Genetic and teratogenic effects of cancer treatments on gametes and embryos

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Male and female germ cells vary in their sensitivity to the mutagenic effects of chemotherapy and radiotherapy, depending on their stage of maturation and the agent used. Although sperm DNA damage exists following treatment, no increase in genetic defects or congenital malformations was detected among children conceived to parents who have previously undergone chemotherapy or radiotherapy. The use of assisted reproductive technologies and micromanipulation techniques might increase this risk; hence caution should be exercised. In female cancer patients, miscarriage and congenital malformations are not increased following chemotherapy. However, when IVF and embryo cryopreservation is practised between or shortly after treatment, possible genetic risks to the growing oocytes exist, and hence the babies should be screened. During pregnancy, the potential teratogenic effects of chemotherapy influence the choice and timing of therapy. Termination is usually recommended in the first trimester. Second- and third-trimester exposure does not usually increase the teratogenic risk and cognitive development, but it may increase the risk of poor obstetric outcome and fetal myelosuppression. During the first two weeks after fertilization of the embryo, radiation is lethal but not teratogenic. High doses of radiation during pregnancy induce anomalies, impaired growth and mental retardation, and there may be an increased risk of childhood leukaemia and other tumours in the offspring.

Key words: cancer/chemotherapy/radiotherapy/mutagenicity/gametes

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Introduction

The two fundamental cancer treatments, chemotherapy and radiotherapy, have long been known to have a multitude of short- and long-term adverse effects. They have been shown to be mutagenic to somatic cells, causing gene mutations, chromosomal breaks, rearrangements and aneuploidy, and their long-term effects have included treatment-related secondary tumours in cancer survivors (Witt and Bishop, 1996). With a growing population of young cancer survivors (Boring *et al.*, 1994), significant concerns have been raised regarding the adverse effects of these treatments on the offspring of treated individuals.

That includes offspring conceived after completion of treatment, and fetuses exposed *in utero* to cancer treatments. Studies indicate that chemotherapy and radiotherapy treatments can be mutagenic to both animal and human germ cells. Damage to the genetic composition of the human germ cell might influence fertilization, increase the rate of abortions, or cause malformations in children conceived by men or women previously exposed to cancer treatments.

Cancer occurs in ~2–10 in 10 000 pregnancies (Kennedy *et al.*, 1993). If treatment is indicated during pregnancy, and the chemotherapeutic agents cross the placenta, the fetus can be exposed. In such cases, the potential teratogenic effects of therapy on the fetus influence the choice of therapy, the timing, or whether a pregnancy should be terminated. These decisions can lead to a conflict between optimal maternal therapy and fetal well-being, and are usually complicated by emotional, ethical and religious beliefs. Unfortunately, much of the information upon which therapeutic decisions are made are based on anecdotal reports.

This review focuses on the mutagenic and teratogenic potential of the different modalities of cancer treatment. The possible risk

of assisted reproductive technologies in patients exposed to radio or chemotherapy is discussed. The management of different cancers during pregnancy is detailed in another contribution to this mini symposium (Weisz *et al.*, 2001).

Mutagenicity of cancer treatment

Most existing data on the mutagenic effects of chemotherapeutic agents have been derived from animal studies, as outlined below. In humans, there is less information on the effect of individual drugs, as most reports are obtained from exposures to multiple drug administration for common malignancies.

The data collected indicate that chemotherapy and radiotherapy treatments are mutagenic to both male and female germ cells at various stages of maturation. However, human studies monitoring the offspring of treated individuals have shown that the observed increase in mutations in germ cells has not translated into an increase in fetal abnormalities. Whether the treated parent was male or female, the incidence of fetal chromosomal or congenital abnormalities remained the same as for the general population (Holmes and Holmes, 1978; Li *et al.*, 1979; Green *et al.*, 1991; Sanders *et al.*, 1996). A number of factors exist which might explain this apparent discrepancy. First, mutated spermatozoa may not be able successfully to fertilize the oocyte. Second, if fertilization does occur, then dominant lethal mutations may result in an undetected miscarriage at a very early stage. It is also possible that some correction mechanism exists within the oocyte to correct sperm chromosomal aberrations and to correct induced mutational damage to the oocyte (Generoso *et al.*, 1979; Matsudo and Tobari, 1989; Host *et al.*, 2000). As presented by the following data, there is a trend of decreased frequency of genetic abnormalities a long time after exposure to chemotherapeutic agents. This may be due to there being a correction mechanism within the spermatogonia, or to removal of the damaged spermatogonia.

The mutagenic effects of chemotherapy by classes

Chemotherapeutic agents can be divided into six different classes according to their mechanism of action; alkylating agents, cisplatin and its analogues, vinca alkaloids, antimetabolites, topoisomerase interactive agents, and newer agents (Devita *et al.*, 1997). All these drugs act by interrupting vital cell processes by causing an arrest of the normal cellular proliferation cycle. Some have a specificity for one or more phases of the cell cycle, especially during cell division. The mechanism of action of each of the different classes is summarized in Table I.

Alkylating agents (AA)

Alkylating agents (AA) are the most commonly used agents for antineoplastic treatment, and have a very wide spectrum of activity. AA are also the most widely researched class of chemotherapy because of their severe effect on human fertility. In men, AA cause depletion of germinal epithelium in the testes and aplasia of germinal cells, resulting in severe oligozoospermia or azoospermia within 90–120 days of treatment (Byrne *et al.*, 1987), with poor long-term recovery (Meirow and Schenker, 1995). In a study on the return of spermatogenesis after cyclophosphamide treatment, those men who regained spermatogenesis (4 years later, most had not) did so after an average

interval of 31 months (Buchanan *et al.*, 1975). AA are mutagenic to all stages of maturation of male human germ cells; however, these agents do not cause transmissible chromosomal translocation or aneuploidy in stem cells, which is a very significant parameter for genetic risk assessment (Witt and Bishop, 1996). In women, AA cause ovarian fibrosis, and follicular and oocyte depletion (Familiari *et al.*, 1993), resulting in a high ovarian failure rate. In studies on mice, AA have been shown to be mutagenic to preovulatory oocytes (Becker and Schoneich, 1982). A recent study on mice showed an increase in abortions and fetal malformations in pregnancies resulting from oocytes exposed at different stages of oocyte maturation. The study showed that abortion rate was highest in oocytes exposed at the preovulatory stage—up to one week before ovulation (56% compared with between 19 and 31% for oocytes exposed at earlier growth stages). The malformation rate was at least 10-fold higher in oocytes exposed at any stage, compared with controls (1.2%). The highest malformation rate (33%) was found in oocytes exposed at the earliest stage of maturation. Thereafter, malformation rate decreased gradually as the time interval increased between exposure and ovulation (Meirow *et al.*, 2001).

Cisplatin and analogues

These are commonly used in the treatment of testicular, endometrial and ovarian tumours, as well as some other solid tumours. In studies on male mice, cisplatin induced chromosomal aberrations in spermatocytes, as well as differentiating spermatogonia. The aberrations (mostly chromatid breaks and fragments) were induced immediately after treatment. A significant number of aberrant spermatocytes showed autosomal and/or XY univalents, tetravalents, and with extra elements (Adler and el-Tarras, 1990; Choudhury *et al.*, 2000). However, long after exposure the transmission of such effects was found to be decreased substantially by the time the exposed spermatogonia matured. Nonetheless, some abnormal spermatozoa were still present (Katoh *et al.*, 1990; Choudhury *et al.*, 2000). However, this might be because the damaged spermatocytes did not survive to full maturation.

