REVIEW

Preservation of fertility in children treated for cancer

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As treatment for childhood cancer has become increasingly successful, adverse effects on reproductive function are assuming greater importance. Preservation of fertility before treatment must be considered in all young patients at high risk of infertility, and provision of such services requires collaboration between oncology centres and assisted conception units. The UK Children's Cancer Study Group is planning to audit current management of preservation of reproductive function in young patients with cancer, and the British Fertility Society is preparing a voluntary code of best practice to guide and inform clinicians and scientists. Limitation of radiation exposure by shielding of the testes and ovaries should be practiced where possible and sperm banking should be offered to all sexually mature boys at risk of infertility. The rapidly advancing experimental techniques for harvesting of gonadal tissue must be considered and embarked on without unrealistic expectations, although future utilisation of the tissue is unlikely to be realised until the next decade.

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Improvements in the diagnosis, management, and treatment of childhood cancers have made the prospect of survival into adulthood a realistic expectation for the majority of children. However, it is a recognised complication that certain forms of treatment may compromise fertility.[1 2] Consideration of fertility preservation is a quality of life issue at a time of intense stress for young patients and their families. Nevertheless, in our experience, open discussion is embraced and often potentially therapeutic for the vulnerable family facing treatment for cancer. Discussion of fertility issues at the time of diagnosis provides the family with the reassurance that the oncology team believe in a future when these issues will become important.

Treatment of childhood cancer with chemotherapy and radiotherapy may damage gonadal tissue and result in permanent sterility in both males and females.[3–18]

RADIOTHERAPY INDUCED GONADAL DAMAGE

The nature of radiotherapy induced gonadal damage depends on the field of treatment, total dose, and fractionation schedule.[3–5] In males, doses as low as 0.1–1.2 Gy can damage dividing spermatogonia and disrupt cell morphology, resulting in oligozoospermia.[3 4] Permanent azoospermia has been reported following single fraction irradiation with 4 Gy or 1.2 Gy fractionated.[3 4] Leydig cells are more resistant to damage from radiotherapy than the germinal epithelium, and progression through puberty with normal potency is frequent, despite severe impairment of spermatogenesis. Testicular irradiation with doses of greater than 20 Gy is associated with Leydig cell dysfunction in prepubertal boys, while Leydig cell function is usually preserved up to 30 Gy in sexually mature males.[5]

Total body, abdominal, or pelvic irradiation may cause ovarian and uterine damage.[6–9] The human oocyte is sensitive to radiation, with an estimated LD_{50} of less than 2 Gy.[6] The younger the child at the time of radiotherapy the larger the number of primordial follicles present, hence for a given radiation exposure the longer the "window" of fertility before a premature menopause ensues. Ovarian failure will require oestrogen replacement for pubertal induction and cyclical hormone replacement to relieve the symptoms of oestrogen deficiency (vaginal dryness, hot flushes, and irritability), provide cardiovascular protection, and optimise bone density. However, even where ovarian function is preserved, this does not guarantee fecundity as radiation damage to the uterus may have occurred. Uterine irradiation in childhood increases the incidence of nulliparity, spontaneous miscarriage, and intrauterine growth retardation.[8 9] The mechanism underlying these findings remains unclear, but reduced elasticity of the uterine musculature and uterine vascular damage have been suggested.[8 9]

CHEMOTHERAPY INDUCED GONADAL DAMAGE

The impact of combination cytotoxic chemotherapy on gonadal function is dependent on gender and age of the child undergoing treatment, and the nature and dosage of the drugs received.[9–18] Drugs known to cause gonadal damage include procarbazine, cytosine arabinoside, and the alkylating agents, particularly cyclophosphamide, chlorambucil, mustine, melphalan, busulphan, and the nitrosoureas.[9–18] Treatment for Hodgkin's disease in the UK with "ChlVPP" (chlorambucil, vinblastine, procarbazine, prednisolone) is known to cause gonadal damage, particularly in the male, and the agents implicated are chlorambucil and procarbazine. In a recent long term follow up study, 89% of the males treated before puberty had evidence of

