. Younger patients may, therefore, be found to have an increased risk of POI if monitored for longer periods after treatment. There is also a substantial variation in follicle complement between women, perhaps as much as fifty-fold [16], which physiologically results in the near 20 year age spectrum of the normal menopause. This has led to research investigating biomarkers of the ovarian reserve, i.e. the number of non-growing primordial follicles in the ovary. There are no direct markers available, but recent research has highlighted the potential value of measurement of serum anti-Müllerian hormone (AMH) which is produced by small growing follicles, which in turn reflects primordial follicle numbers [25]. AMH in the healthy female rises to a peak at age 24.5 years then declines towards the menopause [26] and has been proposed as an indirect marker of ovarian reserve in young women [11]. AMH was first shown to be reduced in some female survivors of childhood cancer despite preservation of regular menstrual cycles [27]. In general, AMH declines rapidly during chemotherapy in both adult women and girls and adolescents [28, 29] with recovery thereafter dependant on the treatment received [30, 31]. For example, there is little recovery of AMH levels in women who have received high doses of alkylating agents [32, 33], with a similar pattern seen in girls and adolescents [29]. Thus girls who have received high-risk therapy will often have undetectable AMH concentrations at the end of therapy with no recovery thereafter, in contrast to the recovery seen with lower risk therapies. In prospective analyses correlating pre-treatment reproductive biomarkers with ovarian activity after chemotherapy, AMH has been shown to be a valuable predictor of long term ovarian function in women with early breast cancer, although age remains an important stratifier [34-36] (Figure 2). Long-term studies are needed to assess the predictive value of both pre- and post-treatment AMH measurement in girls, to evaluate its usefulness in predicting future fertility, and for the detection of those who do not develop POI in the immediate post-treatment period, but who none-the-less may have a shortened reproductive lifespan and hence a reduced timeframe in which to have children. Current evidence suggests that young women can retain fertility despite markedly reduced ovarian reserve (as reflected in very low AMH concentrations) after cancer treatment [37], although there is also evidence of increased prevalence of infertility in both adult [23] and childhood cancer survivors [38] without POI.

Age, in relation to pubertal status, is also an important intrinsic factor for males as this will

determine the stage of testicular development for the patient which may have relevance in terms of the susceptibility of the gonad to the effects of cancer treatment. There are three important phases of postnatal gonadal development in males [39]. During the fetal and early postnatal life the hypothalamo-pituitary gonadal (H-P-G) axis is active and the germ cells undergo an important period of differentiation from gonocyte to spermatogonia. This is followed by a childhood period where the H-P-G axis is regarded as relatively quiescent. However, as demonstrated in non-human primates, the testis is not inactive during this period with functional maturation of Sertoli cells and proliferation in germ cells [40]. Although it has been suggested that the testis is less susceptible during pre-puberty [41] it remains sensitive to the damaging effects of chemotherapy and radiotherapy during childhood and recent evidence indicates that the testis may even be at greater risk in the pre-pubertal period than in adulthood as indicated by studies in non-human primates [42, 43]. This may relate to effects on proliferating Sertoli cells resulting in failure of outgrowth of the seminiferous tubules [44, 45]. Damage, either direct or indirect, to the spermatogonial stem cells (SSCs) is the most important factor in determining whether fertility will be preserved: if the SSCs are lost then establishment or restoration of spermatogenesis will not occur. Assessment of spermatogenesis post-treatment can only be reliably performed by semen analysis. FSH and inhibin B are both serum biomarkers of spermatogenic function, and are of value for example in comparison of treatment effects [46]. However in assessing the individual cancer survivor, neither have sufficient accuracy for clinical use [47].