In rats, administration of cisplatin leads to the formation of cisplatin–DNA adducts in spermatozoa. When cisplatin-treated male rats were bred with untreated females, no adverse developmental effects or decreases in body weight were seen in the offspring, although there was a trend towards increased early embryo mortality (Hooser *et al.*, 2000).

Cisplatin[®] is a specific inducer of dominant lethal mutations in female mice, and causes different types of chromosomal damage and DNA adducts (Blommaert *et al.*, 1995) that induce genetic effects in oocytes resulting in early embryonic mortality (Katoh *et al.*, 1990). The latter study also showed that exposure of maturing oocytes to cisplatin resulted in significantly increased embryonic mortality. In another study performed to determine the effects of cisplatin on murine oocytes, exposure of mature oocytes to cisplatin before ovulation led to marked (treatment-induced) aneuploidy (Higdon *et al.*, 1992).

Vinca alkaloids

These are also known as aneuploidy inducers, and are used in the treatment of leukaemia, lymphoma, testicular and breast cancer. In men, they cause the arrest of spermatogenesis, and may also

Table I. A brief description of the six classes of chemotherapy and their mechanisms of action (Devita *et al.*, 1997)

Class of agent	Name of drugs	Mechanism	Cell cycle
Alkylating agents	Cyclophosphamide, Nitrogen mustard, Chloroethyl nitrosurea, Busulfan, Chlorambucil, Melphalan, Thiotepa,	Cross-link DNA strand, interrupt RNA and protein synthesis	Non-specific
Cisplatin and analogues	Cisplatin, Carboplatin	Interferes with DNA synthesis without affecting normal RNA and protein synthesis	Possibly specific (G_2 arrest)
Vinca alkaloids (aneuploidy inducers)	Vincristine, Vinblastine	Bind tubulin and cause dissociation of the microtubule apparatus	Specific: G_1 and S phase
Antimetabolites	Methotrexate, Aminopterin, 5-Fluorouracil, Cytarabine	Inhibit cellular metabolites by acting as false substrates for reactions required in DNA or RNA synthesis.	Non- specific
Topoisomerase (top) interactive agents (radiomimetics)	Bleomycin, Actinomycin, Doxorubicin, Daunorubicin	Interact with enzyme–DNA complex. Prevents resealing of the top I-mediated DNA single strand breaks	Specific: G_2 arrest/ S-phase apoptosis
Newer agents	Paclitaxel	Acts on microtubule system	Non-specific

affect motility of mature spermatozoa. The administration of vincristine to dogs resulted in transient deterioration of semen characteristics during treatment, though these subsequently returned to normal. Such changes are attributed to a direct effect of vincristine on the extragonadal spermatozoal reserves (Saratsis *et al.*, 2000). Vinblastine is a highly potent spindle poison that produces a high number of shortened and monopolar spindles as well as multiple chromosomal loss (Gassner and Adler, 1995). Vinblastine is cytotoxic to primary spermatocytes, while spermatogonia and preleptotene spermatocytes are relatively resistant (Sjoblom *et al.*, 1995).

The effects of vinblastine sulphate on in-vivo and in-vitro meiosis in male rats showed an increased rate of detached chromosomes. Cell proliferation was inhibited, as shown by the number of cells arrested at metaphase during the first meiotic (MI) or second meiotic (MII) division. Vinblastine was found to be a potent inducer of cell death (Kallio *et al.*, 1995).

When mice oocytes were exposed to vinblastine before the first meiotic division, high levels of aneuploidy were observed (Russo and Levis, 1992). Significant meiotic-arresting activity following administration of vinblastine results in an increased frequency of ovulated oocytes arrested in MI phase, hyperhaploid oocytes, and undegenerated chromosome sets of the first polar body (Mailhes *et al.*, 1993). These aneuploid cells can be fertilized to form zygotes (Albanese, 1987a), which means that these damaged oocytes could produce malformed fetuses.

Antimetabolites

These are used to treat paediatric leukaemia, breast, ovarian and gastrointestinal cancers. These agents act on rapidly dividing

cells, i.e. the later stages of spermatogenesis, and induce dominant lethal mutations. It has been shown that 5-flourouracil and 6-mercaptopurine cause chromosomal aberrations (Generoso *et al.*, 1975; Albanese, 1987b). Stem cells, which undergo slow division, are much less sensitive to these agents. Long-term administration of 6-mercaptopurine in low doses to male mice induced a high embryonic resorption rate in pregnant females mated with the exposed males (Meirow *et al.*, 2000). These results indicate a high rate of sperm damage which induced lethal embryonic damage. Insufficient research data are available on the effects of antimetabolites on female germ cells.

Topoisomerase interactive agents

These are commonly used in the treatment of haematological malignancies and for the treatment of solid tumours. It was found that these agents are cytotoxic to all spermatogonial stages. The mutagenic effects of adriamycin (doxorubicin) was demonstrated in mice spermatocytes (Liang *et al.*, 1986). Also, administration of bleomycin to male mice induced chromosomal anomalies in spermatogonia and spermatocytes (Van-Buul and Goudzwaard, 1980). Other studies, however (Meistrich *et al.*, 1990; Russell, 1990), have not demonstrated mutagenic effects on any stage of mice spermatogenesis. This apparent contradiction could be explained by selection against the affected and abnormal germ cells, resulting in the loss of chromosomally damaged cells (Tease, 1992). The mutagenicity of bleomycin was studied in the different stages of spermatogenesis in *Drosophila*, with bleomycin significantly increasing the proportions of both complete and mosaic lethals at the meiotic and pre-meiotic stages. Genetic instabilities induced by bleomycin were transformed into actual

mutations in late zygotic divisions, and it was shown that bleomycin-induced instabilities could be transmitted to future generations.

Adriamycin and bleomycin are female-specific mutagens, and have both been shown to induce dominant lethal mutations in maturing/preovulatory oocytes of female mice (Katoh *et al*., 1990; Sudman *et al*., 1992). Etoposide (VP-16), which is used as an antineoplastic drug in humans, inhibits topoisomerase II activity by forming a complex that blocks the DNA strand-rejoining activity of topoisomerase II. This results in DNA-strand breaks and the formation of structural chromosome aberrations. A study in mice demonstrated preferential pericentric lesions and aneuploidy induced in oocytes by the topoisomerase II inhibitor, etoposide (Mailhes *et al*., 1995).

