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Teratogen Update: Paternal Exposures—Reproductive Risks

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The potential of many drugs and chemicals to cause prenatal harm is well-established. The types of effects seen vary and include spontaneous abortions, stillbirths, congenital malformations present at birth, and conditions detected only months to years after birth. Most studies designed to determine whether agents cause malformations have examined the effects of exposure of the embryo or fetus at various times during gestation. Thus the effects studied are *maternally-mediated*. Despite these drug studies and those on other causes of birth defects, such as chromosomal abnormalities, the cause of approximately 60% of congenital malformations is unknown. Fewer studies have examined the possibility that exposure of the male to a drug or chemical could lead to abnormalities in his offspring, i.e., be male- or *paternally-mediated*. Over the last 10–20 years, there has been more interest in addressing this question as increasing numbers of patients and workers are exposed to agents that alter fertility. A number of animal studies as well as more recent human epidemiological studies have demonstrated that exposure of males to various agents can result in abnormal reproductive, pregnancy, and/or progeny outcomes (Olshan and Mattison, '94).

MECHANISMS OF MALE-MEDIATED EFFECTS

Drug treatment of the male prior to conception could affect the outcome of subsequent progeny due to a drug-induced defect in the spermatozoon itself, such as an effect on the DNA or chromosomal proteins, or due to an effect caused by the presence of the drug in the seminal fluid. There are three main mechanisms of male reproductive toxicity: nongenetic (e.g., due to the presence of a drug in seminal fluid), genetic (e.g., gene mutation or chromosomal abnormality), and epigenetic (e.g., an effect on gene expression, genomic imprinting, or DNA methylation). The male reproductive system has a number of unique properties that help us interpret some of the mechanisms underlying male-mediated drug effects. Germ cells in the testis show one of the highest mitotic activities of any tissue in the body, so that in the human adult about 100 million new cells are produced each day (Amann, '81). Spermatogenesis is highly regulated, starting with spermatogonial stem cells and ending with differentiated, motile spermatozoa. It is one of the few examples in the adult of a system where undifferentiated cells pass through a number of distinctly different developmental phases, i.e., mitosis (spermatogonia), meiosis (spermatocytes), differentiation (haploid-phase spermiogenesis), and maturation (in the epididymis). In man and other animals, the continued proliferation of germ cells throughout life, from puberty to death, is maintained by a process of stem-cell renewal and differentiation. Stem-cell spermatogonia are located at the base of the epithelium, where they give rise to new stem cells or to more differentiated spermatogonia (Hermo and Clermont, '95). Another population of stem cells rarely divides in adults and is tentatively classed as dormant reserve stem cells. In rodents, germ cells start to proliferate and proceed through spermatogenesis in the first month of life; in contrast, in humans there is a long juvenile period with spermatogenesis being initiated in the second decade, at puberty. Following proliferation, germ cells enter meiotic prophase (including leptotene, zygotene, and pachytene phases), and subsequently undergo two meiotic divisions to become haploid spermatids. During spermiogenesis, spermatids undergo a dramatic series of morphological changes, prior to being released into the epididymis. Within the epididymis spermatozoa pass through a final process of maturation whereby they become motile and able to fertilize the egg.

The kinetics of spermatogenesis have been worked out in detail for a number of species; it is well-established that the timing of each of the four phases mentioned above is constant for a given species. In man, it takes approximately 64 days (Heller and Clermont, '63) for germ cells to develop from spermatogonia to spermatozoa; a further 2–5 days (Rowley et al., '70) is required for spermatozoa to pass through the epididymis. The germ-cell stage(s) affected by a given drug can be determined by examining the time between drug exposure and conception (Table 1). For instance, an abnormal reproductive outcome occurring 5 days after treatment indicates a drug effect on maturation of

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TABLE 1. Germ cell type affected in man at different times after exposure to a drug or chemical*

Time of exposure (days)	Germ cell type affected	Activity affected
1	Drug in semen	Direct toxicity
1–5	Spermatozoa	Epididymal transit/storage
6–29	Spermatid	Differentiation
30–53	Spermatocyte	Meiosis
54–69	Spermatogonia	Mitosis

*Adapted from Courot, '70.

sperm in the epididymis. A drug that causes DNA damage during synapsis of chromosomes during meiotic prophase would affect the progeny conceived approximately 40 days later.

Drug effects on any of the steps in the production of the mature spermatozoon could change any one of the components of this highly specialized cell. For example, both an alteration in the flagellum, which results in lower motility, or an effect of the drug on the plasma membrane could result in lower fertilization rates, whereas damage to the chromatin could lead to fetal death or heritable effects in the offspring. Epigenetic alterations, involving changes in gene expression without a change in nucleotide sequence, should also be considered. A number of human teratogens have been tentatively classed as having evidence of epigenetic activity (Bishop et al., '97). However, assays to determine whether a given paternal exposure has a direct epigenetic effect have not yet been developed. As for teratogens, it is often difficult to determine whether an alteration in gene expression is a direct effect or an indirect effect of a given chemical or drug on a different target. For the purposes of this review, no attempt has been made to separate genetic and epigenetic effects; however, exposures where epigenetic effects should be considered have been indicated. For paternal exposures, effects on imprinted genes may be particularly important. Imprinted genes are only expressed from either the maternal or paternal allele. For imprinted genes expressed from the paternal allele, inactivation of the paternal allele will result in loss of gene function, since the maternal allele is silent (Tycko et al., '97). Although genomic imprinting is not yet precisely defined at the molecular level, the process is initiated during gametogenesis and plays a role in regulating the growth of the conceptus during development. Alterations in imprinting can cause human genetic diseases and have been associated with the development of childhood tumors (Tycko et al., '97). A drug that alters the normal imprinting process during spermatogenesis could be expected to alter development of the resulting offspring. Additional epigenetic effects of paternal exposures on testis-specific gene expression might affect sperm number, morphology, and/or function.

At the DNA level, there are major differences among the various germ-cell types in their sensitivity and responses to mutagens (Witt and Bishop, '96). Slow-dividing, long-lived stem cells might be expected to face the greatest risk from chronic exposure to exogenous agents due to the potential accumulation of DNA damage, especially during the long human prepubertal period. Effects of a drug on the DNA of spermatogonial stem cells are of particular concern as they may persist throughout the reproductive life span of an individual, with the mutant stem cell serving as a long-lasting source of abnormal spermatozoa. To date, there are very few agents that have been shown to produce heritable damage in spermatogonial stem cells, perhaps due to efficient repair or selection mechanisms or the short prepubertal period in mice and rats (Shelby, '96). Differentiating spermatogonia are rapidly dividing cells and are most sensitive to killing by radiation and chemotherapy. The last round of DNA synthesis of spermatogenesis occurs in preleptotene spermatocytes. Preleptotene-leptotene and meiotically dividing spermatocytes are susceptible to killing by irradiation (Clermont and Harvey, '65; Henriksen et al., '96) and other agents. During spermiogenesis in postmeiotic germ cells, histones are replaced by protamines, nuclear condensation occurs, and DNA repair capability ceases. Damage to spermatids may pose a significant risk to the progeny because, despite DNA damage, these haploid cells can still develop into spermatozoa capable of fertilizing an egg (Meistrich, '93; Witt and Bishop, '96).

EPIDEMIOLOGIC FINDINGS

Human exposures are often chronic in nature. If male-mediated effects are suspected, the physician needs first to rule out a direct effect of drug(s) present in semen as well as maternal exposure preconception. As outlined in this section, a number of epidemiologic studies over the last 10 years have started to identify exposures with suspected male-mediated effects.

