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Paternal Exposures: Impact on Reproductive and Developmental Outcome. An Overview

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FRIEDLER, G. *Paternal exposures: Impact on reproductive and developmental outcome. An overview.* PHARMACOL BIOCHEM BEHAV 55(4) 691–700, 1996.—Experimental and epidemiologic investigations document the adverse consequences of an array of paternal exposures on the development of subsequent offspring. Male-mediated abnormalities have been reported after exposure to therapeutic and recreational drugs, to chemicals in the workplace and environment and to ionizing radiation. The impact on progeny outcome includes: an increase in congenital malformations, spontaneous abortions; fetal resorptions; low birth weight; increase in childhood cancers; developmental, neurobehavioral, neuroendocrine, neurochemical abnormalities; effects in F_2 generation progeny. Fertility is often unaffected. The comparative influence of genetic, epigenetic and nongenetic mechanisms in the etiology of paternally-mediated adverse outcomes is unknown. There is no a priori reason to assume that male-mediated effects are limited to the agents studied to date. The broad spectrum of alterations recorded after exposure to a variety of unrelated agents suggests the need for a more focused effort and multidisciplinary exploration of the potential impact of the male parent on reproductive outcome. **Copyright © 1996 Elsevier Science Inc.**

Male-mediated Progeny Neurobehavioral Reproductive outcome Paternal drugs
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INTRODUCTION

A significant concern associated with exposure to potential chemical or physical toxicants is their effect upon parents' ability to produce healthy offspring. It has been estimated that over 75% of all conceptions do not result in a successful reproductive outcome (128). Each year approximately 8% of all babies born in the U.S. have defects detectable at birth (130) and there is no identifiable cause in over sixty percent of these cases (54). The extent to which the male contributes to these reproductive failures is not known but is believed to be substantial (134). Yet until recently, investigations of developmental sequelae of parental exposure to pharmacologic or other chemical and physical agents have focused almost exclusively on maternal factors.

Historical references to male-mediated effects on progeny date from antiquity, with the prime concern of these reports on the harmful effects of alcohol drinking. An extensive review of this literature (126) cites several references to paternal drinking: for example, Carthage and Sparta had laws prohibiting the use of alcohol by newlyweds; Anatomy of Melancholy, published in 1621, cited Gellius: "if a drunken man get a child it will never likely have a good brain"; the gin epidemic in England, in the first half of the eighteenth century, evoked deep concern over the harm caused by drinking of either parent (126). The concern that conception during intoxication was a particular hazard continued until the early part of the twentieth century. Animal studies reported adverse effects of paternal alcohol inhalation on offspring including: malformations, low birth weight, retarded growth and high neonatal mortality over several generations (112). These studies were controversial and conflicting findings were reported by others (126).

The first systematic observations to link adverse effects on reproduction with occupational exposure appeared in the latter part of the nineteenth century (12). High rates of infertility, spontaneous abortions and infant deaths were recorded in lead-working communities in many parts of Europe (95); the reports suggested that exposure of either parent adversely affected reproductive outcome but only women were banned from work in the heavy lead trades (12). Although the prime emphasis was placed on maternal exposure, interest in paternal lead continued into the twentieth century. Several studies identified a positive association between male workplace exposure to lead and reproductive failure (69,95).

Except for protecting pregnant women from excessive radiation exposure and occasional references to women in the

chemical industry during World War II, the early history of the deleterious effects of industrial chemicals on reproductive outcome was largely ignored prior to Minimata (12). It was this tragedy, where exposure to methyl mercury in fish resulted in neurologic abnormalities in many adults and in 6% of all births, which finally led to greater emphasis on the vulnerability of the embryo and fetus to environmental toxins following maternal exposure (12).

Male-mediated effects were not systematically examined until the early seventies when independent studies reported adverse effects in both experimental animals and human offspring after paternal exposure to several drugs or to paternal endocrine interventions. Common findings across studies in rodents were low birth weight, delayed appearance of early postnatal developmental landmarks, growth retardation, altered endocrine function, cross-generational effects (11.36, 37,46,109). Behavioral measures were not routinely employed in these studies. Epidemiologic studies reported an increased incidence of spontaneous abortions and congenital malformations (27,28).

These investigations received little attention or credence prior to the report of infertility in male workers exposed to the nematocide dibromochloropropane (DBCP) (127). Although rat studies in the early sixties had reported that DBCP profoundly decreased spermatogenesis (118), it was the 1977 DBCP report of human male infertility which finally directed attention to the deleterious effects of chemicals on male reproduction (68). As noted by Lamb (68), the DBCP incident aptly illustrated the failure to be alerted by animal data identifying a potential reproductive hazard. It was the DBCP-exposed workers, not trained observers, who first suggested the possible relationship of their low fertilty to occupational exposure (68).

In 1991, a case reviewed by the Supreme Court addressed occupational exposure to lead and contained amicus briefs on the deleterious effects of paternal exposures on reproductive outcome (61). This case, and a scientific symposium on male-mediated effects convened shortly before the Court's decision (107), were widely publicized and may have contributed to the recent refocus of attention, in both the scientific and public arena, to potential male-mediated influences on the development of progeny (17,107,125).

ASSESSMENT OF MALE REPRODUCTIVE FUNCTION

Focus on the singular role of the pregnant female in the health of the fetus and neonate has tended to minimize concern for possible male-mediated effects. Studies in the male have primarily explored the effects of toxic agents on fertility rather than on developmental outcome. This may reflect past emphasis on fertility as the sine qua non of successful conception, and by inference normal birth. Fertility alone is an inadequate indicator of a toxic insult to either developing or mature spermatozoa (71). Which of the most frequently used measures of fertility (sperm concentration, motility, morphology) are the best predictors of reproductive success or failure in humans is unknown as is the relationship of these and other measures of sperm structure and function to reproductive outcome, should fertility occur (130,132,134).

In the past, the primary sources for information on chemicals affecting male reproduction were: spermatotoxic side effects of various therapeutic agents, drugs used to treat subfertile men, potential male contraceptives (134). The recent report of the marked decrease in male sperm production over the past half century has renewed concern over the effect of environmental exposures on male reproductive potential (22).