Combination chemotherapy

The majority of studies investigating the genetic effects of a single chemotherapeutic agent have been conducted in animals. In clinical practice, however, men and women are rarely subjected to just one chemotherapeutic agent, and therefore the results of a single agent administration cannot be determined. Some drug combinations are commonly used together, such as MOPP (methylchloroethamine, vincristine, procarbazine and prednisone) for Hodgkin's disease. Following MOPP treatment, as many as 90% of men remain azoospermic 4 years after therapy (Chapman, 1982). An increased frequency of aneuploidy following MOPP treatment was demonstrated up to 18 years post treatment (Genesca *et al*., 1990). Another study on the spermatozoa of patients who had undergone MOPP therapy (Brandriff *et al*., 1994) reported an increased frequency of hyperhaploidy (indicating chromosomal non-dysjunction) over 3 years after treatment, indicating that the treatment had had a sublethal and not correctable mutagenic effect on stem cells. Sperm chromosomal abnormalities were also assessed in testicular cancer patients before, during and after BEP (bleomycin, etoposide, cisplatin) chemotherapy (Martin *et al*., 1999). In these patients, fluorescence in-situ hybridization (FISH) analysis was used to detect aneuploidy for chromosomes 1, 12, X, and Y, and diploidy detected a significant increase in the frequency of 24,XY spermatozoa during (0.33%) and after (0.34%) chemotherapy compared with pretreatment (0.14%). The study results suggested that there may be a significantly increased risk of chromosomal abnormalities in the spermatozoa of patients during and immediately after treatment, similar to that seen in animal models. Sperm aneuploidy was evaluated (using FISH) in male patients with Hodgkin's disease who were treated with NOVP (novanthrone, oncovin, vinblastine, prednisone) chemotherapy (Robbins *et al*., 1997); ~5-fold increases in spermatozoa with disomies, diploidies and complex genotypes involving chromosome X, Y and 8 were found. The increases in sex chromosome aneuploidies arose from segregation errors at MI as well as MII. However, the aneuploidy effects were transient, declining to pretreatment levels within about 100 days after cessation of therapy. No significant increase was demonstrated in either aneuploidy or structural aberrations in spermatozoa collected more than 9 months after cessation of treatment with other drug combinations (Jenderny *et al*., 1992; Martin *et al*., 1995). However, this might have been due either to a particular sensitivity of stem cells to the cytolethal effects of treatment, or

to very efficient DNA repair mechanisms. Large doses of a particular chemical can overwhelm repair mechanisms in stem cells (Russell, 1987), and this can serve as a possible explanation for the differences in long-term genetic effects following chemotherapy.

Assisted reproduction in cancer patients

Each year, hundreds of thousands of children and young persons of reproductive age are exposed to known mutagens in the form of chemotherapy and/or radiotherapy for cancer (>20 000 annually in the USA alone). As an increasing number of these treatments is effective, and many cancers can now be cured, one of the major prices paid for success is infertility and the growing concern that genetic defects are introduced to the germ cells. In adult patients, semen cryopreservation is offered before treatment begins, but in many cases the quality of the spermatozoa is poor (Meirow and Schenker, 1995). In many centres, frozen semen banking is also offered during radio/chemotherapy if cryopreservation was not performed previously. Not surprisingly, insemination with frozen–thawed semen in these cases is often unsuccessful. In order to increase the fertilization rate and the potential number of embryos that can be created from a single cryopreserved specimen, the technique of intracytoplasmic sperm injection (ICSI) is often used. If spermatogenesis recovers partially after chemotherapy or radiotherapy, then fresh spermatozoa can be used to establish a pregnancy by ICSI. Indeed, assisted reproductive technologies promises much to former cancer patients, and now enables previously 'infertile' patients successfully to conceive offspring. Until now, there has been no increase in malformations among children conceived to parents who have previously undergone chemotherapy or radiotherapy. However, the use of new reproductive technologies and micromanipulation might increase this risk, and has focused concern on this issue. Animal and human studies have shown that administration of chemotherapy or radiotherapy before mating can cause genetic damage in the germline at the spermatogenic cell stage treated. Moreover, ICSI can bypass natural selection processes that exist to screen out genetically damaged germ cells and prevent fertilization and growth of malformed fetuses (Baschat *et al*., 1996). A recent study (Chatterjee *et al*., 2000) detected sperm DNA damage in patients during and following chemotherapy (fludarabin) using single cell 'comet assays'. The authors have suggested that caution be exercised if the ejaculates are used for in-vitro fertility treatment, and further experiments are needed to assess the biological significance of these DNA changes. We suggest that pregnancies of previous cancer patients are closely monitored, and that any incidence of abnormalities reported. At present, we must show extreme caution when counselling these patients.

In former female cancer patients, most studies have shown that miscarriage and congenital malformations are not substantially increased; however, these pregnancies were established long after treatment had ceased. In order to overcome future female sterility, some centres offer IVF and embryo cryopreservation to patients following first-line chemotherapy and before administration of sterilizing treatment. The full span of follicle growth from the primordial to Graafian stage is of the order of 6 months (Gougeon, 1996; Wasserman, 1996). When growing follicles are exposed to chemotherapy, and then stimulated and fertilized, the possibility

of adverse pregnancy outcomes should be considered. Several key questions should be investigated in the near future: Are there more adverse outcomes in pregnancies following oocyte exposures to cytotoxic treatment at different stages of maturation? Do they carry increased genetic risk? If so, what is the safe period between cessation of treatment and oocyte retrieval for IVF? Clearly, we need to monitor the pregnancy outcome of all these patients and, until more data are available, the fetuses and babies should be screened for chromosomal aberrations and birth defects.

Teratogenicity of cancer treatment

The potential teratogenic effect of cancer treatment depends upon the developmental stage of the fetus at the time of exposure. Those developmental stages can be divided into the preimplantation and early post-implantation periods, the embryonic period or major organogenesis period (3rd to 8th week post conception) during which most of the organs develop (Sadler, 1995), and the fetal period (9th completed gestational week to term). During the predifferentiation period, the conceptus is mostly resistant to teratogenic insult (Schardein, 1993). Any embryonic damage occurring at this point would most likely lead to death of the conceptus (Buekers and Lallas, 1998). During organogenesis, damage to any developing organ would most likely lead to major malformations. Although organogenesis is defined until the end of week 8, a number of organs continue to develop until the end of week 10, such as closure of the palate, and the definite kidney (metanephros). During the fetal period, the damage is less extensive. Subtle impairments of fetal growth and development occurring later in gestation, and disturbances of neurological maturation in particular, may not be apparent at delivery but may manifest early in life (Garber, 1989).

The risk of teratogenesis following cancer treatment appears to be significantly lower than is commonly appreciated, probably because developmental stage at exposure, dose, duration and frequency of drug administration are important variables.

Most drugs reach the fetus in significant concentrations after maternal administration, because the placenta is not an effective barrier. Drugs with a molecular weight <600 Da usually cross the placenta (unless strongly protein bound), while those with weights >1000 Da do not cross. Many drugs have a molecular weight of 250–400 Da, allowing easy passage to the fetus (Pacifici and Nottoli, 1995). Of the chemotherapeutic agents examined, cisplatin (Koc *et al.*, 1994) and cyclophosphamide (D'Incalci *et al.*, 1982) easily cross the placenta, while epirubicin has limited transplacental passage (Gaillard *et al.*, 1995). Teratogenic effects of cytotoxic agents have been identified primarily on the basis of observable anomalies in infants and abortuses. First-trimester exposures may be most often implicated because disruptions in organogenesis are relatively easy to detect.

A number of reviews have summarized the incidence of malformations after first-trimester exposure to chemotherapy; an estimate of 10–20% of fetuses exposed to chemotherapy at this time would have major malformations (Caligiuri and Mayer, 1989). The malformations reported were from all organ systems, with no discernible pattern. A review of 82 articles and citations of 139 cases of first-trimester exposure to chemotherapy reported a total of 24 infants born with malformations (17%) after single-agent exposure, and 25% after combination-agent exposure (Doll

et al., 1989). The incidence declined to only 6% in the single-agent exposures if folate antagonists were not included. In 1985, the National Cancer Institute started a registry for in-utero exposure to chemotherapeutic agents. Of the first 210 children monitored, 29 had abnormal outcomes, and 27 of these resulted from first-trimester exposure (Randall, 1993).