Occupational and environmental exposures

Most epidemiological studies have examined the effects of paternal occupational exposures on offspring. For most, paternal occupational/industrial exposure involves multiple agents, and it is difficult to identify the causative agent(s) (Olshan and Faustman, '93; Olshan and Schnitzer, '94). However, some suggestive associations have been reported and provide direction for future epidemiologic studies. An increased incidence of spontaneous abortion or miscarriage has been linked to paternal exposures to anesthetic gases, metals (mercury and lead), solvents, pesticides, and hydrocarbons (Savitz et al., '94). In the case of mercury, a dose-response relationship between urinary mercury concentrations and the rate of spontaneous abortions has been reported (Cordier et al., '91). An increased risk of stillbirth, preterm delivery, and small-for-gestational-age babies has been found for fathers employed in the art and textile industries (Savitz, '94). Fathers employed as janitors, woodworkers, firemen, electrical workers, printers, and painters have been reported to be at increased risk of having a child with a birth defect

(Olshan et al., '90, '91; Schnitzer et al., '95). Exposures related to these occupations include solvents, wood and wood products, metals, and pesticides (Olshan et al., '91); for most studies, quantitative exposure estimates have not been identified. Epidemiological studies have also suggested a link between childhood cancers and occupational exposures; however, the specific etiologic agents involved are not yet known (Savitz and Chen, '90; O'Leary et al., '91). Interestingly, some of the same exposures or occupations are associated with a number of outcomes, e.g., painters and welders with both birth defects and childhood cancer. Animal studies to explore mechanisms may be useful once repeat studies are done and information is available on specific agents or combinations that are associated with male-mediated effects on the progeny.

Recreational exposure

Paternal exposures to most "recreational drugs" such as caffeine, cocaine, and methadone have not been studied in detail. However, among these agents, there is little consistent evidence that either paternal smoking or alcohol results in birth defects (Little and Vainio, '94). For example, although an initial positive association between paternal drinking and low birth weight was reported (Little and Sing, '85), a more recent large retrospective analysis, albeit with lower alcohol exposure than that in the study of Little and Sing ('85), found no adverse effect of paternal alcohol consumption on progeny outcome (Savitz et al., '92).

Therapeutic drugs

With respect to male-mediated effects of most commonly used drugs, little work has been done. Among the therapeutic agents, there has been particular concern with the father's exposure to anticancer drugs. Cancer therapies have increased survival in young adults and children with cancers such as Hodgkin's disease, testicular cancers, and leukemia. Many of the drugs used in cancer treatment cause DNA damage, result in temporary or permanent infertility, and could theoretically alter the sperm genome. Drugs that are commonly used include doxorubicin, cyclophosphamide, vincristine, chlorambucil, melphalan, bleomycin, and 6-mercaptopurine, all of which are potent germ-cell mutagens and somatic cell clastogens in rodents (Shelby, '94; Witt and Bishop, '96). Unlike occupational exposures, exposures to anticancer drugs are relatively well-controlled and carried out in circumstances where dose-response relationships can be determined. To date, no increase in birth defects or in cancer or genetic disease in the offspring has been found (Hawkins, '91; Mulvihill, '94; Sankila et al., '98). However, relatively few children have been born to male cancer survivors, and it is estimated that many thousands of patients will be needed to rule out a relative risk for a germ-cell mutation in the range of 1.5. Further epidemiological studies of the offspring of males treated with anticancer drugs are ongoing. In North America, the Childhood Cancer Survivor Study, a large multicenter study of 25,000 long-term survivors of childhood and adolescent cancer, is currently underway and is looking for evidence of induced genetic disease in offspring as well as other health effects (Mulvihill, '94). Due to the risk of infertility, many cancer patients cryopreserve their sperm prior to treatment. For men who are infertile after therapy, cryopreservation of sperm gives them an opportunity to father children. For men in whom fertility returns, cryopreservation allows a comparison of pre- and posttreatment sperm samples for genetic damage.

Large-scale exposures

One of the largest epidemiologic studies has been carried out on survivors of ionizing radiation from the atomic bombs at Hiroshima and Nagasaki. For the offspring of exposed men, no significant increase was found for a number of endpoints, including stillbirths, congenital abnormalities, low birth weight, cancer, and cytogenetic abnormalities (Neel and Schull, '91). Regarding paternal exposures, some limitations to this study (Neel and Schull, '91) have been reviewed, including inadequate statistical power to detect weak to moderate radiation effects, such as an increase in specific birth defects, incomplete ascertainment for fetal losses or severe congenital malformations due to the time lag between the bombing and the start of the study, and the fact that the spermatogenic cell type affected was spermatogonial stem cells, cells that are known from rodent studies to be more resistant to the induction of mutations by radiation than the later cell types (Olshan, '95). Taking into account these limitations, atomic bomb studies provide evidence that exposure of man to a single dose of ionizing radiation does not result in a detectable increase in genetic disease. The question of chronic radiation exposure was raised more recently when an excess risk of leukemia and non-Hodgkin's lymphoma was reported for children whose fathers were employed at the nuclear reprocessing plant near Sellafield, England (Gardner et al., '90; Gardner, '92). The Sellafield findings generated a lot of interest but remain controversial in the light of several factors, including alternative explanations for the data, the lack of an increase in genetic disease or congenital malformations in the area, and the lack of evidence from other studies for increased cancer risks in children of nuclear plant workers (Doll et al., '94; Olshan, '95). Nevertheless, continued studies of men working in nuclear facilities are useful, since exposure histories are well-documented and may help resolve questions raised by the Sellafield data.

More recently, Dubrova et al. ('96) reported an increased minisatellite mutation frequency in children born in contaminated areas of the Mogilev district of Belarus after the Chernobyl nuclear power station accident in 1986. The results suggest that the accidental release of radioactive material at Chernobyl may have resulted in induction of germline mutations. How-

ever, a similar study examining minisatellite mutations in the children of atomic bomb survivors did not find evidence of mutation induction (Kodaira et al., '95). Clearly, more work is needed to document effects of other chronic exposures, such as the Chernobyl accident, and to determine why effects of such chronic exposures apparently differ from acute exposures, like those following atomic bomb explosions.

Early attention to the area of male-mediated effects on offspring came from concerns that American veterans were exposed during military service in Vietnam to the herbicide Agent Orange, containing dioxin contaminants. It has been difficult to draw firm conclusions from the studies on reproductive outcomes among men who served in Vietnam due to difficulties in establishing exact levels of exposure (Erickson et al., '84; Aschengrau and Monson, '89; Centers for Disease Control, '88; Stellman et al., '88). There has been concern that men serving in the more recent Persian Gulf War were exposed to agents that affected their reproductive health and resulted in birth defects in their children. Although more studies are underway, an analysis by Cowan et al. ('97) found no evidence of an increase in birth defects among children of Gulf War veterans.

EVIDENCE OF MUTATION INDUCTION IN HUMANS

In this section, several examples will be used to illustrate that paternal exposure can lead to sperm abnormalities, infertility, somatic chromosomal defects, and sperm chromosomal defects. In some cases, the effects may have an epigenetic origin. To date, there is no direct evidence for induced inherited genetic disorders in man (Robbins, '96). Only further study will help determine whether paternal exposures such as those mentioned above also lead to genetic abnormalities or abnormalities in offspring.

Somatic chromosome abnormalities

A wide range of occupational exposures is associated with chromosomal mutations (Ashby and Richardson, '85). Evidence from occurrence of second treatment-related tumors in cancer survivors indicates that mutations in somatic cells are involved in the induction of cancer (Tucker et al., '88). Some common occupational exposures, such as 1,3-butadiene, have been linked to an increased risk of leukemia (Macaluso et al., '96), lymphosarcomas, and reticulosarcomas (Ward et al., '96). That many chemicals induce mutations in somatic cells suggests that mutations can also be induced in human germ cells. However, at present, there are no reliable, valid methods to link somatic and germinal mutations with the resultant phenotypes. At least one study has suggested that, following anticancer therapy, cytogenetic damage in somatic cells may not correlate with cytogenetic damage in sperm (Genesca et al., '90a).