Germ Cell Development

The development of spermatozoa involves highly complex cellular processes which may be impacted by environmental factors at several stages of their development (68). Development of mature sperm from primordial sperm cells is a cyclical process which begins at sexual maturation and continues throughout the reproductive life of the male (68). Each cycle takes 74 days in man, 35 days in mice, 48 days in rats (68).

The first stage of spermatogenesis involves the proliferation and renewal of undifferentiated stem cells (spermatogonia): some spermatogonia (Type A) will remain to replenish precursor cells at each new cycle; others (Type B) will undergo additional rapid mitotic divisions and eventual differentiation (68). Similar to other actively replicating cells, spermatogonia are particularly susceptible to environmental insult during this phase of cell proliferation (31). This process is in marked contrast to females, where all oocytes capable of participating in the fertilization process are produced before birth and are no longer dividing (54).

Following the proliferative stage, spermatogonia will undergo meiosis, a process which will eventually result in chromosome reduction from diploid to haploid state. The lengthy phase between primary and secondary spermatocyte production is characterized by accelerated RNA and protein synthesis (68). During this period there is marked cell atresia and only half of the participating stem cells survive (134). The reasons for this high level of cell loss is unknown; this period is also thought to be highly susceptible to environmental insult (134).

The now haploid spermatids undergo a complex morphologic transformation (spermiogenesis) during which DNA and nuclear proteins condense, mitochondria form the midpiece and the tail is formed (68). Testicular spermatozoa are released into the lumen of the seminiferous tubules of the testis and carried by seminal fluid within a lengthy system of ducts (the epididymis) where they undergo a further process of maturation and become motile (68). The ability of spermatozoa to actually fertilize the ovum does not occur until they enter the female reproductive tract; this process (capacitation) involves changes on both the surface of the sperm and within the gamete itself (134).

Germ Cell Injury

Mechanisms that govern many of the complex structural and functional transformations that occur during spermatogenesis are unknown (68). Toxic agents can disrupt these processes at any stage of sperm development, maturation and fertilization; the effect and prognosis for recovery depends upon the stages affected (134). The duration of each stage of spermatogenesis, spermiogenesis and storage in the epididymis prior to release is known for man and several test species (54). Consequently, by controlling the duration of exposure prior to mating, experiments can be designed to determine stage-specific effects of potential toxicants.

Heritable genetic defects in sperm may be due to either gene mutations or chromosomal abnormalities (131). Susceptibility to mutation varies with both stage of sperm development and the mutagen itself; germinal mutations have been identified at all stages of sperm development in mice (130-132). In addition, manifestation of sperm-induced prelesions depends upon the repair ability of the ovum (131).

Chromosomal abnormalities may be either structural (e.g. chromosomal translocations or deletions) or aneuploid (numerical) (54). The dominant lethal test in mice can infer these effects by the extent of pre- or postimplantation loss following mating with treated male mice; chromosomal aberrations can also be identified in treated males by cytologic or fertility testing of their surviving offspring (54).

Epigenetic changes refer to non-mutational changes (in DNA of gametes) which affect gene expression (131–132). Genetic imprinting is a type of epigenetic alteration in which, for some genes, only one allele (either paternal or maternal) is expressed during development (114). Although both parental genomes are essential for normal mammalian development and contribute a similar number of chromosomes, recent evidence indicates that the two pronuclei (male and female) which form the zygote at fertilization are not functionally identical (83,114). Certain genes are expressed in only one of the alleles. Any perturbations of this imprinting process can affect gene expression and subsequent developmental outcome (92).

In addition, the sperm is surrounded by a perinuclear matrix containing hundreds of "novel" proteins whose function is unknown (14). The proteins may serve as receptors and provide a mechanism for the regional distribution of genes within the sperm nucleus (14). Any interference with the structure or function of these matrical proteins could adversely impact birth outcome.

POTENTIAL MECHANISMS

Male-mediated effects have been recorded after paternal exposure at specific stages of spermatogenesis and spermiogenesis or throughout the spermatogenic cycle. Given the varying profile of agents which have been been implicated and the wide spectrum of deficits which have been reported, no single mechanism can adequately explain male-mediated changes (42,131).

Effects of xenobiotic agents on sperm function may result from: direct exposure of germ cells at any stage of sperm development and maturation; indirect effects on the testis, epididymis, accessory sex organs; complex physiologic interactions of spermatozoa within the female reproductive tract at copulation and fertilization; direct transmission of toxicants to the female in seminal fluid or by contaminated clothing (58,87,93,117,134).

Relevant exposures may occur at any time between conception of either parent and the production of their gametes which will eventually participate in the fertilization process; this includes each parent's development in utero, during childhood and at puberty (131). An integrated approach for assessing male-mediated effects requires the development of "bridging biomarkers" to both evaluate animal data for risk assessment and discriminate between genetic, epigenetic and nongenetic mechanisms of abnormal reproductive outcomes of paternal etiology (131). A preliminary model to discriminate the comparative role of genetic, environmental and nonlinear epigenetic processes in underlying phenotypic differences, both somatic and behavioral, has been proposed (79).

The comparative influence of genetic and environmental variables in the etiology of childhood cancers has yet to be established (20). While it is known that humans contribute to the baseline frequency of mutations in their progeny, methods of evaluation in human sperm or in offspring lack sensitivity (132). A cogent illustration is the present inability to detect human germinal mutations above background for either ionizing radiation or chemotherapeutic agents (131) although in animals, the induction of germinal mutations by these agents is well documented (6,73,82,131).

With known gametotoxic agents, mutagens or carcinogens, it is plausible to invoke a genetic basis for male-mediated changes. However, several of the reported paternally-mediated effects involve agents which are probably not either mutagens or carcinogens. These chemicals often induce subtle but significant functional alterations in physiologic, neurochemical, neuroendocrine and behavioral parameters. Alternative hypotheses are required to explain these observations.