The risk of anomalies after administration of chemotherapy in the second and third trimester is probably not greater than the background rate, but there may be a greater risk of stillbirth, fetal growth restriction, premature birth, and maternal and fetal myelsosuppression (Garcia *et al.*, 1999)

A summary of some of the reports on teratogenic effects of individual agents is given in Table II. The antimetabolites methotrexate and aminopterin have been associated with birth defects more frequently than any other agents. There is one case report of multiple congenital anomalies after 5-fluorouracil exposure, but this was probably not related because of the timing of the exposure (Stephens *et al.*, 1980). Alkylating agents are apparently less teratogenic than antimetabolites (Doll *et al.*, 1989). Vinca alkaloids are potent teratogens in animals, although most cases of human exposure resulted in normal infants (Doll *et al.*, 1989).

Neurodevelopmental studies

Delayed effects of in-utero exposure to chemotherapeutic agents are basically undefined. Concerns for potential late manifestations come at least in part from evolving information on the experience of cancer survivors exposed to these agents in childhood.

A major concern is intellectual and neurological function following in-utero exposure to cancer treatments. The central nervous system continues to develop after the first trimester, and is sensitive to insult during the entire period of gestation. Exposure after the first trimester would probably not cause anatomical defects, but may result in neurodevelopmental consequences.

Few data exist on children's cognitive functions or long-term development following in-utero exposure to maternal cancer and its associated treatment. Two neurodevelopmental studies were carried out which assessed for growth and development, school performance, intelligence testing according to Wechsler Intelligence and Bender–Gestalt cognitive tests and neurological function of children born to mothers treated with chemotherapy during pregnancy. The results were compared with those in non-related children of similar age and environmental background. In the first study (Aviles and Niz, 1988), 17 children aged between 4 and 22 years who were born to mothers treated for acute leukaemia during pregnancy were examined. In a later study (Aviles *et al.*, 1991), 43 children ranging in age from 3 to 19 years born to mothers treated for haematological malignancies during pregnancy were examined. In both studies, all the values obtained for children exposed to chemotherapy *in utero* were comparable with control values.

In considering the above factors, we recommend that if treatment cannot be delayed, and is given in the first trimester (especially if folate antagonists are used), then termination of the pregnancy is recommended. When possible, chemotherapy should be delayed until the second trimester, to lessen the risk of anomalies in the fetus. However, chemotherapy started in the second and third trimester may increase the risk of stillbirth, fetal

Table II. Cases of the teratogenic effects of chemotherapeutic agents

Agent	First-trimester exposure	Second- and third-trimester exposure	References
Busulfan	31 exposures – 6 malformed infants[a]	No apparent adverse effects[b]	[a]Briggs et al. (1998); [b]Wiebe et al. (1994)
Chlorambucil	Unilateral renal agenesis (2 cases), ureter agenesis (1 case)[a,b] Retinal defect (1 case)[c]	No adverse effect[d]	[a]Shotton and Monie (1963); [b]Steege and Caldwell (1980); [c]Rugh and Skaredoff (1965); [d]Nicholson (1968)
Cisplatin	No data	10 women treated IUGR (5 cases), moderate bilateral hearing loss (1 case)	Tomlinson et al. (1997)
Cyclophosphamide	Cyclophosphamide embryopathy (7 cases)[a] Normal outcomes (14 cases)[b,c]	No adverse effects[b,c]	[a]Enns et al. (1999); [b]Aviles et al. (1991); [c]Pizzuto et al. (1980)
Vinka alkaloids	Vinblastine – normal outcome[a,b] Vincristine – normal outcomes[b–e]	Normal outcome (>10 cases)[f]	[a]Aviles et al. (1991); [b]Mulvihill et al. (1987); [c]Caliguiru and Mayer (1989); [d]Schardein (1993); [e]Pizzuto et al. (1980); [f]Doll et al. (1989)
Aminopterin	Aminopterin syndrome – cranial dysostosis, hypertelorism, micrognathia, cleft plate, mental retardation[a,b]	Normal outcomes[c]	[a]Warkany (1978); [b]deAlvarez (1962); [c]Nicholson (1968)
Methotrexate[*]	Pattern of anomalies – skull, skeletal, ocular, hypertelorism, limb and CNS defects[a,b] No malformations[b,c]	No anomalies[d]	[a]Buckley et al. (1997); [b]Schardein (1993); [c]Aviles et al. (1991); [d]Nicholson (1968)
6-Mercaptopurine	Normal outcomes[a,b]	Normal outcomes[a]	[a]Briggs et al. (1998); [b]Gilland and Weinstein (1983)
Paclitaxel	No information	–	–
Daunorubicin	No malformations (4 cases)[a]	Normal outcomes[b]	[a]Briggs et al. (1998); [b]Gilland and Weinstein (1983)
Doxorubicin	Multiple anomalies (1 case, combination chemotherapy)[a]	No adverse outcomes[b]	[a]Murray (1984); [b]Briggs et al. (1998)
Bleomycin	No information	Transient neonatal leukopenia and neutropenia[a]	[a]Raffles et al. (1989)
Cytosine arabinoside	Many cases of normal outcomes[a,b] Two cases of malformations[c]	Normal outcomes Myelosuppression and chromosomal abnormalities[d,e]	[a]Schardein (1993); [b]Wagner et al. (1980) [c]Schleuning and Clemm (1987); [d]Zemlickis et al. (1992); [e]Volkenandt et al. (1987)

[*]Risk probably dose-dependent, smaller or non-existent with lower doses <10 mg/week (Feldkamp and Carey, 1993).
[a–f]See References.
CNS = central nervous system; IUGR = intrauterine growth retardation.

growth restriction, premature birth, and maternal and fetal myelosuppression. In spite of the increased teratogenic risk, in-utero exposure to chemotherapeutic agents does not appear to affect neurological and cognitive development.

Radiotherapy

Radiotherapy is used to treat such malignancies as Hodgkin's disease, lymphosarcoma and testicular cancers, many of which strike patients of young or childbearing age. Radiation doses used are high, in the range of 3000 to 7000 cGy—doses that are thousands of times higher than those used in diagnostic radiology. The effects of such high doses can be mutagenic, embryotoxic, embryolethal and teratogenic. Where possible, the radiation field

does not include the gonads, and when indicated the ovaries are surgically relocated away from the radiation field (oophoropexy).

Effects of high doses of irradiation on the gonads

Ionizing radiation has adverse effects on gonadal function in men of all ages, the degree and persistence of the damage being dependent on the dose. In many cases, men are azoospermic after treatment, and those who do regain spermatogenesis some time after treatment exhibit low sperm counts, motility and an increased rate of chromosomal abnormalities (Martin et al., 1986). In comparison, the ovary appears more resistant to radiation than the testis. A suggested reason for this difference is the constant cell division occurring during sperm production in the testes, in contrast to the germ cells within the ovary that do not

divide until completion of the first meiotic division at ovulation (Sadler, 1995). However, in a retrospective cohort study (Byrne *et al.*, 1987), the effects of radiotherapy were seen to be similar in men and women (relative risks of infertility 0.89 and 0.85 respectively for radiation above the diaphragm, and 0.76 and 0.78 below the diaphragm). The differences between the two studies may have been due to differences in shielding and sample size.