Infertility and sperm abnormalities

There are numerous well-known adverse reproductive effects of paternal treatment, including altered fertility and decreased sperm counts as well as abnormal sperm motility and morphology. Approximately 50 agents have been shown to affect the numbers, motility, or morphology of human sperm (Wyrobek et al., '83). Such effects indicate that germ-cell exposure has occurred and suggest the possibility of germ-cell mutations if the agent involved is mutagenic. One of the most well-known drug effects on sperm is the oligospermia and azoospermia seen in men following treatment with anticancer drugs, in particular, alkylating agents such as cyclophosphamide (Byrne et al., '87). In a well-publicized example, workers exposed to the pesticide dibromochloropropane (DBCP) reported infertility and were found to have decreased sperm counts (Whorton et al., '77). Lead exposure has been associated with abnormal sperm morphology and decreased fertility (Lancranjan et al., '75); both genetic and epigenetic mechanisms may be involved in the effects of lead on the male (Gandley and Silbergeld, '94).

Chromosome aberrations in sperm

Two of the most widely used methods to detect cytogenetic damage in sperm are the human sperm/hamster egg technique pioneered by Rudak et al. ('78) and, more recently, fluorescence in situ hybridization (FISH). In the human sperm/hamster egg technique, capacitated human sperm are allowed to fuse with zona-free hamster oocytes, leading to decondensation and reconfiguration of the human sperm chromatin, permitting examination of human sperm metaphase chromosomes for structural and numerical abnormalities. The technique has been used by a number of investigators to study the effects of anticancer treatment on human sperm chromosomes. Martin et al. ('86) showed that sperm of men exposed to ionizing radiation contained a significant proportion of chromosomal aberrations up to 36 months after the termination of treatment, providing the first demonstration of induced chromosomal aberrations in functional human sperm, i.e., postfertilization survivability of radiation-induced mutations. For chemotherapy, although not all studies are positive, a number of different laboratories have reported elevated levels of structural aberrations and aneuploidy in the sperm of treated men (Brandriff et al., '94; Genesca et al., '90b; Jenderny et al., '92; Jenderny and Rohrborn, '87; Martin et al., '95). Interestingly, as with radiation, chromosome damage has been found a number of years after the cessation of treatment, suggesting effects on spermatogonial stem cells.

The human sperm/hamster egg procedure is difficult and labor-intensive. More recently, FISH has been used to assess disomy frequencies for specific chromosomes in individual human spermatozoa. FISH allows many thousands of sperm to be screened quickly for numerical chromosomal abnormalities; however, in the studies done to date, the assay used was unable to detect

structural aberrations. There are few large studies that have been carried out, and negative and positive results have been reported in studies on the effects of anticancer drugs on human sperm chromosomes (Robbins, '96; Martin et al., '97). A recent study in which pre-, during, and posttreatment sperm samples were available, found evidence of sperm aneuploidy in patients treated with chemotherapy for Hodgkin's disease; interestingly, damage decreased to pretreatment levels within 3–4 months after the end of therapy (Robbins et al., '97).

The cytogenetic studies mentioned above provide initial data on direct chromosomal damage in human sperm. Further studies are needed using both assays in homogeneous populations of cancer patients, on the same chemotherapy regimen, before, during, and after treatment. Whether the induced genetic damage is transmissible or not is unknown and will require studies in which sperm are examined and in which for the same patients, partners' pregnancies are monitored. It has been argued that we need to be concerned, since there appears to be no selection against chromosomally abnormal sperm in humans, and cytogenetically abnormal sperm can fertilize eggs (Martin, '89; Martin et al., '90).

LIMITATIONS OF HUMAN STUDIES

Epidemiologic studies have identified a number of different types of paternal exposures, including environmental, occupational, and lifestyle exposures that result in a variety of abnormal pregnancy outcomes. Many findings await repeat studies for confirmation of the initial results. A number of questions arise for future human studies: What is the association of paternal exposures and postnatal abnormalities in children, e.g., behavioral deficits, increased cancer risk, or altered reproductive potential? Can alterations in spermatozoa be used to monitor human exposures or stem-cell damage? Are there ways to protect male germ cells from damage? Problems that have been encountered in human studies include limited sample size, incomplete documentation of exposure, high background rates (e.g., for birth defects, control rates in man are 3–7%), the inability to study subgroups such as spontaneous abortions and birth defects due to small sample size, the presence of unknown confounders, the lack of repeat studies with similar exposures by different investigators, and the endpoints studied, many of which (e.g., behavioral abnormalities) have complex etiologies, with the specific genetic components being varied or unknown.

LESSONS FROM ANIMAL STUDIES

There are limitations to human epidemiologic and clinical studies, including the inability to identify the specific chemicals involved, as well as to control the timing of exposure and dosing. Studies performed in animals may avoid these problems, and give an indication of potential for risk in humans. Similarities between man and rodents in the process of spermatogenesis, as well as in response to injuries such as with radiation (Clifton and Bremner, '83), indicate that studies in animals can help us understand the mechanisms of male-mediated effects in man. In animal studies paternal exposure to numerous agents, including environmental chemicals, recreational substances, and therapeutic drugs, has been shown to cause adverse reproductive outcomes, including congenital malformations. Several examples will be used here to illustrate how well-controlled animal studies have contributed to a more mechanistic understanding of male-mediated developmental effects.

Seminal fluid exposure

Drugs or environmental chemicals, present in the seminal fluid, could enter the female reproductive tract during intercourse and directly interfere with fetal development or may interfere with spermatozoa prior to fertilization. Many compounds have been shown to enter the semen, a fluid that is derived in large part from the secretions of the sex accessory glands and the epididymis (Pichini et al., '94). In animal experiments, methadone, morphine, thalidomide, and cyclophosphamide are examples of drugs that can cause increases in perinatal mortality and decreases in fetal weight through their presence in semen. In addition, in rabbit studies, the presence of thalidomide in semen has been linked with malformations in the offspring (Lutwak-Mann, '64), and in rat studies cyclophosphamide in semen resulted in increased preimplantation loss (Hales et al., '86). Since humans continue to have intercourse during pregnancy, there is the possibility that the conceptus may be exposed to drugs or chemicals in semen at various critical times during development. However, although many drugs will appear in semen, most will be present at such low levels that there would be little concern in humans. Drugs known to be teratogenic at low levels warrant further study. The 5-alpha reductase inhibitor, finasteride, is an example of such a drug, where the possibility of teratogenic effects of semen transmission was considered and tested experimentally in a primate model (Prahalada et al., '97). Pregnant female monkeys were administered, throughout pregnancy, daily doses of finasteride, within and above the range of semen levels of the drug, and effects on the offspring were assessed. No abnormalities were observed in the offspring, even at doses 60–750 times levels found in the semen of men treated with recommended doses of finasteride, suggesting a large safety margin for potential human exposures. Similar studies could be considered for other compounds where there is a concern that indirect exposure of the fetus to low levels of a drug through semen may occur.

Hormonal effects

Alternatively, drugs could alter the male's hypothalamic-pituitary-testicular axis, leading to oligospermia. Do quantitative abnormalities, such as oligospermia,

affect fetal development? Studies to date indicate that quantitative decreases alone, with no qualitative abnormalities, induced by hormonal manipulations, do not adversely affect the progeny of rats (Robaire et al., '87). The issue of hormones and hormonal modulators in the environment and of potential effects on development and reproduction is currently very controversial, and beyond the scope of this review. Possible links between environmental estrogen exposure and testicular cancer are of particular concern. However, although hormonal exposure of developing male rodents has been linked to cryptorchidism, decreases in testis size, and other reproductive tract abnormalities (Gill et al., '79; Sharpe et al., '95), there are no data linking paternal exposure to hormones and birth defects in the fathers' offspring.