Age and pubertal stage are also an important factor in terms of the potential strategies that may be employed for fertility preservation in these patients. For pubertal patients in whom complete spermatogenesis has occurred, there is the well established option of semen cryopreservation. Current recommendations are that all adult men and teenage boys should be offered semen cryopreservation [48]

, [49]. The decision in younger patients may be aided by a clinical assessment of pubertal stage and emotional maturity. However, for pre-pubertal patients and pubertal patients that are not able to produce a semen sample, approaches for fertility preservation remain experimental and are only available in a limited number of centres worldwide. Establishing whether spermatogenesis has commenced in individual patients is important in this context because this will determine the requirements for handling and storage of testis tissue. Although the use of age, Tanner staging, testicular volumes and serum hormonal evaluation may provide some indication, there currently is no definitive way to predict the likelihood of sperm in these patients. Spermarche has been shown to occur over a wide age range and to be associated with an extremely variable testicular volume [50-52]. This includes individuals with testicular volumes <5ml and/or pubic hair stage I [51, 52]. As a result it has been suggested that intra-operative assessment of the biopsy at the time of tissue retrieval may be useful for allocation of tissue to a particular freezing protocol [53].

The patient's general health status may also determine the potential for fertility preservation strategies. In some circumstances the patient may be too unwell and the need for immediate treatment may override other considerations. The patient's health may also impact on the likelihood of success of fertility preservation. It has long been recognised that men with a range of cancers often have severely impaired spermatogenesis at presentation [54] and there is now a growing body of evidence that women with cancer also have impaired ovarian function. This translates into lower markers of the ovarian reserve at presentation (either AMH or ultrasound based antral follicle count), and fewer oocytes obtained than from age matched otherwise healthy infertile women [55, 56]. AMH is also reduced in girls with cancer compared with age matched controls [57], with the deficit related to markers of the degree of ill health. Specific health conditions that are associated with compromised male reproductive function (e.g. cryptorchidism) [58] may also affect the potential success of any fertility preservation strategies.

Individual beliefs and wishes relating to the importance of fertility, and the risk/benefit of procedures for fertility preservation, will vary between patients [59]. Some patients will be extremely concerned irrespective of the assessed risk being low, medium or high and will be keen to attempt semen cryopreservation, while others will be less concerned and more anxious to commence treatment without delay [60]. Informed consent is a pre-requisite for any medical intervention, and in this context is particularly pertinent to children for whom the proposed procedure for fertility preservation remains experimental. The interests of the young patient must always be the priority and issues relating to consent/assent must be carefully evaluated. In adults too, accurate assessment of the degree of risk of loss of gonadal function is central to the patient being able to make a truly informed

decision. The risks of future infertility and also of the proposed fertility preservation procedure must be carefully balanced against the chance of future success of preserving fertility, particularly when the options remain experimental and speculative [59]. It is not in the interest of a patient with very low risk disease to undergo a procedure if there is no real prospect of the tissue being needed for future fertility. Undoubtedly, discussion of fertility issues that will only become apparent and relevant later in life is important in conveying the message of anticipated long-term survival or cure, but the need for invasive, and especially experimental, procedures must be clearly justified as being in the individual patient's interests.

How is fertility lost?

The infertility experienced by some patients after cancer treatment is most often due to a loss of germ cells, but whether that loss is a primary consequence of treatment or an indirect effect is less clear, with such information important for the design of protective treatment. With the mechanisms of action varying across different chemotherapy drug classes, and between chemotherapy and radiotherapy treatment, and with the majority of patients receiving combination treatments, mechanistic examination of damage is complex.

For females, there is a substantial body of evidence pointing to a direct loss of oocytes (including within the NGF pool) in patients who have had ovarian exposure to radiotherapy. The precise cellular effects of chemotherapy treatment are less clear, but it is apparent that different drug types induce different patterns of ovarian damage (Figure 3) [10]. Alkylating agents directly damage oocytes [61, 62], but many other classes of chemotherapy drugs first damage ovarian somatic cells, with germ cell death a secondary, downstream effect [62, 63]. The stage(s) of ovarian follicle most susceptible to damage by chemotherapy treatment will also impact how fertility is affected (Figure 3). Long-term reproductive health requires maintenance of the ovarian NGF pool, but current evidence indicates that it is the growing ovarian follicles that are particularly susceptible to chemotherapy drug damage: death of these developing ovarian follicles leads in turn to accelerated recruitment of primordial follicles into the growing pool. Hence the number of NGFs decreases as a consequence of an increased rate of growth initiation, in addition to direct primordial follicle death [64]. Ovarian follicle death can also occur due to initial damage to extra-follicular ovarian tissue, and stromal and blood vessel damage in response to chemotherapy have been reported [65].