Mutagenic effects of radiotherapy

In males, studies on animals and humans have shown that radiation has direct mutagenic effects on germ cells in relation to dose. High doses may lead to dominant lethal effects, point mutations and chromosomal abnormalities (Brent, 1999). In mice, irradiation has been shown to cause chromosomal aberrations at all stages of sperm development (Tease, 1992). In humans, most men are azoospermic for at least a year following radiation therapy, and studies on sperm samples taken 3 years later found an increased frequency of sperm chromosomal abnormalities (both numerical and structural), 20.9% compared with a control rate of 8.5% (Martin *et al.*, 1986; Martin, 1993). These studies have also found a greater frequency of hypohaploidy relative to hyperhaploidy, suggesting that radiotherapy causes chromosomal loss rather than non-dysjunction. These effects seem to be dose-related, with an apparent threshold (Brent, 1999; Fattibene *et al.*, 1999). There is also a possibility that preconceptional high-dose paternal irradiation may result in increased induction of lymphomyeloid malignancy in the offspring if they are exposed after birth to a recognized inducer of leukaemia (Lord, 1999).

Women previously treated with high-dose abdominal radiotherapy are also at increased risk during subsequent pregnancies for spontaneous abortions (occurring in 38% of patients who had previously undergone irradiation compared with 12% of the population), preterm labour (62 versus 9%) and low birth weight infants (62 versus 6%) (Li *et al.*, 1987; Hawkins and Smith, 1989; Sanders *et al.*, 1996). These effects could be due in part to genetic damage to the follicles during radiotherapy, but much more likely to be due to effects of radiation on the uterus (Critchley, 1999).

In studies on rodents, radiation has been shown to cause destruction of oocytes in primordial follicles, followed by follicular atresia, and stromal hypertrophy which leads to amenorrhoea and sterility (Jarrell *et al.*, 1986). Of those cells which survive treatment, many suffer mutagenic damage. Animal studies have indicated that irradiation can cause chromosomal damage at all stages of oocyte maturation. According to one report (Tease, 1992), the oocyte is most sensitive immediately pre-ovulation, and most structural chromosomal abnormalities induced at that stage are embryo lethal and therefore not transmissible. Others (Kirk and Lyon, 1982) found that the highest proportion of dead and abnormal fetuses resulted from oocytes exposed to radiotherapy at the earliest stages of maturation. In both studies, the greatest damage was caused to growing follicles, of all stages, compared with dormant primordial follicles. This seems to stem from the fact that at these stages the oocytes are metabolically active.

Studies on women exposed to the atomic bomb, and on the offspring conceived and born to them following that exposure, have shown that the incidence of spontaneous abortion is greater, but that these children suffered no increase in mutation or in major congenital anomalies above the normal population

(Damewood and Grochow, 1986). This corroborates other studies on the effects of radiotherapy which found that fetal abnormalities are not increased in the offspring of women exposed to radiotherapy before pregnancy (Li *et al.*, 1987; Hawkins and Smith, 1989; Hawkins, 1991; Sanders *et al.*, 1996).

After reviewing the above literature, and based upon our cumulative experience, we recommend that in women who were exposed to high doses of irradiation prior to conception, if reproduction is not disturbed, there seems to be very little danger to the fetal well-being. The adverse effects on subsequent pregnancies are likely to be due to the direct effects of radiation on the uterus.

High-dose irradiation during pregnancy

Ionizing irradiation is a widely studied, proven 'universal teratogen' affecting all animal species (Brent, 1989, 1999). There is a threshold phenomenon regarding the effects of ionizing irradiation on the human conceptus, supported by extensive experimental data, and exposures to doses below 0.1–0.2 Gy do not seem to cause an increase in the rate of congenital malformations (Brent, 1999).

Radiation is also carcinogenic, with a lower threshold. Classic effects of radiation on the developing mammal are embryonic death, gross congenital malformations and intrauterine growth retardation (IUGR). Each of these effects has a dose–response relationship. In most studies where significant effects on the growth and cognitive development of the human fetus were observed, the exposure was to doses well above 1.0 Gy (Brent, 1999). In these cases, the newborn infants suffered from microcephaly, eye abnormalities, skeletal malformations and stunted growth, as well as mental retardation.

During the first 2 weeks post fertilization, the embryo is highly sensitive to the lethal effects of irradiation and is insensitive to the teratogenic effects of radiation (Brent and Bolden, 1967; Brent, 1999). In weeks 3–10 post fertilization, radiation may be teratogenic, cause growth retardation, and very high doses (at least 1.0 Gy) may be lethal to the embryo (Fattibene *et al.*, 1999). During the later fetal stages, radiation does not result in major anatomical anomalies, but can cause permanent cell depletion of various organs and tissues if the radiation exposure is high enough (Brent, 1989, 1999). In the post-partum period, the infant has the ability to recover partially from the growth-retarding effects of high-dose irradiation. Chromosomal abnormalities and somatic mutations have been invoked to explain radiation-induced anomalies. Irradiation of the zygote may cause chromosomal abnormalities which may be lethal and result in increased preimplantation death, but point mutations are less likely to be a contributing factor to abnormal morphogenesis (Brent, 1999; Pils *et al.*, 1999).

The central nervous system develops throughout gestation, and may therefore be sensitive to radiation at all stages of pregnancy. High doses of ionizing irradiation, mainly in therapeutic doses, were found to induce skeletal, eye and brain anomalies in the human fetus. The main defects were microcephaly, and mental retardation, microphthalmia, cataract, iridal defects and skeletal anomalies. Similar damage was observed in the offspring of mothers exposed to the atomic bomb in Hiroshima and Nagasaki. Analysis of the outcome of children exposed while *in utero* to the atomic bomb indicated that the fetal brain is most susceptible to

high doses of ionizing irradiation during weeks 8–15 after conception, and to a lesser extent during weeks 16–25 (Otake *et al.*, 1996; Schull and Otake, 1999), and apparently least susceptible prior to the 8th week and after the 25th week. The effects were mainly microcephaly and mental retardation. These children also suffered from retardation of growth in stature and in head circumference.

There is concern about the carcinogenic effects of irradiation on the developing embryo and fetus, as several epidemiological studies have demonstrated an increased risk of childhood leukaemia and other childhood tumours (Stewart *et al.*, 1958; McMahon, 1962; Mole, 1979). The overall additional risk is estimated to be about 40% (Harvey *et al.*, 1985). The risk for malformations and cancer following low-dose intrauterine irradiation is relatively low (Mole, 1993; Doll and Wakeford, 1997), being between zero and one case per thousand fetuses irradiated per rad of irradiation.

We recommend that during pregnancy, high-dose irradiation should be avoided, as it may induce central nervous system, eye and skeletal anomalies, impaired growth and mental retardation.

If the embryo was exposed to radiation in the first 2 weeks post fertilization, then the effect is embryolethal but not teratogenic. At any point during the pregnancy, maternal exposure (to the abdomen) of <0.10–0.20 Gy does not seem to cause teratogenic effects, although in-utero exposure to radiation causes 40% increased risk of childhood leukaemia and other tumours. If there was very early or low dose exposure to radiation, these do not justify termination of the pregnancy.