Abbreviations: GnRH, gonadotrophin releasing hormone; HFEA, Human Fertilisation and Embryology Authority; ICSI, intracytoplasmic sperm injection; S1P, sphingosine-1-phosphate

severe damage to the germinal epithelium and recovery of spermatogenesis would be unlikely.[16] Around 50% of girls treated for Hodgkin's disease prepubertally with six or more courses of ChlVPP had raised plasma gonadotrophin concentrations, but longer follow up is needed to determine whether these women have recovery of function or go on to develop a premature menopause.[16] The use of ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), which does not contain alkylating agents or procarbazine, is significantly less gonadotoxic.[17] Current regimens with hybrid protocols are likely to preserve fertility in women and in approximately 50% of men.

Chemotherapy does not appear to have any significant lasting adverse effect on uterine function. Successful pregnancy, with no increased risk of miscarriage, and healthy offspring have been reported following treatment with multiagent chemotherapy regimens.[18]

PRESERVATION OF FERTILITY

Current practice

Potential strategies for preservation of fertility are dependent on the sexual maturity of the patient. The only established option for preservation of male fertility is cryopreservation of spermatozoa.[19] Spermatozoa are usually obtained from the ejaculate by masturbation. When it is not possible to obtain an ejaculate, sperm can be retrieved by epididymal aspiration or testicular biopsy in sexually mature males. Not infrequently, sperm produced by cancer patients at the time of diagnosis is of poor quality. However, with advances in assisted reproduction techniques, in particular intracytoplasmic sperm injection (ICSI), which involves the injection of a single spermatozoon directly into an oocyte, the problems of low numbers and poor motility sperm may be circumvented.[20] In our view any spermatozoa retrieved, either from the ejaculate or surgically, should be cryopreserved irrespective of the perceived quality of the material. Data on the health of offspring born after ICSI are broadly reassuring.[21] A recent study showed that although the conventional criteria of semen quality are frequently abnormal in long term survivors of childhood cancer, the sperm produced do not appear to carry a greater burden of damaged DNA. This observation goes some way to providing reassurance about the use of ICSI, which will circumvent the problems associated with severe oligozoospermia and offer cancer survivors the possibility of paternity in adulthood.[22]

A number of strategies to protect the ovaries and preserve fertility during cancer therapy have been attempted with limited success. In contrast to males, the gamete pool in females is fixed at birth and collection is technically more difficult. In young sexually mature females with partners, collection of mature oocytes for fertilisation and subsequent embryo cryopreservation is possible.[23] Cryopreservation of oocytes is an alternative possibility for women without a partner but is less successful. On average 5–10 oocytes may be harvested per patient with fewer than one baby born per 100 oocytes stored.[24] The main disadvantage of embryo or oocyte storage is the requirement for superovulation with gonadotrophins, which will inevitably delay the commencement of cancer therapy. Limitation of radiation dose to the ovary is sometimes practiced in adult women, but in children is technically difficult.[25]

Experimental strategies

For prepubertal children, lacking in haploid gametes, there are no options currently available to preserve fertility, and any potential strategies must be considered entirely experimental. One approach to preserving fertile potential was based on the original idea that the prepubertal gonads are quiescent and therefore protected from the cytotoxic effects of chemotherapy and radiotherapy which destroy rapidly dividing cells. It was hypothesised that suppression of the hypothalamic-pituitary-gonadal axis by administration of gonadotrophin releasing hormone (GnRH) analogues would render the gonads less susceptible. While it has since become clear that the prepubertal gonads are susceptible to the deleterious effects of cytotoxic therapy, there is significant evidence for the success of protection/recovery strategies in rats. However, clinical studies in man have to date been inconclusive.[26 27] In men treated with sterilising radiotherapy and chemotherapy for childhood cancer, effective gonadotrophin suppression with medroxyprogesterone acetate for at least three months did not result in restoration of spermatogenesis.[27] A number of studies have shown that GnRH analogues inhibit chemotherapy induced ovarian follicular depletion in rodents by blocking gonadotrophin induction.[28] These findings are supported by clinical studies which showed that co-treatment of GnRH analogues and chemotherapy resulted in primary ovarian failure in 1 of 44 (2.3%) compared with 26 of 45 (58%) in the group treated with chemotherapy (with or without mantle field irradiation) only.[28] The judicious use of GnRH analogues may play a role in the appropriate patient group, such as young women and children subjected to alkylating agent based chemotherapy for Hodgkin's disease.

