PERSONAL PRACTICE

Fertility preservation for children treated for cancer (1): scientific advances and research dilemmas

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Accepted 21 November 2000

Most children treated for cancer can now expect to be cured and to be fertile. However, in a significant minority, future fertility may be compromised by their disease or its treatment.[1] Although the primary objective of treating cancer is cure, this should be seen in the context of promoting and protecting the child's overall wellbeing. Infertility may have significant psychological consequences in adulthood, and strategies aimed at ameliorating this "cost of cure" provide new challenges to professionals in many different disciplines.

Cryopreservation of semen is well established for sexually mature boys,[2] but there are currently few options for peri- or prepubertal children. The use of donated gametes, sperm or eggs, has recently become a realistic possibility in many centres and provides an option if treatment has provoked premature ovarian failure or azoospermia. Finally, gametes, germ cells, and tissues can be collected and stored with the ultimate aim of enabling an individual to become a parent of a child that is genetically theirs. Possibilities for safeguarding future fertility vary from no medical intervention to invasive procedures carrying more than negligible risk in order to harvest gonadal tissue. It is also important to recognise that although young cancer survivors are less likely to have children, this is not solely a result of infertility. Many are unable to form long standing peer relationships, others fear relapse of their disease, and some fear the prospect of leaving their child parentless.[3 4]

Rapid developments in assisted reproduction techniques (ART) now raise the possibility of cryopreserving gonadal tissue to conserve the fertility of young cancer patients. These advances raise major practical, scientific, and ethical issues which are addressed in this and a subsequent article.

Summary box 1—agents with proven gonadotoxicity

Alkylating agents
- Cyclophosphamide
- Chlorambucil
- Melphalan
- Busulfan
- Carmustine
- Lomustine
- Mechlorethamine
- Procarbazine
- Cisplatin
- Nitrosoureas

Vinca alkaloids
- Vinblastine

Antimetabolites
- Cytosine arabinoside

The problem: gonadal damage induced by chemotherapy in boys and girls

Damage to the gonads by irradiation or chemotherapy depends on the patients' gender, age at the time of treatment, radiation dose and fractionation schedule, and total dose and nature of chemotherapy delivered.[1 5] Overall a reduction in fertility is seen. Most chemotherapy protocols use multiple agents whose effects may be synergistic.[6–14] Biochemical detection of gonadal damage is rarely possible before puberty, so treatment induced gonadal damage in childhood may present with infertility or premature menopause in adulthood.

Effect of radiotherapy on gonadal function in boys and girls

Sperm production is susceptible to damage at very low doses of irradiation (>1.2 Gy), but as Leydig cell function is usually preserved up to 12 Gy, it is possible for males who have sustained damage to the germinal epithelium to progress through puberty and retain potency.[15 16]

Abdominal, pelvic, and total body irradiation may all result in ovarian and uterine damage.[17 18] The human oocyte is sensitive to radiation, with an estimated LD_{50} of less than 4 Gy.[19] Less than 2% of children receiving total body irradiation subsequently became pregnant or fathered a child,[13] although there may be some protection of ovarian function in prepubertal girls.[17 19 20] Uterine radiation increases the incidence of nulliparity, fetal loss, and small for dates infants,[21] and reduces the success of assisted reproduction.[18 22]

Summary box 2—radiotherapy induced damage to gonads
Boys
- Azoospermia following low dose radiotherapy (>1.2 Gy)
- Leydig cell function preserved up to 12 Gy

Girls
- Oocytes LD_{50} < 4Gy

Total body irradiation—less than 2% of survivors become parents[13]

Summary box 3—options for fertility preservation (boys)
Established
- Sperm banking

Experimental
- Rectal electrostimulation
- Testicular/epididymal aspiration of spermatocytes
- Testicular biopsy cryopreservation and future in vitro manipulation
- Hormonal manipulation

Fertility preservation and assisted reproduction strategies for boys and girls

Advances in ART are particularly relevant to prepubertal children who are currently excluded from a number of strategies available to sexually mature patients.

STRATEGY FOR BOYS

Spermatogenesis and steroidogenesis are functions of the adult male testes, but spermatogenesis starts prepubertally.[23] It depends on the capacity of the totipotential stem cells to undergo self renewal and provide progeny that mature into viable spermatocytes. Meiosis, with reduction to haploid chromosomal complement, is a relatively early event and is completed by the time of maturation to spermatids; post-meiotic spermatocytes may occasionally be seen in children as young as 4 years.[23 24]

Peri- or postpubertal males who are Gillick competent and sexually mature, may wish to undertake sperm banking before gonadotoxic chemotherapy.[2] (In English law the validity of a child's consent to medical treatment depends on their capacity or competence to do so. Under the Family Law Reform Act (1969) children over 16 years can consent to medical treatment provided they are not incompetent. Children under the age of 16 may consent to treatment if they have sufficient understanding and intelligence to enable them to understand fully what is proposed. A child who can show this ability is referred to as "Gillick competent" after the legal case in which judgement was given.[25]) However, this is not "routine" practice across UK Children's Cancer Study Group centres, nor are there adequate adolescent "friendly" facilities.

