PERGAMON

European Journal of Cancer 38 (2002) 1634–1644

European Journal of Cancer

www.ejconline.com

Paediatric Update

Fertility and progeny

A.B. Thomson[a], H.O.D. Critchley[b], W.H.B. Wallace[a,*]

[a]*Section of Child Life and Health, Department of Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, UK*
[b]*Section of Obstetrics and Gynaecology, Department of Developmental and Reproductive Sciences, University of Edinburgh, Edinburgh, UK*

Received 25 September 2001; received in revised form 11 February 2002; accepted 25 February 2002

1. Introduction

Survival rates for childhood cancer have improved dramatically over the last 30 years such that approximately 70% of children can expect to become long-term survivors [1,2]. However, the successful treatment of childhood cancer with multi-agent chemotherapy in combination with surgery or radiotherapy causes significant morbidity in later life [3]. Infertility is one of the more commonly encountered and psychologically distressing complications of treatment and strategies to preserve fertile potential need to be developed. Preservation of fertility is dictated by sexual maturity of the patient with the only available established options being cryopreservation of spermatozoa for the male and of embryos for the female. Options in children are limited and still experimental, but advances in assisted reproduction techniques have focused attention on preserving gonadal tissue for future use [4–10]. Testicular or ovarian tissue can be harvested and stored before sterilising cancer therapy. Following cure, the stored tissue could be autotransplanted, with restoration of natural fertility, or these stored cells could be matured *in vitro* until they reach a sufficiently mature stage for fertilisation via assisted reproduction [4–10]. The future use of harvested gonadal tissue is an exciting new area of gamete biology which raises a wide range of ethical and legal issues. We review the impact of treatment for childhood cancer on future fertility, explore the available clinical and experimental options and the effects such interventions may have on the offspring.

2. Gonadal damage

Treatment of childhood cancer with chemotherapy and radiotherapy may damage gonadal tissue and result in permanent sterility in both males and females [11–18]. The prevalence of gonadal failure in survivors of childhood cancer is difficult to ascertain because there are relatively few prospective studies. This is further compounded by the fact that treatment regimens are continually evolving.

2.1. Males

2.1.1. Chemotherapy

Cytotoxic chemotherapy agents may produce permanent damage to the germinal epithelium of the testis at all ages resulting in oligozoospermia or azoospermia in adulthood [11–15,19–26]. The extent of the damage is dependent upon the agent administered and dose received, although it can be difficult to determine the relative contribution of each individual drug as most treatments are administered as multi-agent regimens. A number of agents, including procarbazine, cisplatin and the alkylating agents, such as chlorambucil and cyclophosphamide have been identified as being gonadotoxic (Table 1) [12,13,19–23]. Treatment of Hodgkin's disease with combination chemotherapy regimens such as MOPP (mechlorethamine, vincristine, procarbazine and prednisolone), ChlVPP (chlorambucil, vinblastine, procarbazine and prednisolone) or COPP (cyclophosphamide, vincristine, procarbazine and prednisolone) have been reported in a number of studies to result in permanent azoospermia in more than 90% of patients [12,24]. The adriamycin, bleomycin, vinblastine, dacarbazine (ABVD) combination, which does not contain alkylating agents, chlorambucil or procarbazine, has been shown to be much less gonadotoxic, resulting in temporary azoospermia in only 33% of patients and oligozoospermia in 21%, with 'full' recovery after 18

* Corresponding author at: Department of Haematology/Oncology, Royal Hospital for Sick Children, 17 Millerfield Place, Edinburgh, EH9 1LW, UK. Tel.: +44-131-536-0420; fax: +1-131-536-0430.
E-mail address: hamish.wallace@luht.scot.nhs.uk (W.H.B. Wallace).

PII: S0959-8049(02)00093-X

months reported in most patients [24]. 'Hybrid' regimens (e.g. alternating cycles of ABVD with ChlVPP or MOPP) are also likely to be less gonadotoxic [25] than MOPP, Ch1VPP or COPP given alone.

Cyclophosphamide, either alone or in combination with other agents, is known to damage the germinal epithelium. In a follow-up study of 30 men for a mean of 12.8 years after treatment with cyclophosphamide (total dose 560–840 mg/kg) for childhood nephrotic syndrome, azoospermia as reported in 13%, oligozoospermia in 30% and normal semen analysis in the remaining 57% [22]. The threshold for impaired spermatogenesis was a total cumulative dose of 10 g. However, when used in combination with doxorubicin and dacarbazine or vincristine—which have not been shown to be gonadotoxic—for the treatment of solid tumours, azoospermia was permanent in 90% of men treated with cyclophosphamide doses >7.5 g/m^2 [26]. Treatment of acute lymphoblastic leukaemia (ALL), the commonest childhood malignancy, usually includes cyclophosphamide or cytarabine. A study of testicular histology in 44 boys treated for ALL demonstrated a 50% reduction in tubular fertility index (TFI) (percentage of tubules containing identifiable spermatozoa), with severe impairment (TFI $<40\%$) in 18 patients. The severity of the damage was influenced by previous chemotherapy with cyclophosphamide and cytarabine (>1 g/m^2), although the TFI improved with increasing time from treatment [27]. 'Full' recovery of spermatogenesis was observed in 3 of 7 of the patients with severe depression of TFI followed-up for a median 10.8 years (range 5.5–15.9 years) off treatment [20]. Current treatment of ALL in the UK includes cytarabine (total cumulative dose: 2 or 4 g/m^2) and cyclophosphamide (total dose: 1.2 or 2.4 g/m^2). Although these doses are unlikely to be sterilising, long-term follow-up is necessary.

Table 1
Gonadotoxic chemotherapy agents

Gonadotoxic chemotherapy
Alkylating agents
Cyclophosphamide
Ifosfamide
Nitrosoureas, e.g. BCNU, CCNU
Chlorambucil
Melphalan
Busulphan
Vinca-alkaloids
Vinblastine
Antimetabolites
Cytarabine
Platinum agents
Cisplatin
Others
Procarbazine

2.1.2. Radiotherapy

The degree and duration of radiotherapy-induced testicular damage depends on the field of treatment, total dose and fractionation schedule [15,16]. Doses as low as 0.1–1.2 Gy damage dividing spermatogonia and disrupt cell morphology resulting in oligozoospermia [16,28–30]. Permanent azoospermia is reported following single fraction irradiation with 4 or 1.2 Gy fractionated [16,28]. 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 (Table 2) [31].

2.2. Females

2.2.1. Ovary

2.2.1.1. Chemotherapy. Although females are less susceptible to the deleterious effects of chemotherapy than males the gonadotoxic agents are similar (Table 1). Ovarian damage is drug- and dose-dependent and is related to age at the time of treatment, with progressively smaller doses required to produce ovarian failure with increasing age [32–35].