References

Adler, I.D. and el-Tarras, A. (1990) Clastogenic effects of cis-diamminedichloroplatinum. II. Induction of chromosomal aberrations in primary spermatocytes and spermatogonial stem cells of mice. *Mutat Res.*, **243**, 173–178.

Albanese, R. (1987a) Induction and transmission of chemically induced chromosome aberrations in female germ cells. *Environ. Mol. Mutagen.*, **10**, 231–243.

Albanese, R. (1987b) Mammalian male germ cell cytogenetics. *Mutagenesis*, **2**, 79–85.

Aviles, A. and Niz, J. (1988) Long term follow up of children born to mothers with acute leukemia during pregnancy. *Med. Pediatr. Oncol.*, **16**, 3–6.

Aviles, A., Diaz-Maqueo, J.C., Talavera, A. *et al.* (1991) Growth and development of children of mothers treated with chemotherapy during pregnancy: current status of 43 children. *Am. J. Hematol.*, **36**, 243–248.

Baschat, A.A., Schwinger, E. and Diedrich, K. (1996) Assisted reproductive techniques – are we avoiding the genetic issue? *Hum. Reprod.*, **11**, 926–928.

Becker, K. and Schoneich, J. (1982) Expression of genetic damage induced by alkylating agents in germ cells of female mice. *Mutat. Res.*, **92**, 447–464.

Blommaert, F.A., Van Dijk-Knijnenburg, H.C., Dijt, F.J. *et al.* (1995) Formation of DNA adducts by the anticancer drug carboplatin: different nucleotide sequence preferences *in vitro* and in cells. *Biochemistry*, **34**, 8474–8480.

Boring, C.C., Squires, T.S., Tong, T. and Montgomery, S. (1994) Cancer statistics, 1994. *Cancer J. Clin.*, **44**, 7–26.

Brandriff, B.F., Meistrich, M.L., Gordon, L.A. *et al.* (1994) Chromosomal damage in sperm of patients surviving Hodgkin's disease following MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) therapy with and without radiotherapy. *Hum. Genet.*, **93**, 295–299.

Brent R.L. (1989) The effects of embryonic and fetal exposure to x-ray: microwaves, and ultrasound: counseling the pregnant and non-pregnant patient. About these risks. *Semin. Oncol.*, **16**, 347–368.

Brent R.L. (1999) Utilization of developmental basic science principles in the evaluation of reproductive risks from pre- and postconception environmental radiation exposures. *Teratology*, **59**, 182–204.

Brent, R. and Bolden, B.T. (1967) The indirect effect of irradiation on embryonic development. III. The contribution of ovarian irradiation, oviduct irradiation, and zygote irradiation to fetal mortality and growth retardation in the rat. *Radiat. Res.*, **30**, 759–773.

Briggs, G.G., Freeman, R.K. and Yaffe, S.J. (eds) (1998) *Drugs in Pregnancy and Lactation*, 5th edition. Williams & Wilkins, Maryland., pp. 119–120, 678–681, 300–302, 357–359.

Buchanan, J.D., Fairley, K.F. and Barrie, J.U. (1975) Return of spermatogenesis after stopping cyclophosphamide therapy. *Lancet*, **ii**, 156–157.

Buckley, L.M., Bullaboy, C.A., Leichtman, L. and Marquez, M. (1997) Multiple congenital anomalies associated with weekly low-dose methotrexate treatment of the mother. *Arthritis Rheum.*, **40**, 971–973.

Buekers, T.E. and Lallas, T.A. (1998) Chemotherapy in pregnancy. *Obstet. Gynecol. Clin. North Am.*, **25**, 323–329.

Byrne, J., Mulvihill, J.J., Myers, M.H. *et al.* (1987) Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. *N. Engl. J. Med.*, **317**, 1315–1321.

Caligiuri, M.A. and Mayer, R.J. (1989) Pregnancy and leukemia. *Semin. Oncol.*, **16**, 388.

Chapman, R.M. (1982) Effect of cytotoxic therapy on sexuality and gonadal function. *Semin. Oncol.*, **9**, 84–94.

Chatterjee, R., Haines, G.A., Perera, D.M. *et al.* (2000) Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukaemia. *Hum. Reprod.*, **15**, 762–766.

Choudhury, R.C., Jagdale, M.B. and Misra, S. (2000) Effects of cisplatin in the male germline cells of Swiss mice. *J. Chemother.*, **12**, 352–359.

Critchley, H.O. (1999) Factors of importance for implantation and problems after treatment for childhood cancer. *Med. Pediatr. Oncol.*, **33**, 9–14.

Damewood, M.D. and Grochow, L.B. (1986) Prospects for fertility after chemotherapy or radiation for neoplastic disease. *Fertil. Steril.*, **45**, 443–459.

de Alvarez, R.R. (1962) An evaluation of aminopterin as an abortifacient. *Am. J. Obstet. Gynecol.*, **83**, 1467–1477.

Devita, V.T., Jr, Hellman, S. and Rosenberg, S.A. (1997) *Cancer – Principles and Practice of Oncology*. Lippincott-Raven Publishers, USA.

D'Incalci, M., Sesa, C., Coumbo, N. *et al.* (1982) Transplacental passage of cyclophosphamide. *Cancer Treat. Rep.*, **66**, 1681–1682.

Doll, R.C. and Wakeford, R. (1997) Risk of childhood cancer from fetal radiation. *Br. J. Radiol.*, **70**, 130–139.

Doll, R.C., Ringenberg, O.S. and Yarbo, J.W. (1989) Antineoplastic agents and pregnancy. *Semin. Oncol.*, **16**, 337–346.

Enns, G.M., Roeder, E., Chan, R.T. *et al.* (1999) Apparent cyclophosphamide (cytoxan) embryopathy: a distinct phenotype? *Am. J. Med. Genet.*, **86**, 237–241.

Familiari, G., Caggiati, A., Nottola, S.A. *et al.* (1993) Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. *Hum. Reprod.*, **8**, 2080–2087.

Fattibene, P., Mazzei, F., Nuccetelli, C. and Risica, S. (1999) Prenatal exposure to ionizing radiation: sources, effects and regulatory aspects. *Acta Paediatr.*, **88**, 693–702.

Feldkamp, M. and Carey, J.C. (1993) Clinical teratology counseling and consultation case report: low dose methotrexate exposure in the early weeks of pregnancy. *Teratology*, **47**, 533–539.

Gaillard, B., Leng, J., Grellet, J. *et al.* (1995) Transplacental passage of epirubicin. *J. Gynecol. Obstet. Biol. Reprod.*, **24**, 63–68.

Garber, J. (1989) Long term follow up of children exposed *in utero* to antineoplastic agents. *Semin. Oncol.*, **16**, 437–444.

Garcia, L., Valcarcel, M. and Santiago-Borrero, I.J. (1999) Chemotherapy during pregnancy and its effect on the fetus-myelosuppression: two case reports. *J. Perinatol.*, **19**, 230–233.

Gassner, P. and Adler, I.D. (1995) Analysis of chemically induced spindle aberrations in male mouse germ cells: comparison of differential and immunofluorescent staining procedures. *Mutagenesis*, **10**, 243–252.