Genetic and epigenetic effects

Qualitative defects in sperm may be expected to result in genetic defects or mutations that are transmitted to the offspring. In animal studies, the most frequently used assays for germ-cell mutagenicity are the dominant lethal, heritable translocation, and specific locus mutation assays (Shelby, '96). Dominant lethals allow fertilization but result in embryonic death and are thought to be a result of chromosomal abnormalities (structural or numerical) in germ cells of the treated male; the test does not assess heritable risks. The heritable translocation test measures chromosomal abnormalities (translocations) transmitted to the male offspring of treated males. Visible or biochemical specific locus mutation tests also estimate the frequency of heritable alterations: offspring of treated male mice are analyzed for alterations of visible morphological traits or biochemical parameters, indicative of specific gene mutations in male germ cells.

Numerous different chemicals and drugs produce positive results in these assays in animal studies (Olshan and Faustman, '93). From studies using these assays, a number of interesting points emerge, including the fact that different germ-cell types are sensitive to different chemicals. Most chemicals that are mutagenic induce mutations in postspermatogonial stages, and only a few chemicals to date have induced transmissible mutations in spermatogonial stem cells (Witt and Bishop, '96). For instance, only nine chemicals, of which three are anticancer drugs (mitomycin C, melphalan, and procarbazine), have been shown to induce specific locus mutations in spermatogonial stem cells (Witt and Bishop, '96; Shelby, '96). A large number of chemicals induce mutations in later germ-cell types (Witt and Bishop, '96). Alkylating agents, including the nitrogen mustards, platinum-based drugs, and nitrosoureas, are potent germ-cell mutagens and induce dominant lethals, heritable translocations, and specific locus mutations in poststem-cell stages of germ-cell development, clearly demonstrating that mutations in postspermatogonial germ-cell types can be transmitted. Interestingly, the mechanisms for induction of mutations in germ cells are stage-dependent, e.g., whereas melphalan induces large DNA sequence deletions and other rearrangements in postspermatogonial stages, it produces other types of mutations in spermatogonia (Russell et al., '92).

Germ cells, such as primordial germ cells and spermatozoa, that were traditionally thought not to be susceptible to drugs, are also at risk of transmitting damage. For example, in mouse studies, primordial germ cells were more sensitive than stem-cell spermatogonia to the effects of ethylnitrosourea (Shibuya et al., '93; Wada et al., '94). When ethylnitrosourea was administered to pregnant female mice, their male offspring had reduced fertility and produced offspring with phenotypic anomalies. These results suggest that the male germline may even be vulnerable in utero, e.g., in a woman undergoing chemotherapy during pregnancy. Dominant lethal mutations have been reported after exposure of spermatozoa in the epididymis to a number of agents, including cyclophosphamide (Qiu et al., '92) and acrylamide (Shelby et al., '86; Smith et al., '86), despite the fact that the chromatin is highly condensed in these cells.

Mutagenicity tests detect large chromosomal structural or numerical damage or gene mutations at selected loci. A positive response in a given mutagenicity assay indicates a true hazard; however, the absence of an effect does not mean that the chemical being studied holds no threat for future generations. More subtle effects, such as single or multiple nucleotide changes, errors in genomic imprinting, or altered regulation of gene expression, would not be detected. Much genetic disease in humans, including congenital malformations, results from mutations at poorly defined loci. In rodents, paternal exposures can induce various developmental defects or phenotypic anomalies, including decreased fetal size, increased stillbirth and neonatal death, birth defects, tumors, and behavioral or neurochemical abnormalities. Several diverse, nonspecific phenotypic anomalies, such as growth retardation, hydrocephaly, generalized edema, and micrognathia, have been reported after paternal exposure to known mutagens (Nomura, '82; Kirk and Lyon, '84; Trasler et al., '85; Nagao, '88; Jenkinson and Anderson, '90). Both acute and chronic exposures result in birth defects. For the commonly used anticancer drug cyclophosphamide, chronic low-dose exposure of male rats, using doses similar to those used in clinical regimens, did not affect various measures of male reproductive function but did result in increases in preimplantation and postimplantation loss and an increase in abnormal and growth-retarded fetuses when the males were mated with untreated females (Trasler et al., '85, '86). For individual agents, the type of reproductive outcome, such as preimplantation loss or birth defect, observed after paternal treatment, often depends on the germ-cell type exposed to the drug. For an endpoint such as birth defects, examination of thousands of offspring of paternally treated mice and rats showed 3–8-fold increases over control rates (Table 2). Similarly, large numbers of

TABLE 2. Examples of paternal exposures in rodents that result in increased numbers of malformations in the offspring

			# of malformations	
Reference	Animal	Treatment	Control	Treated
Trasler et al., '85, '87	Rat	Cyclophosphamide	7/1,580 (.4%)	23/2,096 (1.1%)
Kirk and Lyon, '84	Mouse	X-ray	17/2,020 (.8%)	110/5,123 (2.1%)
Nomura, '78, '82, '88	Mouse	X-ray	26/4,867 (.5%)	61/1,588 (3.8%)
		Urethane		75/3,400 (2.2%)
		7,12-Dimethylbenz-(a)anthracene		19/1,321 (1.4%)
		Ethylnitrosourea		29/1,175 (2.5%)
Nagao, '87	Mouse	Methylnitrosurea	28/5,086 (.6%)	79/3,614 (2.2%)

patients with well-defined paternal exposures are likely to be needed to show effects in human studies.

Some of the defects induced by paternal exposures to drugs may occur late in life. For instance, in mice, exposure of germ cells to carcinogens and mutagens leads to the occurrence of heritable tumors in the offspring (Tomatis et al., '92). Functional abnormalities in the progeny, such as behavior, may go undetected but may indicate a change in central nervous system (CNS) function. Male-mediated behavioral abnormalities have been reported in the offspring of males treated with various agents, including methadone (Joffe et al., '90), morphine (Friedler and Wheeling, '79; Cicero et al., '91), cyclophosphamide (Adams et al., '81; Auroux and Dulioust, '85), lead (Brady et al., '75; Gandley and Silbergeld, '94), and ethylene dibromide (Fanini et al., '84).

Genomic imprinting

A subset of mammalian genes is subject to genomic imprinting, an epigenetic process that is thought to be initiated during spermatogenesis and oogenesis and then further modified during embryogenesis. For imprinted genes, the gene on either the maternal or the paternal allele is expressed. A drug-induced alteration in the male germline could lead to two theoretical outcomes in the offspring, i.e., expression from both alleles due to "relaxation" of the paternal imprint, or expression from neither allele due to failure to epigenetically mark the paternal allele for expression. The precise nature of the imprint and the timing during spermatogenesis when the process is complete are not known. Concern has been raised for men undergoing intracytoplasmic sperm injection (ICSI) as a treatment for infertility, where immature germ cells or sperm that may have abnormalities are used (Tycko et al., '97). Site-specific DNA methylation, catalyzed by DNA methyltransferase, has been implicated as an important biochemical modification of DNA underlying imprinting. In keeping with an important role for DNA methylation in imprinting, DNA methyltransferase-deficient mice show abnormal expression of imprinted genes (Li et al., '93). Few animal studies have investigated the possible link between paternal exposures and effects on genomic imprinting. Chronic treatment of male rats with 5-azacytidine, a drug that alters DNA methylation, resulted in abnormalities in male germ cells and early embryo development but no increase in the incidence of congenital malformations (Doerksen and Trasler, '96). This is an important area with potential consequences for the offspring of exposed males, and warrants further study.