For male patients, as with females, radiotherapy and chemotherapy with alkylating agents are particularly gonadotoxic, primarily affecting spermatogenesis (Figure 4) [66]. In post-pubertal males,

the spermatogonia (including SSC) are particularly sensitive to chemotherapy and radiotherapy. This is not surprising since, unlike female germ cells, these are a rapidly dividing population of cells in the pre-pubertal testis [42]. A second key difference in the function of the testis compared to the ovary is that the endocrine function of the testis, residing in the Leydig cells, is not directly linked to gamete generation and thus male fertility can be, and indeed generally is, adversely impacted without effects on endocrine function. Much less is known about the specifics of damage to prepubertal males, although this patient group is the one for which there are no established fertility treatments [39].

For both males and females, loss of fertility will often be temporary, provided there remains a sufficient testicular population of SSCs or ovarian supply of NGFs. Crucially, where sufficient germ cells are still present after treatment, evidence to date does not point to any long term, sustained damage in these cases [67] and likewise evidence regarding potential transgenerational effects is generally reassuring [68-71].

Endocrine consequences of gonadal damage from cancer therapy

While the loss of fertility is a major concern in both male and female cancer survivors, the non-fertility or endocrine consequences of gonadal damage are important for long-term health. In females, the intimate association of the germ cell and endocrine cells of the ovary in the growing follicle means that when one is lost or damaged, then both are. Thus, in the worst case where all follicles are lost, the patient will experience POI and thus estrogen deficiency as well as infertility. This will have important consequences for all estrogen dependent tissues, most obviously the skeleton but will also impact on cardiovascular, uterine and cognitive function. Whilst estrogen deficiency is well recognised to have adverse effects on bone density, it is also clear that chemotherapy can have direct negative effects as well [72]. The symptoms of estrogen deficiency, including hot flushes, joint pain and potentially tiredness all contribute to a significant loss of quality of life in these women. In general, these can be ameliorated by hormone replacement therapy that is recommended to be taken until the age of the natural menopause, i.e. approximately age 50. Where treatment has resulted in POI before puberty, then there will be the need for induction of puberty with graduated sex steroid administration. As in other patients, the aim will be to mimic the timing and key milestones of normal puberty, and the need for such therapy should be anticipated in girls who have undergone high risk therapy [73]. Transdermal estrogen replacement is increasingly used for both pubertal induction and long term hormone replacement, and there is some limited evidence that this may be beneficial for cardiovascular, renal, uterine and bone function [74-77].

The increasing data on the potential value of serum AMH measurement for predicting the menopause in normal women may also be helpful for cancer survivors. It may be useful to use this hormone to identify those young women with very low ovarian reserve and therefore a likely significantly shortened reproductive lifespan [29, 35, 78]. In addition to providing patient information, some may also wish to pursue fertility preservation techniques while they still have some ongoing gonadal function if this was not performed pre-treatment.

The situation in men is rather different as the endocrine and gametogenic functions of the testis are more functionally and anatomically separated. It is well accepted that the Leydig cells and thus testosterone production are relatively resistant to chemotherapy and radiotherapy compared to spermatogenesis [66, 79]. As a result, many boys treated for cancer can expect to undergo a normal puberty and maintain normal testosterone production even though they will not be fertile as adults. In some instances, partial Leydig cell damage may be compensated for by elevated LH concentrations [79]. Recent data suggest that Leydig cell dysfunction is under-recognised, with an overall shift to slightly reduced testosterone concentrations in childhood cancer survivors [80]. Similarly, there is a general shift to higher LH concentrations in such men. Overall, the risk of endocrine dysfunction in childhood cancer survivors had an odds ratio of 6.7, with some 23% of men in a survey of 150 patients showing such evidence. This was particularly common in men who had had radiotherapy to the testis, in which group 83% had testicular endocrine dysfunction. It was, however, found in over 30% of men with past leukaemia or lymphoma. There are no long term follow up data indicating whether men with a high LH and normal testosterone progressed to overt hypogonadism over time, although it would seem likely that this does occur in a significant proportion. As with girls anticipation of, and prompt treatment for, pubertal delay are appropriate. Induction of puberty should be considered and implemented as for other adolescent males with hypogonadism using escalating doses of testosterone [81, 82]. Given the long-term adverse effects of hypogonadism on bone density, patients should be assessed regularly, and testosterone replacement initiated as for the normal treatment of the hypogonadal male [73].