It has previously been proposed that some paternal exposures may result from selection of a particular population of gametes for participation in the fertilization process (41,108). This could occur by xenobiotic-induced alterations in: the function of the paternal hypothalamic-pituitary gonadal (HPG) axis, the physical or chemical integrity of the sperm during development or maturation, accessory gland secretions and seminal fluid composition. Any such perturbations of the normal function or environment of developing spermatozoa could adversely affect the gametes available for participation in the fertilization process (39,64,134). Active participation of the female in transport of sperm with particular structural or functional characteristics could also play a role in this process (87,118).

Epigenetic changes in gene expression offer an additional explanation for male-mediated effects. Any paternal exposure which alters the normally occurring imprinting process in sperm could adversely impact subsequent development in utero (92,122). The mechanism of genetic imprinting is thought to involve differential DNA methylation during gametogenesis or prior to formation of the zygote nucleus after fertilization (114). Genomic imprinting may be involved in the genesis of some childhood cancers and has been proposed as a mechanism for the paternal induction of Wilms' tumor, associated with several occupational exposures of the male parent (85,92).

The paternal pronucleus is critical for the proliferation of progenitor cells of differentiated tissues (114). As a consequence, paternal exposures could have unique effects on male chromosomes and subsequent reproductive outcome (41). The selective loss of embryonic inner cell mass observed following paternal cyclophosphamide (CP) exposure suggests a direct effect on the function of the male pronucleus (66). Epigenetic chromosomal modifications resulting from paternal exposure could also impinge upon subsequent fetal development by affecting the placenta since the male pronucleus is essential for its normal development (31,41,83).

Since imprinting affects only the F1 generation, and is therefore not heritable, other mechanisms must be invoked to explain male-mediated effects which persist in subsequent generations. A related hypothesis is the induction of chemical changes in the expression of cell surface receptor proteins which, by alterations in DNA, would permanently change cell phenotype (19). In addition, any toxin-induced interference with the function(s) of the sperm perinuclear matrix could alter reproductive outcome.

A direct effect of xenobiotic exposure could also impact offspring development. A number of therapeutic and other exogenous chemicals have been identified in the fluids of all components of the male reproductive system in animals (75) and in human seminal fluid where they are often highly concentrated (51,75). In addition, the vaginal epithelium is permeable to a wide range of organic and inorganic compounds (15,93).

Experimental studies have shown that toxicants in semen

TABLE 1

DEVELOPMENTAL SEQUELAE OF PATERNAL EXPOSURE*¶: AN OVERVIEW

Agent	Effects** on Offspring								References
	Death	Wt	Malf	Beh	Can	Endo	X	Other	
Therapeutic									
Anesthetic gases	±/−§#	+/+	±/o	o/+					27–29, 39, 55, 116
Cyclophosphamide	o/+	o/+	o/+	o/+	+/+	+/±	F_3	o/+	10, 56–58, 60, 62, 66, 119–121
Recreational									
Alcohol	o/±	±/±	−/−	o/±		o/+		o/+	1–4, 8, 24, 26, 40, 43, 70, 98, 129
Smoking	+/o				+/o				94, 135
Opiates	o/±	o/±		o/+		o/+	F_4	o/+	23, 36–39, 43–46, 63, 108
Occupational									
Agent Orange	±/o		±/o						9, 111
Lead	+/+	o/+		o/+			F_2	o/+	19, 85, 106, 112
Fire smoke			+/o						78
Solvents, hydrocarbons	+/o			o/±	+/o				34, 35, 59, 71, 81, 97, 103
Environmental									
Ionizing radiation			±/+	o/+	±/+				50, 82, 88, 102–105
Electromagnetic fields					±/o				110
Endocrine									
Thyroxine (T4)		o/+				o/+	F_1	o/+	11
Parathyroidectomy						o/+	F_1		47
Alloxan						o/+	F_7		109

*To chemical and physical agents or to endocrine intervention; ¶Based upon Table 5.1, modified and updated (41); **Death = resorptions, fetal/neonatal deaths; Wt = reduced birthweight; Malf = congenital malformations; Beh = behavioral alterations; Can = childhood cancer; Endo = endocrine abnormalties; X = cross-generational; Other = miscellaneous functional deficits (immunologic, neurochemical, physiologic); §In humans/animals; #+, effect present; −, effect absent; o, not examined.

can result in exposure of the embryo postconception via vaginal absorption. Paternal thalidomide (rabbits) induced several congenital malformations and ^{14}C thalidomide remained firmly bound to spermatozoa (72). A single injection of ^{14}C CP to male rats just prior to mating was widely distributed in several organs of the female including brain (58). Whether xenobiotic agents in seminal fluid can alter embryonic or fetal development postconception in humans is unknown (93).

Toxic agents may bind to sperm components (133), enter the oocyte at fertilization and affect the normal sequence of genetic events; this potential for direct access of toxins to the oocyte has been largely unexplored (134).

MALE-MEDIATED EFFECTS

Experimental and epidemiologic studies have now identified a number of pharmacologic and other xenobiotic agents which can adversely impact pregnancy outcome following paternal exposure. Chemicals associated with male-mediated effects range from commonly used therapeutic and recreational drugs to known carcinogens, mutagens or other toxic compounds associated with workplace exposures.

Table 1 illustrates the types of agents that have been associated with adverse reproductive outcome following paternal exposure and includes: structurally and functionally unrelated chemicals, physical agents, endocrine interventions. The broad spectrum of recorded abnormalities suggests that paternal exposures have the potential to significantly alter the normal development of progeny.

Pharmacologic Agents

Opioids. Morphine was the first drug identified with long-term neurobehavioral, neuroendocrine and cross-generational effects after prefertilization exposure of either parent (36–38, 44). Following paternal morphine injections (5-8 days, with a similar drug-free period prior to mating), mouse progeny showed growth retardation and a delayed appearance of developmental landmarks. With the exception of lower birth weight, reproductive indices were unaffected (36,37). There was a carryover of growth effects into the F_4 generation (42). However, extensive karyotyping failed to detect any abnormalities in chromosome morphology, number or configuration (46).