Treatment of Hodgkin's disease with mechlorethamine, vinblastine, procarbazine and prednisolone (MVPP), MOPP or ChlVPP results in ovarian failure in 19–63% of patients [12,32–35]. Amenorrhoea is much more commonly encountered in women over 30 years, 50–89%, whereas in younger women ovarian function appears to be preserved in 48–100% of patients [12,33–35]. Long-term follow-up is necessary to determine how

Table 2
Radiotherapy-induced damage to the reproductive tract

Gender	Site	Effect
Males	Cranial/total body irradiation	Endocrine axis disruption
	TBI/pelvic/testicular	Germinal epithelium
		>1.2 Gy—azoospermia
		0.1–1.1 Gy – oligoozospermia
		Leydig cells
		>20 Gy—prepubertal
		>30 Gy—post-pubertal
Females	Cranial/total body irradiation	Endocrine axis disruption
	TBI/abdominal/pelvic	Ovarian failure ($LD_{50}<4$ Gy)
		older women >5 Gy
		younger women >20 Gy
		Uterine damage
		decreased volume
		decreased elasticity

TBI, total body irradiation.

many of these young women subsequently progress to a premature menopause [36].

Ovarian function appears to be preserved following standard treatment for childhood ALL in most cases [37–39]. In a UK study all of 40 girls treated for childhood ALL achieved adult pubertal development and 37 had regular menses [40]. However, a recent study has shown decreased luteinising hormone (LH) secretion and short luteal phases in young women whose treatment for childhood ALL involved low dose cranial irradiation as CNS-directed therapy [41]. Protocols are continually evolving and vigilant long-term follow-up of these patients is vital, to monitor the risk of late effects.

2.2.1.2. Radiotherapy. Total body, abdominal or pelvic irradiation may cause ovarian and uterine damage and the degree of impairment is related to the radiation dose, fractionation schedule and age at time of treatment [17,18,42,43]. The human oocyte is very radiosensitive, with an estimated LD_{50} of less than 4 Gy [18]. The younger the child at the time of radiotherapy the larger the number of primordial follicles present. Thus, for a given radiation exposure the longer the 'window' of fertility before premature menopause. Permanent menopause may be induced in women aged >40 years following treatment with 6 Gy, while significantly higher doses are required to destroy the oocyte pool completely and induce ovarian failure in young women and children [17].

Ovarian failure was observed in 97% (37 of 38) of females treated during childhood with whole abdominal irradiation (20–30 Gy); 71% developed primary amenorrhoea with a premature menopause (median age 23.5 years) in the remainder [17]. Total body irradiation (TBI), either alone or in combination with cyclophosphamide, as conditioning for bone marrow transplantation (BMT), is associated with infertility [43]. In a long-term follow-up of 708 women median 3 years (range: 1–17 years) after bone marrow transplantation, 532 had received TBI (10–15.75 Gy, single exposure or fractionated) and 176 were treated with cyclophosphamide (200 mg/kg) alone or with busulphan (16 mg/kg), as conditioning therapy. Ovarian failure was observed in 90% of patients following TBI and 68% following chemotherapy.

2.2.2. Uterus

2.2.2.1. Radiotherapy and chemotherapy. Uterine function may also be impaired following radiotherapy. Reduced uterine volume and decreased elasticity of uterine musculature, possibly as a consequence of impaired vascularisation, are found in girls receiving pelvic, abdominal and total body irradiation prepubertally [44–47]. Although successful pregnancies following radiotherapy are reported, the incidence of spontaneous abortion, premature delivery and intrauterine growth retardation is significantly increased [17,43,48]. Following whole abdominal irradiation (20–30 Gy) in childhood, all pregnancies occurring in women with preserved ovarian function resulted in midtrimester miscarriage [17]. For patients with ovarian failure, oocyte donation is a possibility, but the success will be dictated by uterine function. There is only one report of a successful pregnancy and healthy offspring with oocyte donation following BMT and TBI for childhood cancer [47]. In an attempt to improve uterine function, a number of studies have explored the role of exogenous sex steroid replacement in women with premature ovarian failure and impaired uterine volumes following TBI and BMT [45,46,49]. In one study, eight girls received hormone replacement therapy sufficient to induce pubertal development and menses, but inadequate to achieve normal uterine growth with mean uterine volumes only 40% of the normal adult volume [46]. By contrast, women with premature ovarian failure following TBI for childhood leukaemia and treated with physiological sex steroid replacement therapy have shown an increase in uterine volume and endometrial thickness comparable to healthy controls [45]. Further studies are required to determine the optimal regimen of sex steroid replacement therapy for these young women who have a life-long requirement for hormone replacement.

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 are reported following treatment with multi-agent chemotherapy regimens [50].

3. Fertility preservation

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 is provided by pioneering experiments in ewes [51], and the report of a successful autologous ovarian graft in a previously oophorectomised women [52]. In addition, live human births have been reported after the transfer of embryos fertilised with immature spermatogenic cells [53–55]. Such issues have inevitably provoked questions from parents and oncologists about their possible application in children undergoing cancer therapies [56,57].

3.1. Males (See Table 3)

3.1.1. Established practice

3.1.1.1. Cryopreservation of mature gametes. Cryopreservation of spermatozoa is the only established option

Table 3
Clinical and experimental strategies for preservation of reproductive function in oncological patients

Males	Females
a. Clinical practice	
Sperm banking	Oophoropexy
Ejaculation	Embryo cryopreservation
Rectal electrostimulation	
Testicular/epididymal aspiration	
b. Experimental strategies	
Cryopreservation of immature Spermatogenic cells	Cryopreservation of oocytes
Gonadotrophin suppression	Gonadotrophin suppression
Cryopreservation of testicular tissue	Inhibition of follicle apoptosis Cryopreservation of ovarian tissue

in current practice for restoration of male fertility. Spermatozoa are usually obtained from the ejaculate by masturbation, but rectal electrostimulation techniques or retrieval by epididymal aspiration or testicular biopsy may also be successful. In the young man with difficulty sustaining an erection, sildenafil, a treatment for erectile dysfunction, may be helpful. This drug is a specific phosphodiesterase type 5 (PDE 5) inhibitor that enhances nitric oxide (NO)-mediated vasodilation in the corpus cavernosum by inhibiting cyclic guanosine monophosphate breakdown.

The problems of low numbers and poor motility sperm often seen in men with newly diagnosed malignancy, may be circumvented [58–60] by recently developed assisted reproduction techniques, especially intracytoplasmic sperm injection (ICSI), which involves the injection of a single spermatozoan directly into an oocyte. More recently, several pregnancies have been achieved with ICSI using immature spermatids and secondary spermatocytes extracted from testicular tissue in men with spermatogenic arrest [53–55]. The use of these immature haploid spermatogenic cells for ICSI, raises the possibility of retrieving haploid cells from testicular tissue in peripubertal boys. A major concern, however, is the large size of the testicular biopsy required. For prepubertal boys, lacking in haploid gametes, no options are available at present to preserve fertility and potential strategies must be considered entirely experimental.