Heritability

An important question with clinical relevance is the heritability in future generations of the initial damage to male germ cells. In rodents, evidence of heritability (for malformations, postimplantation loss, and/or behavioral abnormalities) has been reported for a number of exposures, including radiation, urethane, and cyclophosphamide. Chronic paternal treatment with cyclophosphamide leads to decreases in litter sizes, but some pups survive without noticeable malformations. An increase in postimplantation loss and malformations among progeny resulted when these "normal" F1 animals whose fathers were treated with cyclophosphamide were mated with untreated females (Hales et al., '92). Similarly, another study with cyclophosphamide found behavioral abnormalities in the F2 and F3 generations, with disorders more severe in males than females (Auroux et al., '90). Heritable mutations were also found in mice whose fathers were treated with urethane and ionizing radiation (Nomura, '94), suggesting that drug-induced mutations in germ cells can be passed on to future generations. Tumors in the F1 and later generations have been reported following paternal treatment with ionizing radiation, ethylnitrosourea, and urethane (Nomura, '94; Tomatis et al., '81, '90).

Other exposures

Epidemiological studies in humans have suggested that paternal occupational exposures may be linked to spontaneous abortions, miscarriages, and childhood cancers (McDonald et al., '89; Olshan and Faustman, '93). There are relatively few studies in animals regarding occupational-type exposures. Paternal treatment of mice with chromium chloride, a constituent in welding fumes, resulted in increased numbers of offspring with tumors; however, exposure to six other metal components resulted in no differences from controls (Anderson et al., '94). A comprehensive analysis of data from a

number of studies on the genetic effects of 1,3-butadiene and its metabolites was carried out in an effort to estimate the germ-cell genetic risk to exposed humans (Pacchierotti et al., '98). 1,3-Butadiene, a synthetic organic chemical used in the petroleum industry, tire plants, and polymer production, has been of particular interest as it is carcinogenic in mice at low-exposure concentrations and has been associated with an increased risk of leukemia and other cancers in exposed workers (Macaluso et al., '96; Ward et al., '96). Acknowledging that their conclusions on 1,3-butadiene were based on approximations, Pacchierotti et al. ('98) nevertheless concluded that a genetic hazard for the progeny of exposed workers exists at exposure concentrations still allowed in some countries. Paternal exposures to recreational drugs such as alcohol, opiates, and smoking have been examined in a number of studies. Common findings in offspring following paternal exposure to opiates such as morphine and methadone include low birth weight, and behavioral and endocrine abnormalities (Friedler, '96). In some rodent studies, paternal alcohol exposure was associated with increases in perinatal mortality, decreases in fetal size, and behavioral abnormalities in the progeny (Nelson et al., '96). Unlike drugs that are known mutagens and cause genetic damage, the mechanisms of paternal effects of alcohol and opiates are unclear and may involve epigenetic mechanisms.

Germ-cell protection

Radiation and cancer chemotherapeutic agents can suppress spermatogenesis for prolonged periods of time in rodents and man and are known to be germ-cell mutagens in rodents (Witt and Bishop, '96). It would therefore be clinically useful to protect spermatogenesis from the damaging effects of chemotherapy and radiotherapy, and this has been attempted by several laboratories using animal models. Protection of spermatogenesis from cyclophosphamide was first shown in mice using pretreatment with daily injections of an analogue of gonadotropin-releasing hormone (GnRH) (Glode et al., '81). Pretreatment of rats with various regimens, all of which suppress intratesticular testosterone levels, including gonadal steroids, GnRH agonists, or antagonists, can protect the testis from cancer chemotherapy-induced damage (Meistrich et al., '98). Although the mechanisms are unclear, the hormonal treatments used to date in rodents are thought to protect the survival of spermatogonial stem cells and/or maintain an appropriate paracrine environment, to allow surviving stem spermatogonia to differentiate posttreatment. Hormonal protection of spermatogonial stem cells has been extended to some men undergoing cancer chemotherapy; however, mechanisms of protection and ideal dosing regimens still need to be established.

Another approach to decreasing gonadal injury associated with anticancer therapy is through the production of artificial cryptorchidism. The elevation of the testes into the inguinal canal results in reversible germ-cell loss; testicular injury following artificial cryptorchidism is thought to be due to increased gonadal temperature. In an experiment supporting the potential utility of this approach for male germ-cell protection, cryptorchid rats were protected from the irreversible effects of 2,5-hexanedione-induced germ-cell loss, possibly due to decreased exposure of germ cells in the cryptorchid testes to the compound (Boekelheide et al., '90).

Other potential avenues for future approaches to protecting germ cells include harnessing endogenous cellular protective mechanisms such as heat shock proteins and molecules that regulate apoptosis. Heat shock proteins, including spermatogenic cell-specific forms, are found in abundance in rodent and human germ cells (Miller et al., '92; Dix, '97); some heat shock proteins are induced in response to environmental stress and may play a role in protecting germ cells from various paternal exposures. Some heat shock proteins are essential for spermatogenesis. For instance, in mice homozygous for a targeted deletion in the *Hsp70–2* gene, pachytene spermatocytes fail to complete meiotic prophase and become apoptotic (Dix et al., '97). The *p53* tumor-suppressor gene prevents the propagation of DNA damage to daughter cells by causing cell-cycle arrest or by inducing apoptosis (Smith and Fornace, '96) and may also be involved actively in DNA repair (Smith et al., '95; Li et al., '96). In the mouse, p53 is expressed during meiotic prophase in pachytene spermatocytes (Schwartz et al., '93) and appears to be important for normal spermatogenesis, since the testes of mice with reduced levels of p53 are histologically abnormal, consistent with abnormalities in DNA repair and meiotic divisions (Rotter et al., '93). Interestingly, when homozygous p53-deficient male mice (*p53-/-*) were exposed to irradiation 4 weeks prior to mating, an increased level of exencephaly was found in the homozygous female p53-deficient progeny (Armstrong et al., '95). The results suggest the intriguing possibility that p53 may play a role in suppressing radiation-induced male-mediated teratogenesis.

CONCLUSIONS

In man, there is as yet no documented transmission to the offspring of drug- or chemical-induced heritable changes; however, data are accumulating to suggest caution. Evidence from human studies includes documented decreases in the quality and quantity of sperm after paternal exposure to drugs and toxic chemicals, and needs to be considered in the light of the ability of cytogenetically abnormal sperm to fertilize oocytes. Clinically, a portion of chromosomal abnormalities occurring in embryos and newborns is known to be of paternal origin, and data from epidemiological studies suggest that men in certain occupations have increased risks of fathering children with birth defects or cancer (Savitz and Chen, '90; Olshan and Faustman, '93). In contrast, numerous observations from animal studies

indicate that paternal exposure to drugs and chemicals can result in adverse reproductive outcomes and the transmission of genetic damage. Low birth weight, congenital malformations, behavioral defects, delayed appearance of early postnatal landmarks, growth retardation, endocrine abnormalities, and cross-generational effects are some of the adverse outcomes resulting from paternal exposures in animal studies. Children are born with similar defects, and the available evidence does not allow us to rule out the possibility that some of these defects are caused by paternal environmental or therapeutic exposures.

Human epidemiologic data are very important due to the limitations in extrapolating from animal studies to human exposures. For the future, well-designed epidemiological studies, with large numbers of accurately identified cases, accurate exposure histories, and identification of confounders, are needed. For occupational exposures, parallel studies in animal models may help establish biological plausibility and discern underlying mechanisms. Coordination of international efforts will be important to respond quickly with well-designed studies to follow reproductive outcomes after environmental disasters. With the rapid advances that are occurring in the identification of genes involved in human disease and in the screening of genes for mutations, molecular and DNA-based approaches should be incorporated into these epidemiological studies to search for genetic changes in human germ cells and the resulting offspring.