Several learned and unlearned behaviors were affected including exploration, locomotion, passive avoidance and attenuated responsiveness to drug and non-drug stressors (39,45). There were no overt indications of endocrine malfunction, yet the morphine offspring showed selective abnormalities in reproductive endocrinology: a markedly attenuated serum LH response to castration, indication of altered function of the HPG axis; lower levels of serum testosterone and pituitary LH; depressed seminal vesicle weight (39,43). Similar endocrine abnormalities were present in rat offspring following chronic paternal morphine for 9 weeks (23); in addition, serum LH levels were lower and hypothalamic beta-endorphin levels were slightly elevated. No differences in measures of reproductive endocrine status were apparent in female offspring who showed a significant increase in serum corticosterone and hypothalamic beta-endorphin (23).

When male rats received a single large morphine injection and were mated after 24 hours, there were pronounced effects on litter size, mortality and sensitivity to morphine-induced antinociception (25). Depressed birth weight and elevated postnatal mortality, most pronounced in male rat pups, was also apparent after chronic paternal methadone with concomitant mating (63,108). These studies suggest a direct toxic effect of opioids via seminal fluid or caprophagy. Reproductive indi-

ces, including birth weight, were unaltered in methadone-derived F_2 progeny (108). All behaviors tested in F1 methadone offspring (open field, locomotor activity, passive and active avoidance, rotorod latencies) were significantly changed, with a complex interaction with offspring sex, test order and days of testing (63).

Paternal exposure to either levorphan (a potent opioid) or dextrorphan (its analgesically inactive enantiomorph) affected the physiologic and neurobehavioral development of mouse offspring. These observations suggested that the paternal imprint was not unique to morphine and its congeners (39).

Alcohol. The description of fetal alcohol syndrome in 1973 led to extensive study on the effects of maternal consumption of alcohol during pregnancy on the fetus. It was not accompanied by similar concern for the possible influence of paternal alcohol on developmental outcome. Investigations of the effects of alcohol on male reproduction have primarily focused on alcohol-induced reproductive impairment or genetic contributions to alcoholism (7,13,49,123). Neurophysiologic alterations in unexposed sons of male alcoholics provide strong evidence for a biological predisposition for these changes (13) but a direct impact of paternal alcohol itself on offspring neurobehavioral function has not been excluded.

Early experimental study of male-mediated effects examned reproductive and morphologic abnormalities. Chronic oral paternal alcohol in rats resulted in increased resorptions, lower fetal weight and increased postnatal mortality (67, 74,90). In mice, a similar treatment regimen did not alter reproductive indices or induce malformations (91).

A pronounced decrease in fertility, viability, and litter size was recently reported in offspring of male rats mated 24 hours following a single large injection of alcohol (26). In mice, a marked decrease in fertility and birth weight was observed only when matings were within five days of paternal ethanol (28 day) exposure (8). In contrast, investigations in both species found that mating immediately following chronic (8–9wk) oral alcohol had no effect on fertility (2,3) or birth weight (2–4).

The often subtle effects of paternal alcohol on perinatal and early postnatal development were similar in profile to paternal opiates: effects were induced by short-term or chronic exposure; reproductive indices other than birth weight were seldom affected. However, several functional parameters were compromised in rodent offspring by paternal exposure: immunologic competence (16,53), thermoregulation (40), several behaviors (1,3,40,129) and neuroendocrine function (24).

Behavioral changes in offspring following paternal oral exposure varied with species, strain and test parameters and included: both hypo- and hyperactivity (1,3,40), and impairments in passive avoidance (1,3), in spontaneous alternation in a T-maze (1–3) and in radial maze acquisition and discrimination (129). No behavioral effects were found in rat offspring exposed to paternal ethanol by inhalation but complex changes in levels of brain norepinephrine, 5-hydroxytryptamine and met-enkephalin were detected (80).

Alcohol had profound effects on reproductive endocrinology of rat progeny exposed to chronic alcohol from prepubescence to early adulthood (24). Despite marked reduction of endocrine indices of sexual maturation (decreased testosterone and beta-endorphin, decreased weight of testes, accessory sex organs) in the alcohol group of potential fathers, their fertility was unaffected. Litter size was smaller in alcohol-derived pups but other reproductive and developmental indices were unchanged (24). However, testosterone and hypothalamic beta-endorphin levels and seminal vesicle weights were significantly decreased in the alcohol-derived offspring (24).

In humans, a positive association between heavy paternal drinking and infant low birth weight has been reported (70). A recent extensive retrospective analysis found no negative impact of paternal alcohol on birth outcome (98); however, alcohol consumption in this population was far less than in the earlier report.

Numerous investigations have confirmed the adverse effects on reproductive outcome in drug-dependent pregnant women. With the exception of the alcohol studies above and the identification of drugs of abuse in human semen (51,75) or bound to sperm (133), there is no information available on the potential male-mediated impact of any drug of abuse on human pregnancy outcome.

Cyclophosphamide(CP). This drug is the most widely studied agent for its male-mediated effects on reproductive outcome. Reports of a profound decrease in male fertility after CP chemotherapy first called attention to its profound effects in the male (127). In rats, a single high dose of CP resulted in: decreased testes weight, transient oligospermia, decreased DNA synthesis (in spermatogonia), decreased RNA and protein synthesis (in spermatids) (57,93). In contrast, chronic low dose exposure did not adversely affect several measures of male reproductive function: epididymal sperm counts, reproductive organ weights, serum testosterone, LH and FSH were unchanged (119). Yet, chronic CP exposure had significant effects on pregnancy outcome: a dose- and time-dependent increase in both pre- and postimplantation loss, and an increase in both malformed and growth-retarded fetuses (62, 119–121). Similar effects of chronic CP were also apparent in F_2 progeny (56).

CP significantly delayed the appearance of standard neurobehavioral developmental landmarks (pinna unfolding, eye opening, swimming development), cliff avoidance (longer latency) and affected locomotor activity after either acute or chronic exposure (5,33). Deficits in conditioned avoidance learning occurred in three subsequent generations following paternal exposure to low dose CP for 15 days but activity was unaffected (10). Male progeny exhibited greater sensitivity to CP-induced abnormalities(10), a vulnerability previously noted in opioid- and alcohol- derived progeny (1,3,23,39). In humans, no increase in spontaneous abortions, congenital or behavioral abnormalities have been described after paternal CP but systematic studies are lacking (119).

OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES

Animal Studies

Comparatively few experimental studies on reproductive hazards of paternal exposure to occupational and environmental chemicals have addressed effects on neurobehavioral outcome (71). In an early study in this area, exposure of male rats to lead resulted in decrements in maze learning in their offspring (19). Other investigators reported a decrease in birth weight, litter size, viability (113) and a reduction in dendrites of hippocampal pyramidal cells (106) in rat offspring following paternal lead exposure.

Male-mediated neurobehavioral alterations have been observed in rodent progeny following following paternal exposure to industrial chemicals (71). Alterations in both behavior and neurotransmitter levels were apparent in offspring of male rats mated at 4 weeks after exposure to ethylene dibromide: the interval between exposure and mating indicated that the effect on developing sperm occurred during premeiotic stages of spermatogenesis (34,59). Similar effects on brain neuro-

transmitters in offspring, in the absence of behavioral alterations, occurred after inhalation exposure to 2-methoxyethanol (81). Offspring of triethylene amine (TEM) male mice showed a neurologic impairment associated with an abnormal swimming pattern; cytogenetic study established concordance between the neurologic deficit and a TEM-induced chromosomal translocation (71).

An epidemiologic report of the deleterious effect of paternal exposure to inhalation anesthetics on birth outcome (27) prompted experimental investigation of nitrous oxide (N_2O). In mice, a single paternal inhalation exposure to nitrous oxide (80% N_2O/20% O_2/4 h) resulted in decreased birth weight of male pups. Other reproductive indices were unaffected. Delayed onset of developmental landmarks (pinna unfolding, eye opening) and an attenuated hypothermic response to morphine challenge were apparent in offspring derived from paternal treatment groups (39). No other alterations in pre- or post-weaning behaviors were detected.

Epidemiologic Studies

Anesthetics. The report of a positive association between paternal occupational exposure to inhalation anesthetics and an increase in spontaneous abortions was one of the first studies which systematically addressed male-mediated effects on reproductive outcome (27). Anesthetics in common use at the time were N_2O, halothane and enfluorane; individual agents were not identified (76). Subsequent studies supported this early finding and N_2O, the most commonly used of the inhalation anesthetics, was implicated (28,29). Other investigators were unable to replicate these findings (116). Methodologic limitations of these studies included variable estimates of the magnitude of exposures, failure to identify specific agent(s), recall and response biases (116).

A recent large retrospective study, with improved estimates of exposure and better control of confounding variables, reported an increased risk for both spontaneous abortions and congenital malformations in offspring after chronic paternal or maternal exposure to N_2O (55). In this survey of operating room personnel in several Canadian hospitals, both the level and duration of exposure were much higher than in several prior investigations where no significant effects were detected (76). The results suggested that individuals are at greater risk in settings with inadequate scavenging of waste anesthetic gases (99).

Dental operatories are usually less adequately ventilated than hospital operating rooms (27) and may explain the earlier positive findings in dentists (28,29). N_2O is a known mutagen in some test systems (76) and may increase cancer risk in exposed populations (27). It has also been suggested that some of the adverse effects of N_2O may be due to the increased concentration of NO and NO_2 in operating rooms (52); these chemicals may have teratogenic potential (76).

A possible confounder in studies with dentists was the absence of any control for the possible toxicity from mercury amalgam. These fillings release mercury vapor into the mouth and are the principal source of this exposure in the general population (78). Mercury vapor, released from amalgam fillings in pregnant sheep, has been detected in the fetus (124). In addition, paternal exposure to mercury vapor has been strongly implicated in spontaneous abortions (30,99).

Carcinogens. The relationship between fathers' employment and childhood cancer was first suggested in 1974 (32). Shortly thereafter, a positive association between fathers' exposure to solvents, or to employment in the aircraft industry, and brain tumors in their children was identified (89). Subsequent epidemiologic studies have replicated a significant link between childhood cancers and occupational exposure (35,85, 89,110). Chlorinated hydrocarbon solvents, paints, petroleum products, pesticides and metals have been most strongly implicated; specific etiologic agents have not been identified (35,84,97).

The list of occupations at risk is lengthy and includes: workers in petroleum and chemical industry, hydrocarbon-associated occupations, machinists and factory workers, workers with paints and pigments (84,86,97). There are frequently multiple exposures within and across chemical categories and identification of the causative agent(s) by job classification is often impossible (84).

Two extensive reviews, which examine the putative link between male exposure and childhood malignancies, contain critical analyses of individual study designs for twenty four (97) and thirty two (84) studies. Both reviews concur on the consistent association of the above exposures (based upon occupational title) and outcome. They conclude that limitations of the available data do not permit identification of specific etiologic agent(s), and stress the need for improved methodology in order to furtheridentify relevant exposure risks.

Paternal smoking (recreational/occupational) is another chemical exposure which has been associated with childhood malignancies. Congenital malformations and mental retardation have also been reported in offspring (65,94,97).

Ionizing radiation. Children of fathers exposed to low-level ionizing radiation at the Sellafield, England nuclear plant showed a 6-8 fold increase in leukemia (50). This study conflicted with an earlier extensive report on atomic bomb survivors which concluded that the risk of adverse reproductive outcome was not significantly higher than for nonirradiated parents (102). An earlier report of a significant association between paternal preconception exposure and congenital malformations at the Hanford, Washington site was interpreted as "false positives"; this conclusion was based primarily upon the discordance of these findings with the genetic studies on Hiroshima, Nagasaki survivors (105). In light of the Sellafield report, authors of the Hanford study are reconsidering their conclusions and urge additional study of populations occupationally exposed to ionizing radiation (104). The Sellafield finding remains controversial (88,104,105).