3.1.2. Experimental strategies

3.1.2.1. Gonadotrophin suppression. Strategies to protect spermatogenesis and restore sperm production following gonadotoxic treatment have been investigated. The relatively quiescent prepubertal testis may be less susceptible to the deleterious effects of chemotherapy and radiotherapy [61] and it was postulated that the induction of a prepubertal state by gonadal suppression would afford some protection to the testis during cytotoxic therapy. Although it is clear that cytotoxic therapy is gonadotoxic at all ages [12], manipulation of the testicular milieu by gonadotrophin suppression has yielded encouraging results in animals. Protection of spermatogenesis in rats has been shown using a variety of hormones to suppress the hypothalamic–pituitary–gonadal axis including testosterone alone [62] or in combination with oestrogen [63], gonadotrophin-releasing hormone (GnRH) antagonists with testosterone [64], and GnRH agonists alone [65] or in combination with anti-androgen [66,67] during treatment with procarbazine, cyclophosphamide and radiotherapy. Furthermore, administration of GnRH agonists or testosterone either immediately, or after a delay, following sterilising radiotherapy or procarbazine treatment enhances recovery of spermatogenesis in rats [67,68]. Although the mechanism by which cytotoxic therapy induces azoospermia is uncertain, studies in rats have shown that some stem cells survive cytotoxic insults and that ensuing infertility is a consequence of the inability of spermatogonial stem cells to differentiate. Consequently, it is believed that hypothalamic–pituitary–gonadal axis suppression facilitates differentiation by lowering intratesticular testosterone levels [68].

Stem cell survival is evident clinically from reports of recovery of spermatogenesis and return of fertility many years later [15,20,24]. However, despite the success of hormone suppression in rats, studies in humans have been inconclusive. In one study where cyclophosphamide was administered as immunosuppressive therapy for nephrotic syndrome in adult men, preservation of fertility was achieved [69]. Of the men who received cyclophosphamide alone, 90% remained azoospermic at 6 months posttreatment, whereas sperm concentrations returned to normal in all five of the men who received testosterone therapy during the immunosuppressive treatment but other studies have not confirmed these results. Suppression of testicular function with a GnRH agonist, alone or in combination with testosterone during gonadotoxic chemotherapy treatment for lymphoma, did not confer any protective benefit or enhance recovery of spermatogenesis [70,71]. In men treated with sterilising radiotherapy and chemotherapy for childhood cancer, effective gonadotrophin suppression with medroxyprogesterone acetate for at least 3 months did not result in restoration of spermatogenesis [72]. The absence of histological evidence of spermatogonial stem cells in testicular biopsies from these men before and after suppression suggests complete ablation of the germinal epithelium and irreversible infertility. Endocrine manipulation to enhance recovery of spermatogenesis may be successful in patients in whom the testicular insult is less severe and at least some spermatogonial stem cells are preserved.

3.1.2.2. Harvesting testicular tissue. In theory, testicular tissue could be removed before the start of treatment and cryopreserved for later use. Following cure from his

malignancy the patient's isolated germ cells could be autotransplanted or conserved *in vitro* until they reach a stage sufficiently mature to achieve fertilisation with ICSI. In order to develop autologous germ cell transplantation in humans, techniques have to be developed by which human testicular germ cells can be isolated, stored and reintroduced into the testis. Cryopreservation of testicular tissue and spermatozoa are well established, but freezing procedures will require modification and optimisation, taking into consideration the inherent biological differences between the immature diploid stem cells and mature gametes, if such techniques are to be applied to spermatogonial stem cells [73].

3.1.2.3. Future use of germ cells. The future use of testicular germ cells will be dictated by the options available at that time, but will most likely involve either maturation of the germ cells *in vitro* for use with ICSI, or tissue transplantation. Ideally, tissue transplantation would involve injecting preparations of purified germ cells into the testis with restoration of natural fertility. *In vitro* maturation techniques, although still very much in their infancy, would have the advantage of eliminating any risk of transplanting malignant cells into the patient. Male germ cells can survive *in vitro* for several months and are likely to undergo cell division [73]. Tesarik and colleagues have reported *in vitro* spermatogenesis and healthy offspring using ICSI. However, the maturation process in these studies involved *in vitro* maturation of late stages of spermatogenesis rather than development from germ cells [74,75].

Testicular germ cell transplantation was pioneered by Brinster and colleagues in 1994 [5]. Testicular germ cells isolated from mouse testis and transplanted into the testis of genetically or experimentally sterile mice initiated and sustained normal donor spermatogenesis, restoring fertility and producing healthy offspring [76]. A number of studies have concentrated on developing the most efficient technique for infusing the stem cells into the testis. Injection into the rete testis of bull, monkey and man, under ultrasound guidance proved to be the simplest and most effective method of filling the seminiferous tubules *in vitro* [77]. Injection into the rete allows a much larger volume of cells to be infused compared with the micro-injections involved in re-infusing directly into the seminiferous tubules. Further work is required to perfect this technique for human application.

Immature testicular tissue has been shown to grow and differentiate when grafted into another species [10]. This provides an additional strategy for conserving the male germ line and circumvents the risk of reintroducing malignant cells. However, this technique is unlikely to be ethically acceptable and is compromised by the risk of interspecies transfer of potentially pathogenic micro-organisms.

3.2. Females (see Table 3)

As with males the options available are dependent upon the sexual maturation of the patient, although in contrast to males, the gamete pool is fixed at birth and collection is technically more difficult. A number of strategies to protect the ovaries and preserve fertility during cancer therapy have been attempted with limited success.

3.2.1. Established practices

3.2.1.1. Surgical translocation. Reducing the radiation dose to the ovary by shielding or removing the ovaries from the field of radiation (oophoropexy) may preserve ovarian function [78,79]. Oophoropexy involves laparoscopic transposition of the ovaries (with their blood supply intact) to a position behind the uterus, which acts as a shield, or to the paracolic gutters, away from the field of radiation, to minimise exposure. Experimentally, heterotopic ovarian autotransplantation has involved grafting the ovary to a distant site in the body and anastomosing blood vessels [78,79]. However, even where ovarian function is preserved oocyte retrieval with assisted reproduction and surrogacy may be required to achieve a pregnancy as the uterus may also have been damaged by the radiation therapy and this may compromise the ability of women to carry a pregnancy to term.