Oocyte loss induced by cytotoxic therapy has been shown to occur by apoptosis; consequently inhibition of the apoptotic pathway has been explored as a mechanism for preventing ovarian failure. Sphingosine-1-phosphate (S1P), a metabolite of ceramide, is believed to inhibit apoptosis in somatic cells. Treatment of mice ooctyes with S1P prevents chemotherapy induced apoptosis in vitro. In vivo administration of S1P confers resistance to radiation induced apoptosis in mice, with pregnancy rates of 100%.[29] While S1P may herald promise of a new approach to preservation of ovarian function, further studies are necessary to explore the detrimental effects of such treatment on normal neurological function, as deletion of sphingomyelinase during normal fetal life leads to the development of Neimann-Pick disease-like symptoms in post-fetal life.

Advances in assisted reproduction and increasing interest in gamete extraction and maturation have focused attention on preserving gonadal tissue from children before sterilising chemotherapy or radiotherapy, with the realistic expectation that future technologies will be able to utilise their immature gametes. The impetus for preserving gonadal tissue follows on the heels of pioneering experiments in ewes,[30] together with media interest in the report of an autologous ovarian graft in a previously oophorectomised female with return of, albeit short lived, menstrual cycle.[31] In addition, live human births resulting from the transfer of embryos fertilised with immature spermatogenic cells have been reported.[32] Such issues have inevitably raised questions from parents and oncologists about their possible application in children undergoing treatment for cancer.[33 34]

In theory gonadal tissue could be removed before the start of treatment and cryopreserved, either as a segment of tissue or as isolated germ cells. Following cure from his/her malignancy the frozen thawed gonadal tissue could be used in a number of ways. In males, the isolated germ cells could be autotransplanted to the testis or matured in vitro until they reach a stage sufficiently mature to achieve fertilisation with ICSI. Immature testicular tissue has been shown to grow and differentiate when grafted into another species.[35] This provides an additional strategy for conserving the male germ line and circumvents the risk of reintroducing malignant cells. Clearly this technique is unlikely to be ethically acceptable and is compromised by the risk of interspecies transfer of potentially pathogenic microorganisms. In females, one potential strategy for the future use of the stored material would be to replant the ovarian tissue, with the hope of restoring natural fertility and also maintaining sex steroid production.

Autologous transplantation of fresh and frozen thawed primordial follicles to the ovaries of sterile recipients has restored fertility, resulting in live healthy offspring in mice and sheep.[36] Human primordial follicles survive cryopreservation, and return of ovarian hormonal activity has been achieved with reimplantation.[37] However, no pregnancies have yet been reported and this procedure must be considered experimental. It is likely that ovarian grafts will have a limited life span, in which case transplantation should be delayed until fertility is desired. In females, harvesting ovarian tissue and cryopreservation of cortical slices are practiced in a number of centres, although these procedures remain experimental and future utilisation of stored tissue is uncertain. As with males, in view of the potential risk of transplanting tumour cells, a number of alternative strategies are being investigated. Isolation of follicles and in vitro maturation for assisted reproduction is likely to be the best option and may be the only option when the uterus has been damaged by radiotherapy.

There is a dearth of data reported on the experience of human ovarian tissue cryopreservation. In a study of 51 women, aged 17.9 (2.7–34) years, at risk of infertility secondary to treatment for cancer, ovarian tissue was harvested in 31 patients, 71% of whom had previously received chemotherapy.[38] In 77% of subjects the whole ovary was removed, while in the remainder half of the ovary was removed, either laparoscopically (n = 29) or at laparotomy (n = 2). Evaluation of the number of primordial follicles in the ovarian cortex showed a mean of 20/mm^2 in girls less than 7 years (n = 6), 4/mm^2 in girls aged 10–15 years (n = 8), diminishing to 1.6/mm^2 in girls greater than 15 years old. Of the 31 patients, 11 died, while follow up of the remainder was limited. Eight patients were lost to follow up and of the remaining 12 patients, no data were available on ovarian function. Although this study showed the feasibility of ovarian tissue cryopreservation, clearly structured prospective studies are required.[38]