Implications of Studies

Both epidemiologic and experimental evidence suggest that paternal exposure to a broad range of agents can adversely impact reproductive outcome. Effects range from infertility, malignancies, neurobehavioral and neuroendocrine alterations in offspring to morphologic and behavioral abnormalities in subsequent generations. Extensive studies with CP illustrate the potential impact of paternal exposures on various stages of sperm development. Chronic low dose CP resulted in: a progressive increase in postimplantation loss, cross-generational learning deficits (2–4 wk CP = post-meiotic maturation during spermiogenesis); increased preimplantation loss (4-6 wk CP = spermatocytes, early spermatids); increased malformed and growth retarded fetuses (7–9 wk CP = spermatogonia). The broad spectrum of alterations produced by stage-specific effects of CP (detailed earlier) was unaccompanied by detectable alterations in paternal reproductive function or fertility.

In contrast, profound effects on the HPG axis of both male

parent and offspring were apparent after paternal exposure of spermatids or epididymal spermatozoa (1 wk, morphine) or throughout spermatogenesis (9 wk, ethanol). With both drugs, significant abnormalities in reproductive endocrine function of offspring were accompanied by subtle effects on growth and neurobehavioral development. These examples underline the importance of multisystem assessments in investigations of possible male-mediated influences on reproductive outcome.

The available data on paternal effects suggest that the following groups of chemicals are prime candidates for study: highly reactive chemicals, chemicals that are cytotoxic and affect spermatogenesis, agents identified as potential mutagens or carcinogens, chemicals which alter sperm parameters (morphology, motility), chemicals which affect neuroendocrine regulation, with a particular focus on the HPG axis. These categories of agents comprise several of the alerts raised in an extensive review of chemicals affecting male reproductive potential (101). The authors suggest that although the mechanism of action of many of these agents (e.g. potential teratogens, carcinogens, mutagens) in affecting male human reproductive potential is obvious, the reproductive hazard of greatest concern is probably the chemical that does not share many of these characteristics, ". . . is not generally cytotoxic, acts by disrupting biological processes unique to the reproductive process, and is subtle in onset." Several agents with male-mediated effects fit this description.

SUMMARY AND CONCLUSIONS

Both experimental and epidemiologic investigations document the adverse consequences of paternal exposure on reproductive outcome. Abnormalities in progeny outcome that have been reported in animal studies, with the exception of low birth weight, are usually not accompanied by alterations in fertility or other commonly used indices of reproductive success. Male-mediated effects have been recorded after exposure to: therapeutic and recreational drugs, chemicals in the workplace and environment, ionizing radiation, endocrine interventions.

Paternal exposures can induce a broad spectrum of deleterious effects on the normal course of development. The impact on progeny outcome includes: increase in pre- and postimplantation loss, congenital malformations, increase in spontaneous abortions, fetal and neonatal growth retardation, increased incidence of childhood cancers, neurobehavioral and neurochemical deficits, abnormalities in reproductive endocrine function. Paternal exposures may also induce longterm functional alterations that are expressed as subtle developmental or neurobehavioral abnormalities which may persist in subsequent generations. The comparative role of genetic, epigenetic and nongenetic mechanisms in the etiology of paternally-mediated effects is largely unknown.

There is currently a rapidly expanding body of epidemiologic information on the association between an array of male exposures and reproductive outcome. This active study by epidemiologists sharply contrasts with experimental science where relatively few investigators have examined male-mediated effects in animal models. The obvious advantage of this approach is the ability to control critical variables including exposure and relevant maternal factors. Animal models offer the opportunity to explore hypotheses which elucidate potential mechanisms for paternal effects. It is an intriguing challenge.

There is no a priori reason to assume that male-mediated effects are limited to the agents studied to date. The broad spectrum of changes reported to follow paternal exposures to several classes of toxicants warrants a more concerted effort and multidisciplinary exploration of the role of male-mediated exposures on developmental outcome.

REFERENCES

1. Abel, E. L. Paternal and maternal alcohol consumption: Effects on offspring in two strains of rats. Alcohol Clin.. Exp. Res. 132:533–541; 1989.
2. Abel, E. L. Rat offspring sired by males treated with alcohol. Alcohol 10:237–242; 1993.
3. Abel E. L.; Lee J. A. Paternal alcohol exposure affects offspring behavior but not body or organ weights in mice. Alcohol Clin. Exp. Res. 12:349–355; 1988.
4. Abel E. L.; Tan, S. E. Effects of paternal alcohol consumption on pregnancy outcome in rats. Neurotoxicol. Teratol.10:187–192; 1988.
5. Adams, P. M.; Fabricant, J. D.; Legator, M. S. Cyclophosphamide-induced spermatogenic effects detected in the F1 generation by behavioral testing. Science 211:80–82;1981.
6. Anderson, D. Male-mediated F1 abnormalities. Mutat. Res.229:103–246; 1990.
7. Anderson, R. A.; Willis, B. R.; Oswald, C.; Zaneveld, L. J. D. Ethanol-induced male infertility:impairment of spermatozoa. J. Pharmacol. Exp. Ther. 225:479–486; 1983.
8. Anderson, R. A.; Furby, J. E.; Oswald, C.; Zaneveld, L. J. D. Teratological evaluation of mouse fetuses after paternal alcohol. Neurobehav. Toxicol. Teratol. 3:117–20; 1981.
9. Aschengrau, A.; Monson, R.R. Paternal military service in Vietnam and the risk of late adverse pregnancy outcomes. Am. J. Pub. Health 80:1218–1224;1990.
10. Auroux, M.; Dulioust, E.; Selva, J.; Rince, P. Cyclophosphamide in the F0 male rat: Physical and behavioral changes in three successive adult generations. Mutat. Res. 229:189–200; 1990.
11. Bakke, J. L.; Lawrence, N. L.; Robinson, S. Observations on the untreated progeny of hypothyroid male rats. Metabolism 25:437–44; 1976.
12. Barlow, S.; Sullivan, F. Reproductive hazards of industrial chemicals. New York: Marcel Decker; 1985.
13. Begleiter, H.; Porjesz, B.; Bihari, B.; Kissin, B. Event-related brain potentials in boys at risk for alcoholism. Science 225: 1493–96; 1984.
14. Bellve, A. R.; Chandrika, R.; Martinova, Y.; Barth, A. H. The perinuclear matrix as a structural element of the mouse sperm nucleus. Biol. Reprod. 47:451–465; 1992.
15. Benziger, D. P.; Edelson, J. Absorption from the vagina. Drug Metab. Rev. 14:137–168;1988.
16. Berk, R. S.; Montgomery, I. N; Hazlett, L. D.; Abel, E. L. Paternal alcohol consumption: Effects on ocular response and serum antibody response to Pseudomonas aeruginosa infection in offspring. Alcohol Clin.. Exp. Res. 13:795–798; 1989.
17. Bertin, J. E.; Werby, E. A. Legal and policy issues. In: Paul, M.; ed. Occupational and environmental reproductive hazards: A guide for Clinicians. Baltimore: Williams and Wilkins; 1993: 150–162.
18. Bleich, D.; Polak, M.; Eisenbarth, G. S.; Jackson, R. A. Decreased risk of type I diabetes in offspring of mothers who acquire diabetes during adrenarchy. Diabetes 42:1433–1439; 1993.
19. Brady, K.; Herrera, Y.; Zenick, H. Influence of parental lead exposure on subsequent learning ability of offspring. Pharmacol. Biochem. Behav. 3:561–565; 1975.
20. Buckley, J. Male-mediated developmental toxicity: Paternal ex-