3.2.1.2. Storage of embryos or mature oocytes. The only strategy currently available for preservation of female fertility is cryopreservation of embryos. Embryo cryopreservation and IVF, with partner or donor sperm, is a well established technique routinely offered to women before treatment for cancer, with a success rate of 12.2% per cycle [80]. Cryopreservation of oocytes is an alternative possibility for women without a partner, but is much less successful, with fewer than one baby born per 100 oocytes stored so this remains an experimental technique at present [81]. The main disadvantage of embryo or mature oocyte storage is the requirement for superovulation with gonadotrophins which inevitably delays the start of cancer therapy.

3.2.2. Experimental strategies

3.2.2.1. Gonadotrophin suppression. Suppression of the hypothalamic–pituitary–ovarian axis during cancer therapy appears to reduce follicular cytotoxicity and decrease the risk of premature ovarian failure. A number of studies have demonstrated that gonadotrophin analogues (GnRH-a) inhibit chemotherapy-induced ovarian follicular depletion in rodents. The exact mechanism is uncertain, but may involve direct suppression of GnRH receptors in the ovary, with subsequent inhibition of recruitment of small follicles into the proliferating pool as well as atresia of the already-

developed follicles. Applicability in the human is uncertain, particularly as the human ovary has significantly fewer numbers of GnRH receptors in the ovary [82]. In rhesus monkeys GnRH-a cotreatment protected against cyclophosphamide-induced ovarian damage by significantly reducing follicular decline compared with cyclophosphamide alone [82]. Clinical studies have demonstrated that cotreatment of GnRH-a with chemotherapy resulted in premature ovarian failure (POF) in one out of 28 (3.6%) compared with 26 out of 40 (65%) in the group treated with chemotherapy alone [83]. Adjuvant treatment with GnRH-a, to limit the gonadal toxic effects of otherwise successful treatment regimens, is extremely attractive. However, this approach must be viewed with some caution as although GnRH-a provided some protection against cyclophosphamide, no advantage was conferred against irradiation-induced damage. This may be partly explained by the different mechanism of gonadal damage induced by radiotherapy, namely the destruction of primordial follicles which are not under the influence of gonadotrophins [84]. To summarise, the judicious use of GnRH-a 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.

3.2.2.2. Prevention of follicle atresia. Oocyte loss induced by anticancer therapy has been shown to occur by apoptosis, so inhibition of the apoptotic pathway has been explored as a mechanism for preventing ovarian failure. Ceramide is a sphingolipid second messenger derived both from sphingomyelinase-catalysed hydroloysis and *de novo* synthesis. Ceramide is metabolised and phosphorylated to give sphingosine-1-phosphate, which is believed to inhibit apoptosis in somatic cells. Disruption of the gene encoding acid sphingomyelinase or treatment with sphingosine-1-phosphate attenuates apoptosis of primordial fetal oocytes and increases the number of oocytes present at birth. Treatment of mice oocytes with sphingosine-1-phosphate prevents chemotherapy-induced apoptosis *in vitro*. *In vivo* administration of sphingosine-1-phosphate confers resistance to radiation-induced apoptosis in mice with pregnancy rates of 100% [85]. While sphingosine-1-phosphate may herald promise of a new approach for preserving ovarian function, further studies are necessary to determine any detrimental effects of such treatment on normal neurological function, as deletion of sphingomyelinase during normal fetal life leads to the development of Niemann-Pick disease-like symptoms in post-fetal life [85].

3.2.2.3. Harvesting ovarian tissue. Cryopreservation of ovarian tissue is the only option potentially available for pre-pubertal children and most young women. Harvesting ovarian tissue may take the form of cryopreservation of slices of ovarian cortex, which are rich in primordial follicles, or cryopreservation of immature oocytes. Good survival rates and viability after thawing is similar whether primordial follicles are stored as slices or in isolation. By contrast, poor results are obtained with immature oocytes [8,9,86].

3.2.3. Future use of ovarian tissue

3.2.3.1. Ovarian tissue transplantation. The harvesting of ovarian tissue can readily be achieved laparoscopically before potentially sterilising cytotoxic therapy and cryopreserved until required for future use. At a later date, the ovarian tissue could be reimplanted, with the hope of restoring natural fertility and also maintaining sex steroid production. Autologous transplantation of fresh and frozen-thawed primordial follicles into the ovaries of sterile recipients has restored fertility resulting in live healthy offspring in mice and sheep [49,87–89]. Human primordial follicles survive cryopreservation and return of ovarian hormonal activity has been achieved with reimplantation, but no pregnancies have been reported yet and this procedure must be considered experimental [50,90]. It is likely that the ovarian grafts will have a limited life-span so transplantation should wait until the woman desires to become pregnant.

3.2.3.2. In vitro *culture*. Concern has been voiced that the reimplanted gonads may act as sanctuary sites for cancer cells, particularly the haematological malignancies. Fresh and frozen-thawed ovarian tissue from diseased mice transmitted lymphoma when transplanted into healthy recipients [91]. The risk of transplanting tumour cells can be eliminated by maturing the ovarian follicles *in vitro* followed by assisted reproduction [91–97]. Culture of primordial follicles may be achieved by *in vitro* culture of ovarian cortical slices, which would maintain structural integrity and enable interaction between follicles and surrounding stromal cells. Alternatively, primordial follicles could be isolated and cultured, which would have the advantage of allowing direct monitoring of follicular development. Culture of primary and primordial follicles to pre-ovulatory and pre-antral stages, respectively, has been achieved in mice, with subsequent isolation and maturation of the secondary follicles and a live offspring produced following IVF in one case [98]. Cultures from enzymatically isolated primary and primordial follicles from mice, rats and pigs have yielded similar degrees of development [98,99]. Isolated fresh and frozen–thawed human primordial follicles have been successfully maintained in culture for up to 5 days, but no evidence of growth was observed [9].

3.2.3.3. Xenogeneic transplantation. Heterotopic xenogeneic transplantation is one alternative. Studies have

shown that the renal capsule of immunodeficient mice can successfully serve as a site for transplantation of ovarian slices from various species [100,101]. Ovarian cortical slices from sheep, cats and marmosets have been transplanted into immunodeficient mice and developed to late antral stages, providing a model and a host for follicular development [102]. Studies in which human ovarian slices have been transplanted into severe combined immunodeficient (SCID) mice have produced similar results following administration of follicle stimulating hormone [103].

3.3. Clinical practice for harvesting gonadal tissue

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 report the storage of testicular tissue [104]. 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 for storage conditions [105].

4. Progeny

Overall, there are reassuring reports that there is no increased incidence of either congenital abnormalities or childhood malignancy in children born to long-term survivors of childhood cancer [106,107]. 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 assisted reproduction techniques (ART), nor the effects of ART on the complex cascade of precisely timed molecular interactions during early embryonic development.