What can be done?

Fertility preservation is now part of the UK National Institute for Clinical Excellence (NICE) guidance for the management of people diagnosed with cancer [83], and some options are well established. These include semen cryopreservation from adult men, and embryo and oocyte cryopreservation for women. For both sexes, options for children and adolescents remain

experimental. Direct measures for fertility preservation (gamete and gonadal tissue cryopreservation) are discussed below and outlined in Figure 5; although discussed briefly, space precludes detailed discussion of indirect approaches including ovarian transposition or gonadal shielding, and hormone or other drug therapy to potentially reduce gonadal toxicity; these have recently been reviewed [8].

Existing fertility preservation methods

I. Protection of the gonad in-situ

In patients who are due to undergo radiotherapy in the abdomino-pelvic region, it may be possible to shield the gonad from the radiotherapy beam. In young males, this has been shown to preserve testicular growth and function when used in combination with bone marrow transplantation [84]. However, in females particular care needs to be taken in children to correctly identify the position of the ovaries [85]. Recent improvements in radiotherapy techniques may also result in more specific targeting to the tumour site of solid malignancies, which should reduce the chance of damage to neighbouring gonadal tissue. Similarly, modifications of treatment regimen in order to reduce the effects on fertility are also being investigated. In particular, replacing alkylating agents such as procarbazine with alternative agents such as dacarbazine, as in the recently closed Euronet (Euronet-PHL-C1) study for classical Hodgkin lymphoma, offers a real possibility of reducing gonadotoxicity and preserving fertility in these patients [14].

II. Sperm cryopreservation

Cryopreservation of semen from adult men has long been an established option. It is rapid, non-invasive and widely available. A discussion about fertility should be included in the counselling of all patients with cancer prior to their treatment [48]

, [49], and should cover the potential risk of the proposed cancer treatment regimen, the options for fertility preservation and whether these are established or experimental techniques. Facilities for semen cryopreservation should be available to all patients prior to commencement of their treatment [48] and the subsequent use of stored semen samples for assisted reproduction (e.g. IUI, IVF with ICSI) is well established for adults that have received treatment for cancer [86, 87]. There are a number of hurdles that must be overcome before sperm storage can be achieved. The patient must be physically and emotionally mature enough to produce a sample. Consent should be taken from the patient to store the sample and this should include issues such as what would happen to the sample in the event of the patient's death. Despite the guidance advocating semen cryopreservation for patients, the number of males who choose to store semen remains low and, even for those who do store a sample, the number of patients who subsequently use their sample is also low [86].

III. Oocyte and embryo cryopreservation

The most established method for female fertility preservation is embryo cryopreservation, a long established and routine part of IVF treatment for infertile couples. It does, however, require time, and although current approaches to ovarian stimulation have reduced this [88], some two to three weeks will still be required. Importantly, the creation of embryos requires sperm and the resulting embryos will be the joint property of the man and woman involved (unless donor sperm are used). This will, therefore, not be ideal for women who are not in an established relationship and even where they are, the implications of embryo formation should be very clearly discussed with the woman and her partner beforehand. Historically, oocyte cryopreservation has been relatively unsatisfactory with poor survival of cryopreserved oocytes, but this has markedly changed with the development of vitrification, involving ultra-rapid freezing in high concentrations of cryoprotectant [89]. With current protocols, oocyte survival is high with essentially normal developmental competence. Thus this has now become a viable option for women and is no longer regarded as experimental [90, 91]. There are limited data on usage of cryopreserved oocytes and embryos: a recent report indicates that this may be low [92], as with men returning to use cryopreserved sperm. The reasons underlying this, such as continuing natural fertility, are unclear but the accumulation of samples with low likelihood of utilisation is an important practical consideration for any centre offering this very long-term service.