PRACTICAL CONSIDERATIONS AND COUNSELING

Physicians should be aware that there is increasing concern that both maternal and paternal exposures may be important to consider. Clearly, more basic and clinical research in this area is important (Olshan and Mattison, '94). Detailed histories of mothers' and fathers' exposures should be taken routinely. In man, the relationship between alterations in male fertility (including sperm abnormalities) and birth defects is unclear at present. The findings of increased chromosome aberrations in sperm of patients who have received radiotherapy and chemotherapy suggest that physicians should be cautious in predicting reproductive outcomes in these patients. Cancer patients interested in having children should receive genetic counseling informing them of the available data. Sperm samples for cryopreservation should be collected prior to but not during cancer therapy (Meistrich, '93). For those cancer patients who decide to conceive posttherapy, the data are still scarce; however, it appears that the general recommendation of delaying conception for at least 6 months after all therapy ceases is reasonable (Meistrich, '93; Robbins et al., '97). This timing will ensure that all spermatozoa that fertilize an egg derive from cells that were stem spermatogonia at the time of treatment and are thus expected to carry a lower genetic risk. High-resolution ultrasound and amniocentesis or chorionic villus sampling are the only other screening tools that can be offered to cancer patients at this time. Sperm banking in cancer patients may not only allow these men to have children in the future but may facilitate the comparison of pre- and posttreatment samples for genetic damage. Some of the approaches currently being used in cancer patients, such as delayed conception, sperm storage, and attempts to protect the seminiferous epithelium with hormones, may also be useful for certain occupational exposures in the future.

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LITERATURE CITED

Adams PM, Fabricant JD, Legator MS. 1981. Cyclophosphamide-induced spermatogenic effects detected in the F1 generation by behavioral testing. Science 211:80–82.

Amann RP. 1981. A critical review of methods for evaluation of spermatogenesis from seminal characteristics. J Androl 2:37–60.

Anderson LM, Kasprzak KS, Rice JM. 1994. Preconception exposure of males and neoplasia in their progeny: effects of metals and consideration of mechanisms. In: Olshan AF, Mattison DR, editors. Male-mediated developmental toxicity. New York: Plenum Press. p 129–140.

Armstrong JF, Kaufman MH, Harrison DJ, Clarke AR. 1995. High-frequency developmental abnormalities in *p53*-deficient mice. Curr Biol 5:931–936.

Aschengrau A, Monson RR. 1989. Paternal military service in Vietnam and risk of spontaneous abortion. J Occup Med 31:619–623.

Ashby J, Richardson CR. 1985. Tabulation and assessment of 113 surveillance cytogenetic studies conducted between 1965 and 1984. Mutat Res 154:111–133.

Auroux MR, Dulioust EJ. 1985. Cyclophosphamide in the male rat: behavioral effects in the adult offspring. Behav Brain Res 16:25–36.

Auroux M, Dulioust E, Selva J, Rince P. 1990. Cyclophosphamide in the F0 male rat: physical and behavioral changes in three successive generations. Mutat Res 229:189–200.

Bishop JB, Witt KL, Sloane RA. 1997. Genetic toxicities of human teratogens. Mutat Res 396:9–43.

Boekelheide K, Eveleth J, Hall SJ. 1990. Experimental cryptorchidism protects against long-term 2,5-hexanedione-induced testicular germ cell loss in the rat. J Androl 11:105–112.

Brady K, Herrera Y, Zenick H. 1975. Influence of parental lead exposure on subsequent learning ability of offspring. Pharmacol Biochem Behav 3:561–565.

Brandriff BF, Meistrich ML, Gordon LA, Carrano AV, Liang JC. 1994. Chromosomal damage in sperm of patients surviving Hodgkin's disease following MOPP therapy with and without radiotherapy. Hum Genet 93:295–299.

Byrne J, Mulvihill JJ, Myers MH, Connelly RR, Naughton MS, Krauss MR, Steinhorn SC, Hassinger DD, Austin DF, Bragg K, Holmes GF, Latourette HB, Weyer PJ, Meigs JW, Teta MJ, Cook JW, Strong LC. 1987. Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. N Engl J Med 317:1315–1321.

Centers for Disease Control. 1988. Centers for Disease Control Vietnam Experience Study. Health status of Vietnam veterans, III. Reproductive outcomes and child health. JAMA 259:2715–2719.

Cicero TJ, Adams ML, Giordano A, Miller BT, O'Connor L, Nock B. 1991. Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of the offspring. J Pharmacol Exp Ther 256:1086–1093.

Clermont Y, Harvey SC. 1965. Duration of the cycle of the seminiferous epithelium of normal hypophysectomized and hypophysectomized-hormone treated albino rats. Endocrinology 76:80–89.

Clifton DK, Bremner WJ. 1983. The effect of testicular X-irradiation on spermatogenesis in man: a comparison with the mouse. J Androl 4:387–392.

Cordier S, Deplan F, Mandereau L, Hemon D. 1991. Paternal exposure to mercury and spontaneous abortions. Br J Ind Med 48:375–381.

Courot M. 1970. Spermatogenesis. In: Johnson AD, Vandemark NL, editors. The testis. New York: Academic Press. p 339–432.

Cowan DN, DeFraites RF, Gray GC, Goldenbaum MB, Wishik SM. 1997. The risk of birth defects among children of Persian Gulf War veterans. N Engl J Med 336:1650–1656.

Dix DJ. 1997. Hsp70 expression and function during gametogenesis. Cell Stress Chaperones 2:73–77.

Dix DJ, Allen JW, Collins BW, Poorman-Allen P, Mori C, Blizard DR, Brown PR, Goulding EH, Strong BD, Eddy EM. 1997. HSP70–2 is required for desynapsis of synaptonemal complexes during meiotic prophase in juvenile and adult mouse spermatocytes. Development 124:4595–4603.

Doerksen T, Trasler JM. 1996. Developmental exposure of male germ cells to 5-azacytidine results in abnormal preimplantation development in rats. Biol Reprod 55:1155–1162.

Doll R, Evans HJ, Darby SC. 1994. Paternal exposure not to blame. Nature 367:678–680.

Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ. 1996. Human minisatellite mutation rate after the Chernobyl accident. Nature 380:683–686.

Erickson JD, Mulnaire J, McClain PW, Fitch TG, James LM, McClearn AB, Adams MJ. 1984. Vietnam veterans risks for fathering babies with birth defects. JAMA 252:903–912.

Fanini D, Legator MS, Adams PM. 1984. Effects of paternal ethylene dibromide exposure on F1 generation behavior in the rat. Mutat Res 139:133–138.

Friedler G. 1996. Paternal exposures: impact on reproductive and developmental outcome. An overview. Pharmacol Biochem Behav 55:691–700.

Friedler G, Wheeling HS. 1979. Behavioral effects in offspring of male mice injected with opiates prior to mating. Protracted effects of perinatal drug dependence. Pharmacol Biochem Behav [Suppl] 11:23–28.

Gandley RE, Silbergeld EK. 1994. Male-mediated reproductive toxicity: Effects on the nervous system of offspring. In: Olshan AF, Mattison DR, editors. Male-mediated developmental toxicity. New York: Plenum Press. p 141–152.

Gardner MJ. 1992. Paternal occupations of children with leukemia. Br Med J [Clin Res] 305:715–716.

Gardner MJ, Snee MP, Hall AJ, Powell CA, Downes S, Terrell JD. 1990. Results of a case-control study of leukemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. Br Med J [Clin Res] 300:423–429.

Genesca A, Barrios L, Miro R, Caballin MR, Benet J, Fuster C, Bonfill X, Egozcue J. 1990a. Lymphocyte and sperm chromosome studies in cancer-treated men. Hum Genet 84:353–355.