4.1. Paternal risks to offspring

There is at least the hypothetical possibility of injection of abnormal spermatozoa or immature spermatogenic cells carrying abnormal genomic DNA with the potential to increase congenital and other abnormalities amongst offspring [108]. Studies in animals have shown that exposure of the male germ line to chemotherapy agents may disrupt spermatozoal DNA and result in deleterious effects on embryo development [109,110]. It has become clear that men from subfertility clinic populations, with abnormalities of the conventional criteria of semen quality, also demonstrate elevated levels of damage to the genomic DNA in their gametes. Even amongst normal populations, sperm chromatin damage has been linked with impaired fecundity [111]. It has been shown that sperm DNA damage does not preclude pronucleus formation at ICSI, and that abnormal DNA within the male gamete is detectable in the early embryo [112].

Thus far, evidence on the safety of ICSI has been largely based upon its use in populations of men with deficits in spermatogenesis unrelated to potentially mutagenic cancer treatment. The evidence concerning health risks to the offspring has been broadly reassuring, although it is limited by the relatively short follow-up so far [113]. Although, by conventional criteria, semen quality is frequently abnormal in long-term survivors of childhood cancer, the sperm produced do not appear to carry a greater burden of damaged DNA [114]. This observation goes some way to providing reassurance about the use of ICSI, which will circumvent the problems associated with severe oligozoospermia and asthenozoospermia, and offer cancer survivors the possibility of paternity in adulthood.

As ARTs advance successful pregnancies are achieved with immature spermatogenic cells adding a further unquantified risk to the fetus. Fertilisation of oocytes with immature spermatogenic cells, such as round cells and elongated spermatids, which have not yet completed spermatogenesis, must be pursued with caution. The mechanism by which sperm precursor cells activate the oocyte at fertilisation is uncertain, but suboptimal oocyte activation may confer poor fertilisation, implantation and high early abortion rates [115]. Spermatid transition into spermatozoa is characterised by salient changes in nuclear protein composition and the significance of circumventing these changes is uncertain [116]. Genetic imprinting is reported to play an important role in embryogenesis and in processes leading to the development of paediatric cancers, including Wilms' tumour and embryonal rhabdomyosarcoma, and other human diseases [117]. Although the detailed mechanism of this process is still unclear, it is likely to involve differences in DNA methylation and requires careful consideration when embarking on germ cell maturation. Children born following assisted conception using spermatozoa and immature spermatogenic cells require careful long-term monitoring [118].

4.2. Maternal risk to the offspring

As with males, there is the theoretical risk that exposure to chemotherapeutic agents and irradiation may cause mutations and DNA changes to the oocyte. Animal studies have demonstrated high abortion and malformation rates related to different stages of oocyte maturation at the time of exposure to chemotherapy

[43,119]. The use of assisted reproduction techniques and embryo cryopreservation in patients previously exposed to cancer therapy has caused concern but studies of pregnancy outcome in cancer survivors have not substantiated these worries. There is no increased incidence of chromosomal or congenital abnormalities in offspring born to women exposed to cancer therapy [43,119] (see key points for a summary of this review).

5. Conclusions

As treatment for childhood cancer has become increasingly successful, adverse effects on reproductive function now assumes great importance. All young patients at high risk of infertility should be identified at diagnosis. 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 appropriately used although without unrealistic expectations for these vulnerable patients. Within the next decade, *in vitro* maturation of immature gametes is likely to be a realistic possibility for fertility preservation.

6. Key points

- Successful treatment of childhood cancer may be associated with impaired gonadal function in adulthood.
- Radiation and chemotherapy may damage the testis at all ages.
- Females are less susceptible than males to damage by chemotherapy.
- Total body, abdominal or pelvic irradiation may cause ovarian and uterine damage and the degree of impairment is related to the radiation dose, fractionation schedule and age at time of treatment.
- Cryopreservation of spermatozoa in males and embryos in females is the only available option for preserving reproductive function. All other strategies remain experimental.
- Best practice for the cryopreservation of gonadal tissue, including criteria for providing a service, patient identification and selection, standard operating procedures and requirements for safe storage must be established.
- Concerns that the offspring of patients successfully treated for cancer might have an increased risk of congenital abnormalities and childhood cancer have not been substantiated.

References

1. Wallace WHB. Growth and endocrine function following the treatment of childhood malignant disease. In Pinkerton CR, Plowman PN, *et al*, eds. *Paediatric Oncology: Clinical practice and Controversies,* 2nd edn. London, Chapman and Hall Medical, 1997, 706–731.
2. Bleyer WA. The impact of childhood cancer on the United States and the world. *Cancer* 1990, **40**, 355–367.
3. Wallace WHB, Blacklay A, Eiser C, *et al*. Developing strategies for long term follow up of survivors of childhood cancer. *Br Med J* 2001, **323**, 271–274.
4. Schlatt S, von Schonfeldt V, Schepers AG. Male germ cell transplantation: an experimental approach with a clinical perspective. *Br Med Bull* 2000, **56**, 824–836.
5. Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. *Proc Natl Acad Sci USA* 1994, **91**, 11289–11302.
6. Clouthier DE, Avarbock MR, Maika SD, Hammer RE, Brinster RL. Rat spermatogenesis in mouse testis. *Nature* 1999, **371**, 418–421.
7. Avarbock MR, Brinster CJ, Brinster RL. Reconstitution of spermatogenesis from frozen spermatogonial stem cells. *Nature Med* 1996, **2**, 693–696.
8. Newton H. The cryopreservation of ovarian tissue as a strategy for preserving the fertility of cancer patients. *Hum Rep Update* 1998, **4**, 237–247.
9. Oktay K, Nugent D, Newton H, Salha O, Gosden RG. Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue. *Fertil Steril* 1997, **67**, 481–486.
10. Oktay K, Karlikaya GG, Aydin BA. Ovarian cryopreservation and transplantation: basic aspects. *Mol Cell Enodcrin* 2000, **169**, 105–108.
11. Waring AB, Wallace WHB. Subfertility following treatment for childhood cancer. *Hosp Med* 2000, **61**, 550–557.
12. Mackie EJ, Radford M, Shalet SM. Gonadal function following chemotherapy for childhood Hodgkin's disease. *Med Pediatr Oncol* 1996, **27**, 74–78.
13. Papadakis V, Vlachopapadopoulou E, van Syckle K, *et al*. Gonadal function in young patients successfully treated for Hodgkin's disease. *Med Pediatr Oncol* 1999, **32**, 366–372.
14. Whitehead E, Shalet SM, Jones PH, Beardwell CG, Deakin DP. Gonadal function after combination chemotherapy for Hodgkin's disease in childhood. *Arch Dis Child* 1982, **57**, 287–291.
15. Howell S, Shalet SM. Gonadal damage from chemotherapy and radiotherapy. *Endocrinol Metabol Clin North America* 1998, **27**, 927–943.
16. Speiser B, Rubin P, Casarett G. Azoospermia following lower truncal irradiation in Hodgkin's disease. *Cancer* 1973, **32**, 692–696.
17. Wallace WHB, Shalet SM, Crowne EC, Morris-Jones PH, Gattamaneni HR. Ovarian failure following abdominal irradiation in childhood: natural history and prognosis. *Clin Oncol* 1989, **1**, 75–79.
18. Wallace WH, Shalet SM, Hendry JH, Morris-Jones PH, Gattamaneni HR. Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte. *Br J Radiol* 1989, **62**, 995–998.
19. Shalet SM, Hann IM, Lendon M, Morris Jones PH, Beardwell G. Testicular function after combination chemotherapy in childhood for acute lymphoblastic leukaemia. *Arch Dis Child* 1981, **56**, 275–278.
20. Wallace WHB, Shalet SM, Lendon M, Morris Jones PH. Male fertility in long-term survivors of acute lymphoblastic leukaemia in childhood. *Int J Androl* 1991, **14**, 312–319.
21. Wallace WHB, Shalet SM, Crowne EC, Morris Jones PH, Gattamaneni HR, Price DA. Gonadal dysfunction due to cis-platinum. *Med Pediatr Oncol* 1989, **17**, 409–413.