Generoso, W.M., Preston, R.J. and Brewen, J.G. (1975) 6-mercaptopurine, an inducer of cytogenetic and dominant lethal effects in premeiotic and early meiotic germ cells of male mice. *Mutat. Res.*, **28**, 437–447.

Generoso, W.M., Cain, K.T., Krishna, M. and Huff, S.W. (1979) Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg. *Proc. Natl Acad. Sci. USA*, **76**, 435–437.

Genesca, A., Caballin, M.R., Miro, R. *et al.* (1990) Human sperm chromosomes. Long-term effect of cancer treatment. *Cancer Genet. Cytogenet.*, **46**, 251–260.

Gililland, J. and Weinstein, L. (1983) The effects of cancer chemotherapeutic agents on developing fetus. *Obstet. Gynecol. Surv.*, **3**, 6–13.

Gougeon, A. (1996) Regulation of ovarian follicular development in primates: facts and hypothesis. *Endocr. Rev*., **17**, 121–155.

Green, D.M., Zevon, M.A., Lowrie, G. *et al.* (1991) Congenital abnormalities in children of patients who received chemotherapy for cancer in childhood or adolescence. *N. Engl. J. Med*., **325**, 141–146.

Harvey, E.B., Boise, J.D., Jr, Honeyman, M. and Flannery, J.T. (1985) Prenatal x-ray exposure and childhood cancer in twins. *N. Engl. J. Med.*, **312**, 541–545.

Hawkins, M.M. (1991) Is there evidence of a therapy-related increase in germ cell mutation among childhood cancer survivors? *J. Natl Cancer Inst*., **83**, 1643–1650.

Hawkins, M.M. and Smith, R.A. (1989) Pregnancy outcomes in childhood cancer survivors: probable effects of abdominal irradiation. *Int. J. Cancer*, **43**, 399–402.

Higdon, R.E., Marchetti, F., Mailhes, J.B. and Phillips, G.L. (1992) The effects of cisplatin on murine metaphase II oocytes. *Gynecol. Oncol*., **47**, 348–352.

Holmes, G.F. and Holmes, F.F. (1978) Pregnancy outcome of patients treated for Hodgkin's disease. *Cancer*, **41**, 1317.

Hooser, S.B., van Dijk-Knijnenburg, W.C., Waalkens-Berendsen, I.D. *et al.* (2000) Cisplatin-DNA adduct formation in rat spermatozoa and its effect on fetal development. *Cancer Lett*., **3**, 71–80.

Host, E., Lindenberg, S. and Smidt-Jensen, S. (2000) The role of DNA strand breaks in human spermatozoa used for IVF and ICSI. *Acta Obstet. Gynecol. Scand*., **79**, 559–563.

Jarrell, J., YoungLai, E.V., Barr, R. *et al.* (1986) An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia and serum goandotropin levels. *Am. J. Obstet. Gynecol*., **154**, 306–309.

Jenderny, J., Jacobi, M.L., Ruger, A. and Rohrborn, G. (1992) Chromosome aberrations in 450 sperm complements from eight controls and lack of increase after chemotherapy in two patients. *Hum. Genet*., **90**, 151–154.

Kallio, M., Sjoblom, T. and Lahdetie, J. (1995) Effects of vinblastine and colchicine on male rat meiosis *in vivo*: disturbances in spindle dynamics causing micronuclei. *Environ. Mol. Mutagen*., **25**, 106–117.

Katoh, M.A., Cain, K.T., Hughes, L.A. *et al.* (1990) Female-specific dominant lethal effects in mice. *Mutat. Res*., **230**, 205–217.

Kennedy, S., Yudkin, P. and Greenall, M. (1993) Cancer in pregnancy. *Eur. J. Surg. Oncol*., **19**, 405–407.

Kirk, M. and Lyon, M.F. (1982) Induction of congenital anomalies in offspring of female mice exposed to varying doses of X-rays. *Mutat. Res*., **106**, 73–83.

Koc, O.N., McFee, M., Reed, E. and Gerson, S.L. (1994). Detection of platinum-DNA adducts in cord blood lymphocytes following *in utero* platinum exposure. *Eur. J. Cancer*, **30A**, 716–717.

Li, F.P., Fine, W., Jaffe, N. *et al.* (1979) Offspring of patients treated for cancer in childhood. *J. Natl Cancer Inst*., **62**, 1193–1197.

Li, F.P., Gimbrere, K., Gelber, R.D. *et al.* (1987) Outcome of pregnancy in survivors of Wilms' tumor. *JAMA*, **257**, 216–219.

Liang, J.C., Sherron, D.A. and Johnston, D. (1986) Lack of correlation between mutagen-induced chromosomal univalency and aneuploidy in mouse spermatocytes. *Mutat. Res*., **163**, 285–297.

Lord, B.I. (1999) Transgenerational susceptiblity to leukaemia induction resulting from preconception, paternal irradiation. *Int. J. Radiat. Biol*., **75**, 801–810.

Mailhes, J.B., Aardema, M.J. and Marchetti, F. (1993) Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate. *Environ. Mol. Mutagen*., **22**, 107–114.

Mailhes, J.B., Marchetti, F. and Young, D. (1995) Synergism between gonadotrophins and vinblastine relative to the frequencies of metaphase I, diploid and aneuploid mouse oocytes. *Mutagenesis*, **10**, 185–188.

Martin, R.H. (1993) Detection of genetic damage in human sperm. *Reprod. Toxicol*., **7** (Suppl. 1), 47–52.

Martin, R.H., Hildebrand, K., Yamamoto, J. *et al.* (1986) An increased frequency of human sperm chromosomal abnormalities after radiotherapy. *Mutat. Res*., **174**, 219–225.

Martin, R.H., Rademaker, A.W. and Leonard, N.J. (1995) Analysis of chromosomal abnormalities in human sperm after chemotherapy by karyotyping and fluorescence in-situ hybridization (FISH). *Cancer Genet. Cytogenet*., **80**, 29–32.

Martin, R.H., Ernst, S,. Rademaker, A. *et al.* (1999) Analysis of sperm chromosome complements before, during, and after chemotherapy. *Cancer Genet. Cytogenet*., **108**, 133–136.

Matsudo, Y. and Tobari, I. (1989) Repair capacity of fertilised mouse eggs for

X-ray damage induced in sperm and mature oocytes. *Mutat. Res*., **210**, 35–47.

McMahon, B. (1962) Prenatal x-ray exposure and childhood cancer. *J. Natl Cancer Inst*., **28**, 1173–1191.

Meirow, D. and Schenker, J.G. (1995) Cancer and male infertility. *Hum. Reprod*., **10**, 2017–2022.

Meirow, D., Badaan, S., Ligumski, M. and Lewis, H. (2000) Sperm production and reproductive performance in male mice treated with the immunosuppressive drug used for inflammatory bowel disease, 6-mercaptopurine. *Fertil. Steril.*, **74**, S80.

Meirow, D., Epstein, M., Lewis, H. *et al.* (2001) Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance, and fetal malformations. *Hum. Reprod.*, **16**, 632–637.

Meistrich, M.L., van Beek, M.E., Liang, J.C. *et al.* (1990) Low levels of chromosomal mutations in germ cells derived from doxorubicin, treated stem spermatogonia in the mouse. *Cancer Res*., **50**, 370–374.