Genesca A, Benet J, Caballin MR, Miro R, Germa JR, Egozcue J. 1990b. Significance of structural chromosome aberrations in human sperm: analysis of induced aberrations. Hum Genet 85:495–499.

Gill WB, Schumacher GFB, Bibbo M, Strauss FH II, Schoenberg HW. 1979. Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities. J Urol 122:36–39.

Glode LM, Robinson J, Gould SF. 1981. Protection from cyclophosphamide-induced testicular damage with an analogue of gonadotropin-releasing hormone. Lancet 1(8230):1132–1134.

Hales BF, Smith S, Robaire B. 1986. Cyclophosphamide in the seminal fluid of treated males: transmission to females by mating and effects on progeny outcome. Toxicol Appl Pharmacol 84:423–430.

Hales BF, Crosman K, Robaire B. 1992. Increased postimplantation loss and malformations among the F2 progeny of male rats chronically treated with cyclophosphamide. Teratology 45:671–678.

Hawkins MM. 1991. Is there evidence of a therapy-related increase in germ cell mutation among childhood cancer survivors? J Natl Cancer Inst 83:1642–1650.

Heller CG, Clermont Y. 1963. Spermatogenesis in man: an estimate of its duration. Science 140:184–186.

Henriksen K, Kulmala J, Toppari J, Mehrotra K, Parvinen M. 1996. Stage-specific apoptosis in the rat seminiferous epithelium: quantification of irradiation effects. J Androl 17:394–402.

Hermo L, Clermont Y. 1995. How are germ cells produced and what factors control their production? In: Pryor JL, Robaire B, Trasler JM, editors. The handbook of andrology. Lawrence, KS: American Society of Andrology and Allen Press. p 13–16.

Jenderny J, Rohrborn G. 1987. Chromosome analysis of human sperm. I. First results with a modified method. Hum Genet 76:385–388.

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

Jenkinson PC, Anderson D. 1990. Malformed foetuses and karyotype abnormalities in the offspring of cyclophosphamide and allyl alcohol-treated male rats. Mutat Res 229:173–184.

Joffe JM, Peruzovic M, Milkovic K. 1990. Progeny of male rats treated with methadone: physiological and behavioral effects. Mutat Res 229:201–211.

Kirk KM, Lyon MF. 1984. Induction of congenital malformations in the offspring of male mice treated with X-rays at pre-meiotic and post-meiotic stages. Mutat Res 125:75–85.

Kodaira M, Satoh C, Hiyama K, Toyama K. 1995. Lack of effects of atomic bomb radiation on genetic instability of tandem-repetitive elements in human germ cells. Am J Hum Genet 57:1275–1283.

Lancranjan I, Popescu HI, Gavanescu O, Klepsch I, Serbanescu M. 1975. Reproductive ability of workmen occupationally exposed to lead. Arch Environ Health 30:396–401.

Li E, Beard C, Jaenisch R. 1993. Role for DNA methylation in genomic imprinting. Nature 366:362–365.

Li G, Mitchell DL, Ho VC, Reed JC, Tron VA. 1996. Decreased DNA repair but normal apoptosis in UV-irradiated skin of p53 transgenic mice. Am J Pathol 148:1113–1124.

Little J, Vainio H. 1994. Mutagenic lifestyles? A review of evidence of associations between germ-cell mutations in humans and smoking, alcohol consumption and use of "recreational" drugs. Mutat Res 313:131–151.

Little RE, Sing CF. 1985. Father's drinking and infant birth weight: report of an association. Teratology 36:59–65.

Lutwak-Mann C. 1964. Observations on progeny of thalidomide-treated male rabbits. Br Med J 1:1090–1091.

Macaluso M, Larson R, Delzell E, Sathiakumar N, Hovinga M, Julian J, Muir D, Cole P. 1996. Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. Toxicology 113:190–202.

Martin RH. 1989. Invited editorial: segregation analysis of translocations by the study of human sperm chromosome complements. Am J Hum Genet 44:461–463.

Martin R, Hildebrand K, Yamamoto J, Rademaker A, Barnes M, Douglas G, Arthur K, Ringrose T, Brown I. 1986. An increased frequency of human sperm chromosomal abnormalities after radiotherapy. Mutat Res 174:219–225.

Martin R, Barclay L, Hildebrand K, Ko E, Fowlow S. 1990. Cytogenetic analysis of 400 sperm from three translocation heterozygotes. Hum Genet 86:33–39.

Martin R, Rademaker A, Leonard N. 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 RH, Ernst S, Rademaker A, Barclay L, Ko E, Summers N. 1997. Chromosomal abnormalities in sperm from testicular cancer patients before and after chemotherapy. Hum Genet 99:214–218.

McDonald AD, McDonald JC, Armstrong B, Cherry NM, Nolin AD, Robert D. 1989. Fathers' occupation and pregnancy outcome. Br J Ind Med 46:329–333.