Experimental approaches

I. For males

For pre-pubertal males, strategies for fertility preservation remain experimental and can be broadly classified into those in which the gonad is protected in situ and those in which gonadal tissue is removed for cryostorage and future use in evolving reproductive technologies. Approaches to protecting the gonad in situ include altering the hormonal milieu to render the gonad insensitive to the effects of cancer treatment. Whilst studies in rodents utilising GnRH analogues and/or sex steroids offered much promise (reviewed in [39]) such approaches have failed to offer protection to the gonad in primates [93, 94] and humans (reviewed in [95]). Limited data from rodent studies are also available on the use of pharmacological agents for fertility preservation in males [96]. However, to date no pharmacological intervention study has been shown to offer protection of the pre-pubertal testis from chemotherapy and radiotherapy induced damage in humans.

The alternative approach is to remove gonadal tissue from suitable patients at high risk of infertility (Table 2) and cryopreserve it prior to cancer treatment. This tissue would then be available for future use in initiating/restoring fertility in these patients. Strategies for cryopreservation are required that preserve the survival and functional capacity of the SSC and several methods have been used to assess SSC viability [97-99]. Approaches that utilise such cryopreserved tissue may include autotransplantation of the tissue or SSCs to the patient after the treatment has finished. Both of these approaches for generating full spermatogenesis from pre-pubertal tissue have proved to be successful in a variety of species, including non-human primates [100-102]. However, in the only study to report on the use of a SSC transplantation approach in humans, a return of fertility has not been subsequently reported [103]. An alternative method that has been utilised for generation of mature gametes involves in vitro culture of the tissue/SSCs. These techniques have also shown promise in rodent models with full spermatogenesis and generation of progeny described for sperm generated from culture of intact immature testicular tissue [104]. To date this approach has not been reproduced using human tissue. The methods described thus far involve the differentiation of immature germ cells although there has been much recent interest in the generation of germ cells from re-programmed stem cells. However these approaches remain very much in their infancy [105].

Despite the progress that is being made in this rapidly expanding field, there remain a number of important questions. Areas of significant uncertainty remain regarding the selection of patients most likely to benefit from this service, the efficiency of both transplant related and in vitro methods, and the safety of future use, including in vitro-generated gametes and the potential for tumour cell contamination and inadvertent replacement.

Given the difficulties in translating the results of animal studies to humans and the relative scarcity of pre-pubertal human testis tissue for research, it is important to establish large collaborations to focus research efforts into key areas and prevent duplication of work. In addition there must be well co-ordinated long term follow-up to validate patient selection criteria and the effectiveness of the strategies for fertility preservation.

II. For females

Ovarian stimulation is generally regarded as inappropriate in girls and at least younger adolescents (although it has been reported in a premenarchal girl [106]). The most accepted available option remaining is ovarian tissue cryopreservation. This is highly invasive, involving general anaesthesia and surgical removal of ovarian tissue (either ovarian cortical biopsies or sometimes oophorectomy). Delay can be minimal, and cancer therapy started very shortly after surgery. While this technique is the only one appropriate for very young patients, its use in adult women varies according to health service organisation and relevant national legislation. Subsequent use of the ovarian tissue generally requires a further surgical procedure to replace the tissue. Live births following both natural conception and IVF have been reported, at least 35 at the time of writing [8, 107]. Successful pregnancy has recently been reported following transplantation of ovarian tissue to a site outside the pelvis, i.e. to the anterior abdominal wall [108], although such heterotopic transplantation has previously been less successful than replacement within the pelvis. The success rate, i.e. the chance of live birth following replacement of ovarian tissue, remains unclear but appears to be approximately 20%, although the majority of women will achieve some ovarian function [109]. An evidence base is thus accruing as to the usefulness of this approach in adult women, but remains at the level of case series reports, with no robust and objective trials testing indications, techniques, or success rates. It is regarded as experimental by professional bodies [110], and is undoubtedly so when applied to girls and adolescents.