posures and childhood cancers. In: Olshan, A. F.; Mattison, D. R., eds. Male-mediated developmental toxicity. New York: Plenum Press; 1994:169–175.
21. Campbell, J. H.; Zimmerman, E.G. Automodulation of genes: A proposed mechanism for persisting effects of drugs and hormones in mammals. Neurobehav. Toxicol. Teratol. 4:435–439; 1982.
22. Carlsen, E.; Giwercman, A.; Keiding, N.; Skakkebaek, N. E. Evidence for decreasing quality of semen during the past fifty years. BMJ 305:609–613; 1992.
23. Cicero, T. J.; Adams, M. L.; Giordano, A.; Miller, B. T.; O'Connor, L.; Nock, B Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of their offspring. J. Pharmacol. Exp. Ther. 256:1086–1093; 1991.
24. Cicero, T. J.; Adams,M. L.; O'Conner, L.; Nock, B.; Meyer, E. R.; Wozniak, D. Influence of chronic alcohol administration on representative indices of puberty and sexual maturation in male rats and the development of their progeny. J. Pharmacol. Exp. Ther. 255:707–715; 1990.
25. Cicero, T. J.; Nock, B.; O'Conner, L.; Adams, M.; Meyer, E. R. Adverse effects of opiate exposure on offspring development and sensitivity to morphine-induced analgesia. J. Pharmacol. Exp. Ther. 273:386–392; 1995.
26. Cicero, T. J.; Nock, B.; O'Conner, L.; Adams, M.; Sewing, B. N. Acute alcohol exposure markedly influences male fertility and fetal outcome in the male rat. Life Sci. 55:901–910; 1994.
27. Cohen, E. N.; Brown,B. W.; Bruce, D. L.; Cascorbi, H. F.; Corbett, T. W.; Jones, T. W. Occupational disease among operating room personnel. A national study. Anesthesiology 41:321–40; 1974.
28. Cohen, E. N.; Brown,B. W.; Bruce, D. L.; Cascorbi, H. F.; Corbett, T. W.; Jones, T. W. A survey of health hazards among dentists. J. Am. Dent. Assoc. 90:1291–1296; 1975.
29. Cohen, E. N.; Brown, B. W.; Wu, M. L.; Whitcher, C. E.; Brodsky, J. B.; Gift; H. C. Occupational disease in dentistry and exposure to anesthetic gases. J. Am. Dent. Assoc. 101:21–32; 1980.
30. Cordier, S.; Deplan, F.; Mandereau, L.; Hemon, D. Paternal exposure to mercury and spontaneous abortions. Br. J.. Ind. Med. 48:375–381; 1991.
31. Davis, D. L.; Friedler, G.; Mattison, D.; Morris, R. Male-mediated teratogenesis and other reproductive effects: Biologic and epidemiologic findings and a plea for clinical research. Reprod. Toxicol. 6:289–292; 1992.
32. Fabia, T.; Thuy, T. L. Occupation of father at time of birth of children dying of malignant diseases. Br. J. Prevent. Soc. Med. 28:98–100;1974.
33. Fabricant, J. D.; Legator, M. S.; Adams, P. M. Post-meiotic cell mediation of behavior in progeny of male rats treated with cyclophosphamide. Mutat. Res. 119:185–190; 1983.
34. Fanini, D.; Legator, M. S.; Adams, P. M. Effects of paternal ethylene dibromide exposure on F1 generation in the rat. Mutat. Res. 139:133–138; 1984.
35. Frangos, S. A.; Peters, J. M.; Chlorinated hydrocarbon solvents: Substituting our way toward human carcinogenicity. Am. J. Ind. Med. 24:355–364; 1993.
36. Friedler, G. Long-term effects of opiates. In: Dancis, J.; Huang, J. C. eds. Perinatal pharmacology: Problems and priorities. New York: Raven Press; 1974:207–16.
37. Friedler, G. Morphine administration to male mice: Effects on subsequent progeny. Fed. Proc. 33:515; 1974.
38. Friedler, G. Pregestational administration of morphine sulfate to female mice: Long-term effects on the development of progeny. J. Pharmacol. Exp. Ther. 205:33–39; 1978.
39. Friedler, G. Effects of limited paternal exposure to xenobiotic agents on the development of progeny. Neurobehav. Toxicol. Teratol. 7:739–43; 1985.
40. Friedler, G. Effects on future generations of paternal exposure to alcohol and other drugs. Alc. Health Res. World 12:126–129; 1988.
41. Friedler, G. Developmental toxicology: Male-mediated effects. In: Paul, M., ed. Occupational and environmental reproductive hazards: A guide for clinicians. Baltimore: Williams and Wilkins; 1993:52–59.
42. Friedler, G. Paternal effects of xenobiotics on pregnancy outcome: An overview. Neurotoxicol. Teratol 17:374; 1995.
43. Friedler, G. Cicero, T. J. Paternal pregestational opiate exposure in male mice: Neuroendocrine deficits in their offspring. Res. Commun. Substance Abuse 8:109–116; 1987.
44. Friedler, G. Cochin, J. Growth retardation in offspring of female rats treated with morphine prior to mating. Science 175:654–56; 1972.
45. Friedler, G. Wheeling, H. S. Behavioral effects in offspring of male mice injected with opioids prior to mating. Pharmacol. Biochem. Behav. 11:23–28;1979.
46. Friedler, G.; Wurster–Hill, D. Influence of morphine administration to male mice on their progeny. Proceed.Committee Problems Drug Dependence. 869–875; 1974.
47. Fujii, T.; Sakamoto, M. Parathyroid transplantation in male rats affects the functional differentiation of the testis in their first generation offspring. Biomed. Res. 1:556–59; 1980.
48. Furuhjelm, M.; Jonson, B.; Lagergren, G. G. The quality of human semen in spontaneous abortion. Int. J. Fertil. 7:17–21; 1962.
49. Gavaler, J. S.; VanThiel, D. H. Reproductive consequences of alcohol abuse:males and females compared and contrasted. Mutation Res. 186:269–77; 1987.
50. Gardner, M. J.; Snee, M. P.; Hall, A. J.; Powell, C. A.; Downes, S.; Terrell, J. D. Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. BMJ300:423–429; 1990.
51. Gerber, N.; Lynn, R. K. Excretion of methadone in semen from methadone addicts: Comparison with blood levels. Life Sci. 19:787–792; 1976.
52. Goldstein, B. D.; Paz, J.; Guiffrid, J. G.; Palmes, E. D.; Ferrand, E. F. Atmospheric derivatives of anesthetic gases as a possible hazard in operating room personnel. Lancet 2:235–237; 1976.
53. Gottesfeld, Z.; Abel, E. L. Maternal and paternal alcohol use: Effects on the immune system of the offspring. Life Sci. 48: 1–8; 1991.
54. Grossblatt, N.; Paulson, L. R. eds. Biologic markers in reproductive toxicity. Washington, D.C.:National Academy Press; 1989.
55. Guirguis, S. S.; Pelmear, P. L.; Roy, M. L.; Wong, L. Health effects associated with exposure to anaesthetic gases in Ontario hospital personnel. Brit. Jour. Ind. Med. 47:490–497; 1990.
56. Hales, B. F.; Crossman, K.; Robaire, B. Increased postimplantation loss and malformations among the F 2 progeny of male rats chronically treated with cyclophosphamide. Teratol. 45:671–678; 1992.
57. Hales, B. F.; Robaire, B. Cyclophosphamide (CPA) as a model drug for studies of male-mediated effects on development. Neurotoxicol Teratol. 17:375; 1995
58. Hales, B. F.; Smith, S.; Robaire, B. Cyclophosphamide in the seminal fluid of treated males: Transmission to females by mating and effect on progeny outcome. Toxicol. Appl. Pharmacol. 84:423–430; 1986.
59. Hsu, L. L.; Adams P. M.; Fanini, D.; Legator, M. S. Ethylene dibromide: Effects of paternal exposure on the neurotransmitter enzymes in the developing brain of F1 progeny. Mutat. Res. 147:197–203; 1985.
60. Hsu, L. L.; Adams, P. M.; Legator, M. S. Cyclophosphamide: Effects of paternal exposure on the brain chemistry of the F1 progeny. J Toxicol. Environ. Health 21:471–481; 1987.
61. International Union, UAW v Johnson Controls, U.S. 111 S. Ct. 1196; 1991.
62. Jenkinson, P. C.; Anderson, D.; Gangolli, S. D. Malformed foetuses and karyotype abnormalities in the offspring of cyclophosphamide and allyl alcohol-treated male rats. Mutat. Res. 229:173–184; 1990.
63. Joffe, J. M.; Peruzovic, M.; Milkovic K. Progeny of male rats treated with methadone: Physiological and behavioral effects. Mutat Res. 229:201–11; 1990.
64. Joffe, J. M.; Soyka, L. F. Paternal drug exposure: Effects on reproduction and progeny. Semin. Perinat. 6:116–124; 1981.