22. Watson AR, Rance CP, Bain J. Long-term effects of cyclophosphamide on testicular function. *Br Med J* 1985, **291**, 1457–1460.
23. Heikens J, Behrendt H, Adriaanse R, Berghout A. Irreversible gonadal damage in male survivors of pediatric Hodgkin's disease. *Cancer* 1996, **78**, 2020–2024.
24. Viviani S, Santoro A, Ragni G, Bonfante V, Bestetti O, Bonadonna G. Gonadal toxicity after combination chemotherapy for Hodgkin's disease: comparative results of MOPP vs ABVD. *Eur J Cancer Clin Oncol* 1985, **21**, 601–605.
25. da Cunha MF, Meistrich ML, Fuller LM, *et al*. Recovery of spermatogenesis after treatment for Hodgkin's disease: limiting dose of MOPP chemotherapy. *J Clin Oncol* 1984, **2**, 571–575.
26. Meistrich ML, Wilson G, Brown BW, *et al*. Impact of cyclophosphamide and long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer* 1992, **70**, 2703–2712.
27. Lendon M, Hann IM, Palmer MK, Shalet SM, Morris-Jones PH. Testicular histology after combination chemotherapy for acute lymphoblastic leukaemia. *Lancet* 1978, **ii**, 439–441.
28. Centola GM, Keller JW, Henzler M, Rubin P. Effect of low-dose testicular irradiation on sperm count and fertility in patients with testicular seminoma. *J Androl* 1994, **15**, 608–613.
29. Clifton DK, Bremner WJ. The effect of testicular X-irradiation on spermatogenesis in man. A comparison with the mouse. *J Androl* 1983, **6**, 387–392.
30. Rowley MJ, Leach DR, Warner GA, Heller CG. Effects of graded ionising radiation on the human testis. *Radiat Res* 1974, **59**, 665–678.
31. Shalet SM, Tsatsoulis A, Whitehead E, Read G. Vulnerability of the human Leydig cell to radiation damage is dependent upon age. *J Endocrinol* 1989, **120**, 161–165.
32. Whitehead E, Shalet SM, Blackledge G, Todd I, Crowther D, Beardwell CG. The effect of combination chemotherapy on ovarian function in women treated for Hodgkin's disease. *Cancer* 1983, **52**, 988–993.
33. Clark ST, Radford JA, Crowther D, Swindell R, Shalet SM. Cytotoxic induced ovarian failure in women treated for Hodgkin's disease: a comparative study of MVPP and a seven-drug hybrid regimen. *J Clin Oncol* 1995, **13**, 134–139.
34. Waxman JH, Terry YA, Wrigley PFM, *et al*. Gonadal function in Hodgkin's disease: long-term follow-up of chemotherapy. *Br Med J* 1982, **285**, 1612–1613.
35. Chapman RM, Sutcliffe SB, Malpas JS. Cytotoxic-induced ovarian failure in women treated with MOPP chemotherapy for Hodgkin's disease. *Am J Med* 1981, **71**, 552–556.
36. Byrne J, Fears TR, Gail MH, *et al*. Early menopause in long-term survivors of cancer during adolescence. *Am J Obstet Gynecol* 1992, **166**, 788–793.
37. Siris ES, Leventhal BG, Vaitukaitis JL. Effects of childhood leukaemia and chemotherapy on puberty and reproductive function in girls. *N Eng J Med* 1976, **294**, 1143–1146.
38. Pasqualini T, Escobar ME, Domene H, Muriel FS, Pavlovsky S, Rivarola MA. Evaluation of gonadal function following long-term treatment for acute lymphoblastic leukaemia in girls. *Am J Pediat Haem Oncol* 1987, **9**, 15–22.
39. Green DM, Hall B, Zevon A. Pregnancy outcome after treatment for acute lymphoblastic leukaemia during childhood or adolescence. *Cancer* 1989, **64**, 235–244.
40. Wallace WHB, Shalet SM, Tetlow LJ, Morris-Jones PH. Ovarian function following the treatment of childhood acute lymphoblastic leukaemia. *Med Pediat Oncol* 1993, **21**, 333–339.
41. Bath LE, Anderson RA, Critchley HO, Kelnar CJ, Wallace WH. Hypothalamic-pituitary-ovarian dysfunction after prepubertal chemotherapy and cranial irradiation for acute leukaemia. *Hum Reprod* 2001, **16**, 1838–1844.
42. Sanders JE, Buckner CD, Amos D, *et al*. Ovarian failure following marrow transplantation for aplastic anaemia or leukaemia. *Blood* 1988, **6**, 813–818.
43. Sanders JE, Hawley J, Levy W, *et al*. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood* 1996, **87**, 3045–3052.
44. Critchley HOD, Wallace WHB, Shalet SM, Mamtora H, Higginson J, Anderson DC. Abdominal irradiation in childhood; the potential for pregnancy. *Br J Obstet Gynaecol* 1992, **99**, 392–394.
45. Bath LE, Critchley HO, Chambers SE, Anderson RA, Kelnar CJ, Wallace WH. Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. *Br J Obstet Gynaecol* 1999, **106**, 1265–1272.
46. Holm K, Nysom K, Brocks V, Hertz H, Jacobsen N, Muller J. Ultrasound B-mode changes in the uterus and ovaries and doppler changes in the uterus after total body irradiation and allogeneic bone marrow transplantation in childhood. *Bone Marrow Transplant* 1999, **23**, 259–263.
47. Larsen EC, Loft A, Holm K, Muller J, Brocks V, Nyboe Anderson A. Oocyte donation in women cured of cancer with bone marrow transplantation including total body irradiation in adolescence. *Hum Reprod* 2000, **15**, 1505–1508.
48. Green DM, Hall B, Zevon MA. Pregnancy outcome after treatment for cute lymphoblastic leukaemia during childhood or adolescence. *Cancer* 1989, **64**, 2335–2339.
49. Critchley HOD, Buckley CH, Anderson DC. Experience with a 'physiological' steroid replacement regimen for the establishment of a receptive endometrium in women with premature ovarian failure. *Br J Obstet Gynaecol* 1990, **97**, 804–810.
50. Nicholson HS, Byrne J. Fertility and pregnancy after treatment for cancer during childhood or adolescence. *Cancer* 1993, **71**, 3392–3399.
51. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oopherectomized sheep by ovarian autografts stored at −196 °C. *Hum Reprod* 1994, **9**, 597–603.
52. Radford JA, Leibermann BA, Brison DR, *et al*. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. *Lancet* 2001, **357**, 1172–1175.
53. Tesarik J, Mendoza C, Testart J. Viable embryos from injection of round spermatids into oocytes. *N Engl J Med* 1995, **333**, 525.
54. Fishel S, Green S, Hunter A, *et al*. Human fertilisation with round and elongated spermatids. *Hum Reprod* 1997, **12**, 336–340.
55. Bernadeu R, Cremades N, Takahashi K, Sousa M. Successful pregnancy after spermatid injection. *Hum Reprod* 1998, **13**, 1898–1900.
56. Grundy R, Larcher V, Gosden RG, *et al*. Personal practice: fertility preservation for children treated for cancer (2): ethics of consent for gamete storage and experimentation. *Arch Dis Child* 2001, **84**, 360–362.
57. Wallace WHB, Walker DA. Conference consensus statement: ethical and research dilemmas for fertility preservation in children treated for cancer. *Hum Fertil* 2001, **4**, 69–76.
58. Sanger WG, Olson JH, Sherman JK. Semen cryobanking for men with cancer—criteria change. *Fertil Steril* 1992, **58**, 1024–1027.
59. Tournaye H, van Steirteghem A, Devroey P. Semen cryobanking for men with cancer. *Fertil Steril* 1993, **60**, 197–198.
60. Aboulghar MA, Mansour RT, Serour GI, Fahmy I, Kamal A, Tawab Amin YM. Fertilization and pregnancy rates after intracytoplasmic sperm injection using ejaculate semen and surgically retrieved sperm. *Fertil Steril* 1997, **68**, 108–111.
61. Rivkees SA, Crawford JD. The relationship of gonadal activity and chemotherapy-induced gonadal damage. *JAMA* 1988, **259**, 2123–2125.