Mole, R.H. (1979) Radiation effects on pre-natal development and their radiological significance. *Br. J. Radiol*., **52**, 89–101.

Mole, R.H. (1993) The biology and radiobiology of *in utero* development in relation to radiological protection. *Br. J. Radiol*., **66**, 1095–1102.

Mulvihill, J.J., McKeen, E.A., Rosner, F. and Zarrabi, M.H. (1987) Pregnancy outcome in cancer patients. *Cancer*, **60**, 1143–1150.

Nicholson, H.O. (1968) Cytotoxic drugs in pregnancy: review of reported cases. *J. Obstet. Gynecol. Br. Commonw*., **75**, 307–312.

Otake, M., Schull, W.J. and Lee, S. (1996) Threshold for radiation-related severe mental retardation in prenatally exposed A-bomb survivors: a re-analysis. *Int. J. Irradiat. Biol*., **70**, 755–763.

Pacifici, G.M. and Nottoli, R. (1995) Placental transfer of drugs administered to the mother. *Clin. Pharmacokinet*., **28**, 235–269.

Pils, S., Muller, W.U. and Streffer, C. (1999) Lethal and teratogenic effects in two successive generations of the HLG mouse strain after radiation exposure of zygotes – association with genomic instability? *Mutat. Res*., **429**, 85–92.

Pizzuto, J., Aviles, A., Noreiga, L. *et al.* (1980) Treatment of acute leukemia during pregnancy: presentation of nine cases. *Cancer Treat. Rep*., **64**, 679–683.

Raffles, A., Williams, J., Costeloe, K. and Clark, P. (1989) Transplacental effects of maternal cancer chemotherapy *Br. J. Obstet. Gynaecol*., **96**, 1099–1100.

Randall, T. (1993) National registry seeks scarce data on pregnancy outcomes during chemotherapy. *JAMA*, **269**, 323.

Robbins, W.A., Meistrich, M.L., Moore, D. *et al.* (1997) Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. *Nature Genet*., **16**, 74–78.

Rugh, R. and Skaredoff, L. (1965) Radiation and radiomimetic chlorambucil and the fetal. *Arch. Ophthalmol*., **74**, 382–393.

Russell, L.B. (1987) Positive genetic hazard predictions from short term tests have proved false for results in mammalian spermatogonia with all environmental chemicals so far tested. *Proceedings, International Environmental Mutagenesis Society, Stockholm, 1984*.

Russell, L.B. (1990) Patterns of mutational sensitivity to chemicals in post-stem cell stages of mouse spermatogenesis. *Prog. Clin. Biol. Res*., **340C**, 101–113.

Russo, A. and Levis, A.G. (1992) Detection of aneuploidy in male germ cells of mice by means of a meiotic micronucleus assay. *Mutat. Res*., **281**, 215–221.

Sadler, T.W. (ed.) (1995) *Langmans Medical Embryology*, 7th edition. Williams & Wilkins, Baltimore.

Sanders, J.E., Hawley, J., Levy, W. *et al.* (1996) Pregnancies following high, dose cyclophosphamide with or without high, dose busulfan or total body irradiation and bone marrow transplant. *Blood*, **87**, 3045–3052.

Saratsis, P., Ypsilantis, P. and Tselkas, K. (2000) Semen quality during vincristine treatment in dogs with transmissible venereal tumor. *Theriogenology*, **15**, 1185–1192.

Schardein, J.L. (1993) *Chemically Induced Birth Defects*, 2nd edition. Marcel Dekker, New York.

Schleuning, M. and Clemm, C. (1987) Chromosomal aberrations in a newborn whose mother received cytotoxic treatment during pregnancy. *N. Engl. J. Med.*, **317**, 1666–1667.

Schull, W.J. and Otake, M. (1999) Cognitive function and prenatal exposure to ionizing radiation. *Teratology*, **59**, 222–226.

Shotton, D. and Monie, I.W. (1963) Possible teratogenic effect of chlorambucil on a human fetus. *JAMA*, **186**, 74–75.

Sjoblom, T., Parvinen, M. and Lahdetie, J. (1995) Stage-specific DNA

synthesis of rat spermatogenesis as an indicator of genotoxic effects of vinblastine, mitomycin C and ionizing radiation on rat spermatogonia and spermatocytes. *Mutat. Res.*, **331**, 181–190.

Steege, J.F. and Caldwell, D.S. (1980) Renal agenesis after first trimester exposure to chlorambucil. *South. Med. J.*, **73**, 1414–1415.

Stephens, T.D., Golbus, M.S., Miller, T.J. *et al.* (1980). Multiple congenital anomalies in a fetus exposed to 5-fluoracil during the first trimester. *Am. J. Obstet. Gynecol.*, **13**, 747–749.

Stewart, A., Webb, J. and Hewitt, D. (1958) A survey of childhood malignancies. *Br. Med. J.*, **5086**, 1495–1508.

Sudman, P.D., Rutledge, J.C., Bishop, J.B. and Generoso, W.M. (1992) Bleomycin: female, specific dominant lethal effects in mice. *Mutat. Res.*, **296**, 143–156.

Tease, C. (1992) Radiation and chemically induced chromosome aberrations in mouse oocytes: a comparison with effects in males. *Mutat. Res.*, **296**, 135–142.

Tomlinson, M.W., Tredwell, M.C. and Deppe, G. (1997) Platinum based chemotherapy to treat recurrent Sertoli-Leydig cell ovarian carcinoma during pregnancy. *Eur. J. Gynecol. Oncol.*, **18**, 44–46.

Van-Buul, P.P. and Goudzwaard, J.H. (1980) Bleomycin, induced structural chromosomal aberrations in spermatogonia and bone, marrow cells of mice. *Mutat. Res.*, **69**, 319–324.

Volkenandt, M., Buchner, T., Hiddemann, W. and Van De Loo, J. (1987) Two cases of acute leukemia in pregnancy. *Lancet*, **ii**, 1521–1522.

Wagner, V.M., Hill, J.S., Weaver, D. and Baehner, R.L. (1980) Congenital anomalies in a baby born to cytarabine treated mother. *Lancet*, **ii**, 98–100.

Warkany, J. (1978) Aminopterin and methotrexate: folic acid deficiency *Teratology*, **17**, 353–358.

Wasserman, P.M. (1996) Oogenesis. In Adashi, E., Rock, J. and Rosenwaks, Z. (eds), *Reproductive Endocrinology*, *Surgery and Technology*. Lippincott-Raven Publishers, USA, pp. 341–357.

Weisz, B., Schiff, E. and Lishner, M. (2001) Cancer in pregnancy: maternal and fetal implications. *Hum. Reprod. Update*, **7**, 384–393.

Wiebe, V.J. and Sipila, P.E.H. (1994) Pharmacology of antineoplastic agents in pregnancy. *Crit. Rev. Oncol. Hematol.*, **16**, 75–112.

Witt, K.L. and Bishop, J.B. (1996) Mutagenicity of anticancer drugs in mammalian germ cells. *Mutat. Res.*, **355**, 209–234.

Zemlickis, D., Lishner, M., Degendorfer, P. *et al.* (1992) Fetal outcome after *in utero* exposure to cancer chemotherapy. *Arch. Intern. Med.*, **152**, 533–536.