Meistrich ML. 1993. Potential genetic risks of using semen collected during chemotherapy. Hum Reprod 8:8–10.
Meistrich ML, Wilson G, Kangasniemi M. 1998. Hormonal protection of spermatogenic stem cells against cytotoxic agents. In: Zirkin BR, editor. Germ cell development, division, disruption and death. New York: Springer-Verlag. p 202–213.
Miller D, Brough S, Al-Harbi O. 1992. Characterization and cellular distribution of human spermatozoal heat shock proteins. Hum Reprod 7:637–645.
Mulvihill HJ. 1994. Reproductive outcomes among men treated for cancer. In: Olshan AF, Mattison DR, editors. Male-mediated developmental toxicity. New York: Plenum Press. p 197–204.
Nagao T. 1987. Frequency of congenital defects and dominant lethals in the offspring of male mice treated with methylnitrosourea. Mutat Res 177:171–178.
Nagao T. 1988. Congenital defects in the offspring of male mice treated with ethylnitrosourea. Mutat Res 202:25–33.
Neel JV, Schull WJ. 1991. The children of atomic bomb survivors: a genetic study. Washington, DC: National Academy Press. 461 p.
Nelson BK, Moorman WJ, Schrader SM. 1996. Review of experimental male-mediated behavioral and neurochemical disorders. Neurotoxicol Teratol 18:611–616.
Nomura T. 1978. Changed urethan and radiation response of the mouse germ cell to tumour induction. In: Severi L, editor. Tumours of early life in man and animals. Perugia: Perugia University Press. p 873–891.
Nomura T. 1982. Parental exposure to X-rays and chemicals induces heritable tumours and anomalies in mice. Nature 296:575–577.
Nomura T. 1988. X-ray and chemically induced germ-line mutation causing phenotypical anomalies in mice. Mutat Res 198:301–320.
Nomura T. 1994. Male-mediated teratogenesis: ionizing radiation/ethylnitrosourea studies. In: Olshan AF, Mattison DR, editors. Male-mediated developmental toxicity. New York: Plenum Press. p 117–128.
O'Leary LM, Hicks AM, Peters JM, London S. 1991. Parental occupational exposures and risk of childhood cancer: a review. Am J Ind Med 20:17–35.
Olshan AF. 1995. Lessons learned from epidemiologic studies of environmental exposure and genetic disease. Environ Mol Mutagen [Suppl] 25:74–80.
Olshan AF, Faustman EM. 1993. Male-mediated developmental toxicity. Annu Rev Public Health 14:159–181.
Olshan AF, Mattison DR, editors. 1994. Male-mediated developmental toxicity. New York: Plenum Press. 406 p.
Olshan AF, Schnitzer PG. 1994. Paternal occupation and birth defects. In: Olshan AF, Mattison DR, editors. Male-mediated developmental toxicity. New York: Plenum Press. p 153–168.
Olshan AF, Teschke K, Baird PA. 1990. Birth defects among offspring of firemen. Am J Epidemiol 131:312–321.
Olshan, AF, Teschke K, Baird PA. 1991. Paternal occupation and congenital anomalies. Am J Ind Med 20:447–475.
Pacchierotti F, Adler I-D, Anderson D, Brinkworth M, Demopoulos NA, Lahdetie J, Osterman-Golkar S, Peltonen K, Russo A, Tates A, Waters R. 1998. Genetic effects of 1,3-butadiene and associated risk for heritable damage. Mutat Res 397:93–115.
Pichini S, Zuccaro P, Pacifici R. 1994. Drugs in semen. Clin Pharmacokinet 26:356–373.
Prahalada S, Tarantal AF, Harris GS, Ellsworth KP, Clarke AP, Skiles GL, MacKenzie KI, Kruk LF, Ablin DS, Cukierski MS, Peter CP, vanZwieten MJ, Hendrickx AG. 1997. Effects of finasteride, a type 2 5-alpha reductase inhibitor, on fetal development in the rhesus monkey (*Macaca mulatta*). Teratology 55:119–131.
Qiu J, Hales BF, Robaire B. 1992. Adverse effects of cyclophosphamide on progeny outcome can be mediated through post-testicular mechanisms in the rat. Biol Reprod 46:926–931.
Robaire B, Duron J, Hales BF. 1987. Effect of estradiol-filled polydimethylsiloxane subdermal implants in adult rats on the reproductive system, fertility and progeny outcome. Biol Reprod 37:327–337.
Robbins WA. 1996. Cytogenetic damage measured in human sperm following cancer chemotherapy. Mutat Res 355:235–252.
Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier H-U, Cassel MJ, Wilson G, Eskenazi B, Wyrobek AJ. 1997. Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. Nat Genet 16:74–78.
Rotter V, Schwartz D, Almon E, Goldfinger N, Kapon A, Meshorer A, Donehower LA, Levine QJ. 1993. Mice with reduced levels of p53 protein exhibit the testicular giant-cell degenerative syndrome. Proc Natl Acad Sci USA 90:9075–9079.
Rowley MJ, Teshima F, Heller CG. 1970. Duration of transit of spermatozoa through the human male ductular system. Fertil Steril 21:390–395.
Rudak E, Jacobs PA, Yanagimachi R. 1978. Direct analysis of the chromosome composition of human spermatozoa. Nature 274:911–912.
Russell LB, Hunsicker PR, Cacheiro NLA, Rinchik EM. 1992. Genetic, cytogenetic, and molecular analyses of mutations induced by melphalan demonstrate high frequencies of heritable deletions and other rearrangements from exposure of postspermatogonial stages of the mouse. Proc Natl Acad Sci USA 89:6182–6186.
Sankila R, Olsen JH, Anderson H, Garwicz S, Glattre E, Hertz H, Langmark F, Lanning M, Moller T, Tulinius H. 1998. Risk of cancer among offspring of childhood-cancer survivors. N Engl J Med 338:1339–1344.
Savitz DA. 1994. Paternal exposures and pregnancy outcome: miscarriage, stillbirth, low birth weight, preterm delivery. In: Olshan AF, Mattison DR, editors. Male-mediated developmental toxicity. New York: Plenum Press. p 177–184.
Savitz DA, Chen J. 1990. Paternal occupation and childhood cancer: review of the epidemiologic studies. Environ Health Perspect 88:325–337.
Savitz DA, Zhang J, Schwingl P, John EM. 1992. Association of paternal alcohol use with gestational age and birth weight. Teratology 46:465–471.
Savitz DA, Sonnenfeld NL, Olshan AF. 1994. Review of epidemiologic studies of paternal occupational exposure and spontaneous abortion. Am J Ind Med 25:361–383.
Schnitzer PG, Olshan AF, Erickson JD. 1995. Paternal occupation and risk of birth defects in the offspring. Epidemiology 6:577–583.
Schwartz D, Goldfinger N, Rotter V. 1993. Expression of p53 protein in spermatogenesis is confined to the tetraploid pachytene primary spermatocytes. Oncogene 8:1487–1494.
Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP. 1995. Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environ Health Perspect 103:1136–1143.
Shelby MD. 1994. Human germ cell mutagens. Environ Mol Mutagen [Suppl] 23:30–34.
Shelby MD. 1996. Selecting chemicals and assays for assessing mammalian germ cell mutagenicity. Mutat Res 352:159–167.
Shelby MD, Cain KT, Hughes LA, Braden PW, Generoso WM. 1986. Dominant lethal effects of acrylamide in male mice. Mutat Res 173:35–40.
Shibuya T, Murota T, Horiya N, Matsuda H, Hara T. 1993. The induction of recessive mutations in mouse primordial germ cells with N-ethyl-N-nitrosourea. Mutat Res 290:273–280.
Smith KM, Zenick H, Preston RJ, George EL, Long RE. 1986. Dominant lethal effects of subchronic acrylamide administration in the male Long-Evans rat. Mutat Res 173:273–277.
Smith ML, Fornace AJ. 1996. The two faces of tumor suppressor p53. Am J Pathol 148:1019–1022.
Smith ML, Chen IT, Zhan Q, O'Connor PM, Fornace AJ Jr. 1995. Involvement of the p53 tumor suppressor in repair of UV-type DNA damage. Oncogene 10:1053–1059.
Stellman SD, Stellman JM, Sommer JF. 1988. Health and reproductive outcomes among American Legionnaires in relation to combat and herbicide exposure in Vietnam. Environ Res 47:150–174.
Tomatis L, Cabral JRP, Likhackev AJ, Ponomarkov V. 1981. Increased cancer incidence in the progeny of male rats exposed to ethylnitrosourea before mating. Int J Cancer 28:475–478.
Tomatis L, Turusov VS, Cardis E, Cabral JRP. 1990. Tumour incidence in the progeny of male rats exposed to ethylnitrosourea before mating. Mutat Res 229:231–237.
Tomatis LS, Narod S, Yamasaki H. 1992. Transgeneration transmission of carcinogenic risk. Carcinogenesis 13:145–151.

Trasler JM, Hales BF, Robaire B. 1985. Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting male fertility. Nature 316:144–146.

Trasler JM, Hales BF, Robaire B. 1986. Chronic low dose cyclophosphamide treatment of adult rats: effects on fertility, pregnancy outcome and progeny. Biol Reprod 34:275–283.

Trasler JM, Hales BF, Robaire B. 1987. A time course study of chronic paternal cyclophosphamide treatment in rats: effects on pregnancy outcome and the male reproductive and hematologic systems. Biol Reprod 37:317–326.

Tucker MA, Coleman CN, Cox RS, Varghese A, Rosenberg SA. 1988. Risk of second cancers after treatment for Hodgkin's diseases. N Engl J Med 318:76–81.

Tycko B, Trasler JM, Bestor T. 1997. Genomic imprinting: gametic mechanisms and somatic consequences. J Androl 18:480–486.

Wada A, Sato M, Takashima H, Nagao T. 1994. Congenital malformations in the offspring of male mice treated with ethylnitrosourea at the embryonic stage. Teratogenesis Carcinog Mutagen 14:271–279.

Ward EM, Fajen JM, Ruder AM, Rinsky RA, Halperin WE, Fessler-Flesch CA. 1996. Mortality of workers employed in 1,3-butadiene production units identified from a large chemical workers cohort. Toxicology 113:157–168.

Whorton D, Krauss KM, Marshall S, Milby TH. 1977. Infertility in male pesticide workers. Lancet 2(8051):1259–1261.

Witt KL, Bishop JB. 1996. Mutagenicity of anticancer drugs in mammalian germ cells. Mutat Res 355:209–234.

Wyrobek AJ, Gordon LA, Burkhart JG, Francis MC, Kapp RW Jr, Letz G, Malling HV, Topham JC, Whorton MD. 1983. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. Mutat Res 115:73–148.