62. Delic JI, Bush C, Peckham MJ. Protection from procarbazine-induced damage of spermatogenesis in the rat by androgen. *Cancer Res* 1986, **46**, 1909–1914.
63. Kurdoglu B, Wilson G, Parchuri N, Ye WS, Meistrich ML. Protection from radiation-induced damage to spermatogenesis by hormone treatment. *Radiat Res* 1994, **139**, 97–102.
64. Pogach LM, Lee Y, Gould S, Giglio W, Huang HF. Partial prevention of procarbazine induced germinal cell aplasia in rats by sequential GnRH antagonist and testosterone administration. *Cancer Res* 1988, **48**, 4354–4360.
65. Ward JA, Robinson J, Furr BJ, Shalet SM, Morris ID. Protection of spermatogenesis in rats from the cytotoxic procarbazine by the depot formulation of Zoladex, a gonadotrophin-releasing hormone agonist. *Cancer Res* 1990, **50**, 569–574.
66. Kangasniemi M, Wilson G, Huhtaniemi I, Meistrich ML. Protection against procarbazine-induced testicular damage by GnRH-agonist and antiandrogen treatment in the rat. *Endocrinology* 1995, **136**, 3677–3680.
67. Meistrich ML, Parchuri N, Wilson G, Kurdoglu B, Kangasniemi M. Hormonal protection from cyclophosphamide-induced inactivation of rat stem spermatogonia. *J Clin Androl* 1995, **16**, 334–341.
68. Meistrich ML, Kangasniemi M. Hormone treatment after irradiation stimulates recovery of rat spermatogenesis from surviving spermatogonia. *J Androl* 1997, **18**, 80–87.
69. Masala A, Faedda R, Alagna S, *et al*. Use of testosterone to prevent cyclophosphamide-induced azoospermia. *Annal Intern Med* 1997, **126**, 292–295.
70. Johnson DH, Linde R, Haisnworth JD, *et al*. Effect of luteinizing hormone releasing hormone agonist given during combination chemotherapy on post therapy fertility in male patients with lymphoma: preliminary observations. *Blood* 1985, **65**, 832–836.
71. Waxman JH, Ahmed R, Smith D, *et al*. Failure to preserve fertility in patients with Hodgkin's disease. *Cancer Chemother Pharmacol* 1987, **19**, 159–162.
72. Thomson AB, Anderson RA, Kelnar CJH, Sharpe RM, Wallace WHB. Investigation of temporary suppression of the hypothalamic-pituitary-gonadal axis to enhance recovery of spermatogenesis following cancer therapy in childhood. *Hum Reprod* [in press].
73. Nagano M, Avarbock MR, Leonida EB, Brinster CJ, Brinster RL. Culture of mouse spermatogonial stem cells. *Tissue Cell* 1998, **30**, 389–397.
74. Tesarik J, Bacheci M, Ozcan C, Greco E, Mendoza C. Restoration of fertility by in-vitro spermatogenesis. *Lancet* 1999, **353**, 555–556.
75. Tesarik J, Mendoza C, Greco E. In vitro culture facilitates the selection of healthy spermatids for assisted reproduction. *Fertil Steril* 1999, **72**, 809–813.
76. Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. *Proc Natl Acad Sci USA* 1994, **91**, 11303–11307.
77. Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF, Nieschlag E. Germ cell transfer into rat, bovine, monkey and human testes. *Hum Reprod* 1999, **14**, 1440–1450.
78. Leporrier M, von Theobold P, Roffe JL, Muller G. A new technique to protect ovarian function in young women undergoing pelvic irradiation. Heterotopic ovarian autotransplantation. *Cancer* 1987, **60**, 2201–2204.
79. Thomas PRM, Winstanly D, Peckham MJ, Austin DE, Murray MA, Jacobs HS. Reproductive and endocrine function in patients with Hodgkin's disease: effects of oopheropexy and irradiation. *Br J Cancer* 1976, **33**, 226–231.
80. Human Fertility and Embryology Authority. *9th Annual Report and Accounts*. 2000, www.hfea.gov.uk.
81. Porcu E, Fabbri R, Seracchiolo R, *et al*. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. *Fertil Steril* 1197, **68**, 724–726.
82. Ataya K, Rao LV, Laurence E, Kimmel R. Luteinizing hormone-releasing hormone agonist inhibits cyclophosphamide induced ovarian follicular depletion in Rhesus monkeys. *Biol Reprod* 1995, **52**, 365–372.
83. Blumenfeld Z, Avivi I, Linn S, Epelbaum R, Ben-Shahar M, Haim N. Prevention of irreversible chemotherapy-induced ovarian damage in young women with lymphoma by a gonadotrophin-releasing hormone agonist in parallel to chemotherapy. *Hum Reprod* 1996, **11**, 1620–1626.
84. Gosden RG, Wade JC, Fraser HM, Sandow J, Faddy MJ. Impact of congenital and experimental hypogonadism on the radiation sensitivity of the mouse ovary. *Hum Reprod* 1997, **12**, 2483–2488.
85. Morita Y, Perez GI, Paris F, *et al*. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. *Nat Med* 2000, **6**, 1109–1114.
86. Abir R, Fisch B, Raz A, Nitke S, Ben-Rafael Z. Preservation of fertility in women undergoing chemotherapy: current approach and future prospects. *J Ass Reprod Gen* 1998, **15**, 469–478.
87. Gosden RG. Restitution of fertility in sterilised mice by transferring primordial ovarian follicles. *Hum Reprod* 1990, **5**, 117–122.
88. Carroll J, Gosden RG. Transplantation of frozen-thawed mouse primordial follicles. *Hum Reprod* 1993, **8**, 1163–1167.
89. Guanasena KT, Villines PM, Crister ES, Crister JK. Live births with autologous transplant of cryopreserved mouse ovaries. *Hum Reprod* 1997, **12**, 101–106.
90. Oktay K, Newton H, Gosden RG. Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice. *Fertil Steril* 2000, **73**, 599–603.
91. Shaw JM, Bowles J, Koopman P, Wood EC, Trounson AO. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. *Hum Reprod* 1996, **11**, 1668–1673.
92. Gosden RG, Rutherford AJ, Norfolk DR. Ovarian banking for cancer patients-transmission of malignant cells in ovarian grafts. *Hum Reprod* 1997, **12**, 403.
93. Eppig DG, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization in vitro. *Biol Reprod* 1989, **41**, 268–276.
94. Spears N, Boland NI, Murray AA, Gosden RG. Nouse oocytes derived from in vitro grown primary ovarian follicles are fertile. *Hum Reprod* 1994, **9**, 527–532.
95. Roy SK, Treacy BJ. Isolation and long term culture of human preantral follicles. *Fertil Steril* 1993, **59**, 783–790.
96. Abir R, Franks S, Mobberley MA, Moore PA, Margara RA, Winston RML. Mechanical isolation and in vitro growth of preantral and small antral human follicles. *Fertil Steril* 1997, **68**, 682–688.
97. Hovatta O, Silye R, Krausz T, *et al*. Cryopreservation of human ovarian tissue by using dimethylsulphoxide and propandiol-sucrose as cryoprotectants. *Hum Reprod* 1996, **11**, 1268–1272.
98. Eppig JJ, O'Brien M. Development in vitro of mouse oocytes from primordial follicles. *Biol Reprod* 1996, **54**, 197–207.
99. Torrance C, Telfer E, Gosden RG. Quantitative study of the development of isolated mouse pre-antral follicles in collagen gel culture. *J Reprod Fertil* 1989, **87**, 367–374.
100. Cox S-L, Shaw J, Jenkin G. Transplantation of cryopreserved fetal ovarian tissue to adult recipients in mice. *J Reprod Fertil* 1996, **107**, 315–322.
101. Nugent D, Meirow D, Brooke PF, Aubard Y, Gosden RG. Transplantation in reproductive medicine: previous experience, present knowledge and future prospects. *Hum Reprod Update* 1997, **3**, 267–280.
102. Gosden RG, Boulton MI, Grant K, Webb R. Follicular development from ovarian xenografts in SCID mice. *J Reprod Fertil* 1994, **101**, 619–623.