ETHICAL ISSUES

Harvesting gonadal tissue and its future use is an exciting new area of gamete biology which raises a wide range of ethical and legal dilemmas that must be addressed before embarking on any clinical programme.[33 34 39–41] These include the safety of the tissue harvesting, subsequent use, and possible implications for the progeny, as well as the legal constraints enforced by the Human Fertilisation and Embryology Authority (HFEA), and the common laws defining validity of consent. For clinical research involving potentially harmful interventions, valid consent is necessary. To be valid, consent must be informed, voluntarily obtained, and given by a competent person. Legal competence to consent requires that the individual giving it is able to understand the information given, believes that it applies to them, retains it, and uses it to make an informed choice. The complex issues of fertility preservation and limited time for discussion imposed by therapeutic imperatives, further fuelled by parental and patient anxieties, will inevitably diminish the validity of such consent. Some of these practical difficulties may be alleviated if obtaining consent could be divided into two stages, with part one involving harvesting and cryopreservation of the tissue and part two involving subsequent use of the tissue. Issues relating to the use of the tissue in the event of the death of the patient must also be discussed.[34 39]

The legal framework defines who is eligible to give the consent. Adolescents over the age of 16 years in Scotland, and 18 years in England, may give consent to treatment in accordance with the Family Law Reform Act 1969 s8, while for younger children, "minors", consent is generally obtained by proxy. In exception to this, legally valid consent from "minors" can be obtained if their doctor considers that they are competent to make an informed decision (Gillick competence).[42] However, the field of assisted reproduction is governed by the statute in the UK and is under the jurisdiction of the HFEA (HFEA Act, 1990), which dictates strict guidelines on the requirements for informed consent with respect to the storage of gametes and embryos and their subsequent use.[43] The HFEA grants licences to individuals for certain procedures involving gametes. Proxy consent is specifically excluded and there is a requirement to provide written and verbal information and an offer of independent counselling. A gamete is defined by the HFEA to be "a reproductive cell, such as an ovum or spermatozoon, which has a haploid set of chromosomes and which is capable of taking part in fertilisation with another of the opposite sex to form a zygote".[44] In practice this would mean that non-gametes, or immature germ cells, could be harvested from children, with parental consent, and stored in, at present, unlicensed premises. If this immature material should ever be matured to form gametes, the requirements for storage of such material would then fall under the jurisdiction of the HFEA. In sexually mature boys, sperm may be produced or retrieved surgically, and written consent obtained in accordance with the Gillick principle and cryopreserved in a licensed centre.

Harvesting and storage of ovarian cortical tissue from girls and young women before gonadotoxic chemotherapy has been available in a number of centres since the mid-1990s and more recently, a few centres have reported the storage of testicular tissue.[38 45] The Royal College of Obstetricians and Gynaecologists has provided a report from a working party on the storage of ovarian and prepubertal testicular tissue. This provides standards for best practice in the cryopreservation of gonadal tissue, including the criteria for providing a service, patient identification and selection, standard operating procedures, and requirements for safe storage.[40]

In December 1999 an international conference was held in Cambridge, to develop an ethically acceptable strategy for the practice and research related to preserving fertility in children and adolescents being treated for cancer. From this meeting a consensus statement was drawn up which made a number of recommendations.[39] Integral to these recommendations were the design and implementation of well constructed research strategies, confined to a finite number of specialist centres, with centralisation of data and rapid dissemination of the results. In turn, these protocols and results should be subject to rigorous review to ensure high standards for collection procedures and storage of material. This would involve multidisciplinary teamwork with multicentre collaboration, to ensure that the best interests of the child are met. It was also recommended that prospective studies be set up to gather data on the impact of current treatment strategies on fertility outcomes in prepubertal children treated for cancer. The experimental nature of this work makes it essential to ensure that clinical and research practice develops in a phased and coordinated manner.

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