Spermarche is an early to mid-pubertal event and precedes the ability to produce an ejaculate.[26 27] In peripubertal boys with spermaturia, the possibility of obtaining a sperm sample by electrostimulation should be considered,[2 28] although facilities for this technique are currently limited. Other options include epididymal or testicular aspiration. As spermatocytes are sparsely present in prepubertal testes, relatively large biopsy specimens will be required. The risk of causing damage and compromising future testicular function seriously questions the suitability of prepubertal testicular biopsy.[29] Furthermore, the technology surrounding cryopreservation and in vitro manipulation of prepubertal testicular tissue is entirely experimental. Clearly any such intervention should only be carried out within ethically approved clinical trials.

Intracytoplasmic sperm injection into oocytes (ICSI) can reverse adult male infertility caused by oligospermia, early spermatogenic arrest, or, in the case of patients with cancer, cryopreserved sperm of poor quality.[30 31] Immature spermatids extracted from testicular tissue[32–34] and more recently secondary spermatocytes[35] have been used in ICSI, but the number of successful pregnancies is extremely low.[36 37] Thus it appears possible to circumvent the maturation process from spermatid to spermatozoa by injection of the haploid male nucleus into the oocyte. In theory, haploid spermatids obtained from the testes of prepubescent boys may have reproductive potential, although this has not yet been proven.

Although reduction to the haploid state has occurred by the round spermatid phase it is not clear that epigenetic, nuclear, and cytoplasmic modifications are complete by this stage. The epigenetic phenomenon of imprinting is thought to occur early in gametogenesis and plays an important role in embryogenesis.[38] Disorders of imprinting are now recognised to cause human disease,[39 40] and are implicated in a number of malignancies including Wilms' tumour and embryonal rhabdomyosarcoma.[41–43] Careful clinical monitoring of children born following assisted conception is essential in case unforseen problems arise.[44]

Totipotential germ cells isolated from the testes of mice can repopulate sterilised mouse testes, giving rise to functional sperm that can fertilise oocytes and produce normal offspring.[45 46] Similar results have now been obtained with cryopreserved germ cells.[47] The techniques used are little different from those used for cryopreservation or ICSI, so that cryopreservation of biologically immortal and unique human germline stem cells is a practical possibility, a development which has enormous ethical and legal implications. Furthermore, xenogeneic transplantation of donor germ cells from rats into the testes of mice treated with myeloablative doses of busulfan results in the production of morphologically normal sperm.[48] This suggests that the sterilised testes of lower animals may act as an "incubator" for germ cells of boys about to undergo gonadotoxic treatment. The ability to manipulate and store totipotential germ cells also raises the possibility of germ line gene therapy. These advances raise the question of cryopreserving

Summary box 4—theoretical mutagenicity problems with ART or assisted reproduction techniques

Male gamete carrying genetic anomalies
- Current limitations in detecting chromosomal abnormalities in spermatid DNA[65]
- Altered expression of imprinted genes
- Germline mutations—either heritable or treatment induced—increasing the risk of cancer predisposition

Male gamete with structural defects
- Abnormalities of centrosome function leading to chromosomal abnormalities[66–68]

Incomplete understanding of sperm activating factors, oocyte activation at fertilisation, and early embryogenesis[69]

Potential for incorporating mutated sperm mitochondrial DNA into the oocyte at ICSI[70]

Female gamete anomalies (oocyte aging related). Reduction in the capacity of the oocyte to repair chemotherapy induced DNA damage in human sperm.[71] This ability is cell cycle specific, suggesting that the timing of ICSI is crucial[72]

Summary box 5—theoretical problems relating to assisted reproduction techniques
- Injection of foreign, sperm associated plasmid DNA into the ooplasm and the risk of transgenic offspring or assimilation of infectious particle
- Injection of biochemical contaminants (from the medium)
- Mechanical oocyte activation (parthenogenesis)
- Physical and biochemical disturbance of the ooplasm
- Damage to the separating chromosomes in the second meiotic spindle
- Human error

spermatids present in prepubertal testicular tissue before radical chemoradiotherapy, in the same way that peripheral blood stem cells are harvested and stored before marrow ablation.

STRATEGY FOR GIRLS

Two potential possibilities exist, cryopreservation of slices of ovarian cortex, rich in primordial follicles and, more experimentally, immature oocyte cryopreservation. Fertility can be restored to oophorectomised sheep following an autograft of cryopreserved ovarian tissue.[49] Cyclical ovarian function is maintained for two years following autograft, with ovulation occurring in 50% of the recipient animals.[50] However, it is likely that ovarian grafts will have a limited lifespan, so that transplantation should wait until pregnancy is desired.[50] Human follicles survive cryopreservation as ovarian cortical strips and are viable,[51] but greatly reduced in number.[52] Primordial human follicles can be isolated and cryopreserved with similar efficiency to slices of ovarian tissue.[53] Reported attempts to cryopreserve human fetal ovarian tissue have not been as successful as adult ovarian tissue.[54] Some centres are already offering cryopreservation of human ovarian cortical tissue to girls and women who require treatment that is likely to result in ovarian failure. We urge the consideration of a trial in which girls at intermediate or high risk of infertility are randomised to harvesting and storage of gonadal tissue or to non-intervention, in order to provide an evidence base for future practice.