A key aspect of this approach that requires consideration is the potential for reimplantation of malignant cells or tissue when the cryopreserved ovarian tissue is replaced. This risk appears high in leukaemia, where malignant cells have been detected in a significant proportion of ovarian biopsies analysed [111] [112]. The risk in other malignancies is low, although a high level of vigilance is required: we have detected ovarian deposits of Ewing's sarcoma in a girl without other evidence of metastasis.

We have recently validated criteria for offering ovarian tissue cryopreservation over a 15 year period, with a population basis including the whole of the South-East of Scotland of all paediatric

oncology patients treated at The Edinburgh Cancer Centre (a regional centre) to minimise bias [113]. The criteria, based on multidisciplinary review, are shown in Table 2; these should be regarded as a basis for discussion of individual cases and further development. In this analysis of 410 new referrals, ovarian tissue cryopreservation was only offered to 8% of patients, but the prevalence of POI in that group was 35% vs 1% in those not offered it (Figure 6). This confirms that these criteria can predict those at highest risk of POI with a high degree of accuracy, although with longer follow up it is highly likely that more women in both groups will develop POI.

The ability of the pre-pubertal ovary to support later fertility has not been shown, although there appears no particular reason to suggest that it cannot: replacement has shown evidence of endocrine activity to induce pubertal development [114, 115]. This indication may however be inappropriate [116] as there is rapid and uncontrolled elevation of estradiol and progesterone to adult levels, the graft lifespan may only be short, and the use of the very scarce number of follicles and oocytes available seems wasteful. Autologous ovarian tissue transplantation in adults for hormone replacement at a heterotopic site may be feasible, although careful consideration of the risk of malignant contamination is important as is the potential need for repeated transplants.

Conclusions and future directions

Recent years have seen substantial progress in the techniques and provision of fertility presevation for young people with cancer. Semen and embryo cryopreservation and now oocyte vitrification are established where appropriate, with the latter greatly improving the options for young women. Ovarian tissue cryopreservation is widely used in adult women and in some children and adolescents, although it remains experimental. It is likely to become more widely offered to girls and adolescents, where ovarian stimulation is inappropriate, but the ethical considerations for children are different and more challenging than those involving adults who are competent to provide informed consent for an experimental procedure. Experimental interventions in children can only be ethical if they can be considered to be therapeutic and in the best interests of the child. These considerations particularly apply to the development of techniques for pre and peri-pubertal boys; while testicular tissue can be cryopreserved, we do not at present know how to use it.

The evidence base underpinning the rapid establishment of fertility preservation remains limited, only now progressing from case reports and series to a small number of cohort studies. The effectiveness of the techniques being offered needs to be established, and more accurate information about long-term fertility in cancer patients is necessary to provide the denominator for this. Most

young men and women treated for cancer do not become infertile: the challenge is to develop robust ways to individualise that risk, allowing truly informed decision making by patients and their clinical team at a time of considerable emotional distress.

Figure legends

Figure 1: The effective sterilising dose for age at treatment and POI prediction given age and radiation dose. We make the conservative assumption that the remaining NGF pool declines at a similar rate to that given by the Wallace-Kelsey model for the untreated female.

(A) Above the grey-red boundary, doses to the ovary will cause immediate POI for most patients due to depletion of the NGF population to below one thousand.

(B) Exemplars of the combined effects of a dose of 5 Gy and age at treatment. The green dashed lines show the 25th, 50th and 75th centiles of the Wallace-Kelsey age-related model of NGF population per ovary for healthy females, with menopause (defined as an NGF population below one thousand) occurring at 46 to 53 years for the majority of women. The blue lines show the immediate NGF depletion for patients aged 6 years due to 5 Gy radiotherapy, and the subsequent 25th, 50th and 75th centiles of the Wallace-Kelsey model representing their ovarian reserve; in this case POI is expected at between 24 and 32 years. The red lines illustrate the effects of the same dose on patients aged 22 years, with POI expected to occur at between 33 and 41 years depending on their ovarian reserve at the time of treatment.