103. Oktay K, Newton H, Mullan J, Gosden RG. Development of human primordial follicles to antral stages in SCID/hpg mice stimulated with follicle stimulating hormone. *Hum Reprod* 1998, **13**, 1133–1138.
104. Brook PF, Radford JA, Shalet SM, Joyce AD, Gosden RG. Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. *Fertil Steril* 2001, **75**, 269–274.
105. Royal College of Obstetricians and Gynaecologists. *Storage of Ovarian and Prepubertal Testicular Tissue: Report of a Working Party*. London: Royal College of Obstetricians and Gynaecologists, 2000.
106. Hawkins MM, Draper GJ, Smith RA. Cancer among 1348 survivors of childhood cancer. *Int J Cancer* 1989, **43**, 975–978.
107. Li FP, Fine W, Jaffe N, Holmes GE, Holmes FF. Offspring of patients treated for cancer in childhood. *J Natl Cancer Inst* 1979, **62**, 1193–1197.
108. Sun J-G, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. *Biol Reprod* 1997, **66**, 602–607.
109. Trasler JM, Hales BF, Robaire B. Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting male fertility. *Nature* 1985, **316**, 144–146.
110. Kelly SM, Robaire B, Hales BF. Paternal cyclophosphamide exposure causes decreased cell proliferation in cleavage-stage embryos. *Biol Reprod* 1994, **50**, 55–64.
111. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. *Fertil Steril* 2000, **73**, 43–50.
112. Twigg J, Irvine DS, Aitken R. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at ICSI. *Hum Reprod* 1998, **13**, 1864–1871.
113. Bonduelle M, Camus M, De Vos A, *et al*. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum Reprod* 1999, **14**, 243–264.
114. Thomson AB, Campbell AJ, Irvine DS, Anderson RA, Kelnar CJH, Wallace WHB. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer *Lancet* [in press].
115. Sousa M, Mendoza C, Barros A, Tesarik J. Calcium response of human oocytes after intracytoplasmic injection of leukocytes, spermatocytes and round spermatids. *Mol Hum Reprod* 1996, **2**, 853–857.
116. Tesarik J, Kopeony V. Nucleic acid synthesis and development of human male pronucleus. *J Reprod Fertil* 1989, **86**, 549–558.
117. Hall JG. Genomic imprinting: review and relevance to human diseases. *Am J Hum Genet* 1990, **46**, 857–873.
118. Grundy R, Gosden RG, Hewitt M, *et al*. Fertility preservation for children treated for cancer (1): scientific advances and research dilemmas. *Arch Dis Child* 2001, **84**, 355–359.
119. Hawkins MM. Pregnancy outcome and offspring after childhood cancer. *Br Med* J1994, **309**, 1034–1040.