Summary box 6—options for fertility preservation (girls)

Experimental
- Cortical strip cryopreservation
- Cryopreservation of immature oocytes
- Cryopreservation of mature oocytes
- Hormonal manipulation

Cryopreservation of immature oocytes

Immature oocytes could be cryopreserved pending advances in in vitro culture technology. Murine primary oocytes gave rise to viable oocytes following in vitro maturation and result in normal offspring after fertilisation, although fertilisation rates are very low.[55] So far embryos produced from frozen thawed immature oocytes that have been matured in vitro have not been transferred to women, and it is not clear whether they would undergo development and growth to term. Although based on the premise that advances in cryopreservation will occur, this may be the most apposite option for prepubertal girls.

Risks of harvesting germ cell tissue

The potential for transferring tumour cells within the ovarian or testicular tissue back into the patient is of concern. Ovarian transplantation from mice with lymphoma into normal female recipient mice resulted in their developing lymphoma.[56 57] The act of cryopreserving ovarian tissue from female mice with lymphoma did not reduce the risk of transmitting cancer cells.[56] The risk of reintroducing the cancer into a cured patient along with the germ cell transplantation is difficult to quantify. Children most at risk of transmitting cancer cells include those with haematological malignancies, for example a testicular or ovarian biopsy in newly diagnosed boys/girls with acute leukaemia/lymphoma. However, haematogenous or local invasion from solid abdominal or pelvic malignancies cannot be excluded, particularly as ovarian tissue should be harvested before chemotherapy commences. Significant advances in our ability to detect cancer specific chromosomal or molecular abnormalities will be necessary in order to screen gonadal tissue for malignant cells prior to cryopreservation. The future potential for in vitro maturation of ovarian follicles or sperm from prepubertal testicular biopsy may overcome this problem, but rigorous animal testing would be required.

Practical issues in the collection and storage of gonadal tissue

A recent report from the Royal College of Obstetricians and Gynaecologists has set guidelines for cryopreservation of gonadal tissue, criteria for providing such a service and standard operating procedures.[58] This is an important step in providing standards for best practice in this experimental arena.

Mutagenic risk to the progeny

The recognition that cancer is a genetic disease of somatic cells has led to the concept of "cancer predisposition", the increased risk of developing cancer related to heritable mutations in growth regulating genes.[59] The molecular basis of certain high penetrance cancer predisposition syndromes, for example, Li–Fraumeni syndrome and retinoblastoma, is now appreciated, but these conditions are relatively rare.[60] Our understanding of low penetrance cancer susceptibility genes is more rudimentary, but it is likely that subtle "gene–gene" or "gene–environment" interactions result in sporadic cancers and are a more common cause of paediatric malignancy.[61] The possibility of circumventing the action of important "gatekeeper" or "caretaker" genes, such as TP53, by experimental manipulation of gametes is unknown, but represents a serious theoretical concern.

The mutagenicity of previous cancer chemotherapy and therapeutic irradiation may put a fetus at risk. Available data indicate that babies of surviving patients treated for cancer do not have an increased incidence of congenital malformation or cancer compared to the general population.[62 63] However, these successful pregnancies mostly result from "normally" achieved conception; we do not know the consequences of circumventing the natural selection processes of normal sexual reproduction using ART, nor the effects of ART on the complex cascade of precisely timed molecular interactions of early embryonic development. Early studies have not yet detected increased health risks to the offspring of assisted reproduction.[44] However, the numbers involved are small, limiting the power to detect adverse risk, and follow up is short so that the long term risks are unknown. The possibility that cancer might be induced in the children of women undergoing in vitro fertilisation is still under investigation.[64] The outcome of any successful pregnancies following ART will have to be carefully monitored.

Summary

Currently there is uncertainty over the most effective and appropriate strategies for preserving and/or restoring an individual's fertility. This makes it difficult for health care professionals to advise young patients and their guardians. Gamete preservation is an evolving science and young children who are candidates for immature germ cell harvesting, storage, and in vitro maturation will make the greatest technical demands on these procedures. Despite its hopeful promise, gonadal cryopreservation is still experimental and should be subject to appropriate regulation and ethical scrutiny in order to prevent the exploitation of vulnerable individuals by commercially driven technology. A randomised trial of gonadal tissue cryopreservation and ART strategies versus best current practice in children with an intermediate to high risk of future infertility would provide an evidence base for future practice.

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