(C) Exemplars of the combined effects of a dose of 14.4 Gy and age at treatment. The green, blue and red lines denote the healthy population, 6 year old patients and 22 year old patients respectively as in (B). The increased dose leads to more severe depletion of the ovarian reserve, leading to expected POI at between 6 and 14 years for patients aged 6 years and expected POI immediately or for those on the 75th centile for ovarian reserve at 25 year for patients aged 22 years at treatment.

Figure 2

Classification mosaic chart for ongoing menses (M) or chemotherapy-related amenorrhoea (A) using serum AMH and chronological age as predictor variables. The primary cut-off values are for AMH; at intermediate AMH concentrations there is an age threshold, above which amenorrhoea is predicted and below which ongoing menses are predicted. The classification schema has sensitivity 98·2% and specificity 80·0%. Reprinted with permission from [35].

Figure 3

(A) Cancer treatments could directly affect the resting pool of primordial follicles or the growing follicle population. As growing follicles inhibit the recruitment of primordial follicles, the loss of this growing population will lead to increased activation of primordial follicles and so loss of that reserve. (B) Cancer treatments could be directly targeting the oocyte or the somatic cells. Oocyte death would result from death of the follicular somatic cells, as the oocyte is dependant on these for its survival. From [10].

Figure 4

Cellular targets for testicular damage following cancer treatment. A) Damage to the SSC and subsequent SSC loss will result in permanent azoospermia. B) Damage to the differentiating germ cells will result in transient azoospermia, however, restoration of spermatogenesis may occur from the surviving SSC. C) Damage to the Sertoli cells may result in failure of these cells to support the SSC and/or differentiating germ cells resulting in permanent or transient loss of fertility as described for A) or B) respectively. D) Damage to Leydig cells following cancer treatment results in testosterone deficiency. This usually occurs at higher doses that will also result in germ cell loss and azoospermia.

Figure 5

Pathways to fertility preservation options for children and young adults. In prepubertal boys, prior to the onset of spermatogenesis, testicular biopsy and cryopreservation is an option. In pubertal and post-pubertal males, the ability to produce a sperm-containing ejaculate allows sperm cryopreservation: prior to this, testicular biopsy with cryopreservation of sperm or tissue is required. In prepubertal females, ovarian stimulation is inappropriate thus ovarian tissue cryopreservation can be offered. After puberty, this remains an option but ovarian stimulation allows the recovery of mature oocytes for cryopreservation, or of embryos after fertilisation. Distinction is made between established and experimental options. Recovery of immature oocytes with in vitro maturation is omitted for clarity.

Figure 6

The cumulative probabilities of not having POI in the years following diagnosis for the group offered ovarian cryopreservation (blue line) and the group not offered ovarian cryopreservation (red line).

(15-year probability 35% [95% CI 10–53] *vs* 1% [0–2]; p<0.0001; hazard ratio 56.8 [95% CI 6.2–521.6]. From [113].

Table 1

Intrinsic and extrinsic factors that should be taken into account when considering fertility preservation strategies for children/young adults undergoing treatment (adapted from [9]).

Intrinsic factors

- Health status of the patient
- Psycho-social factors
  - Consent (patient/parent)
  - Assessment of pubertal status
  - Assessment of ovarian reserve (females)

Extrinsic factors

- Nature of predicted treatment (high/medium/low/uncertain risk)
- Time available
- Expertise/technical options available

Table 2

The Edinburgh Selection Criteria for gonadal tissue cryopreservation. These were established with Ethical Committee review and approval as these are experimental procedures, and should be regarded as a starting point for future discussion, research and refinement.

Females (from [113])

Age < 35 years

No previous chemotherapy/radiotherapy if age >15 year at diagnosis, but mild, non gonadotoxic chemotherapy if < 15 years is acceptable

A realistic chance of surviving five years

A high risk of premature ovarian insufficiency (>50%)

Informed consent (parent and where possible patient)

Negative HIV, Syphilis and Hepatitis serology

Not pregnant and no existing children

Males

Age 0-16 years

A high risk of infertility (>80%)

Unable to produce a semen sample by masturbation

No significant pre-existing testicular pathology (e.g.cryptorchidism)

Informed consent (parent and where possible patient)

Negative HIV, Syphilis and Hepatitis serology

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