65. John, E. M.; Savitz, D.; Sandler, D. P. Prenatal exposure to parents' smoking and childhood cancer. Am. J. Epid.133:123–132; 1991.
66. Kelly, S.; Robaire, M.; Hales, B. Paternal cyclophosphamide treatment causes postimplantation loss via inner cell mass-specific cell death. Teratol. 45:313–318; 1992.
67. Klassen, R. W; Persaud, T. V. N. Experimental studies on the influence of male alcoholism on pregnancy and progeny. Exp. Pathol. 12:38–45; 1976.
68. Lamb, J. C. Fundamentals of male reproductive toxicity testing.In: Lamb, J.C.; Foster, P.M.D.; eds. Physiology and toxicology of male reproduction. New York: Academic Press, Inc., 1988:137–153.
69. Lancranjan, I.; Popescu, H. I.; Gavanescu, O.; Klepsch, I; Serbanescu, M. Reproductive ability of workmen occupationally exposed to lead. Arch. Environ. Health 30:396–401; 1975.
70. Little, R. E.; Sing, C. F. Father's drinking and infant birth weight: Report of an association. Teratology.36:59–65; 1985.
71. Lowery, M. C.; Au, W. W.; Adams, P. M.; Whorton, E. B.; Legator, M.S. Male-mediated behavioral abnormalities. Mutat. Res. 229:213–229; 1990.
72. Lutwak–Mann, C. Observations on progeny of thalidomide-treated male rabbits. BMJ 1:1090–1091; 1964.
73. Lyon, M. F. Sensitivity of various germ-cell stages to environmental mutagens and carcinogens. Mutat. Res. 87:23–45; 1981.
74. Mankes, R. F.; LeFevre, R.; Benitz, K.-F.; Rosenblum, I; Bates, H.; Walker, A.I.T.; Abraham, R. Paternal effects of ethanol in the Long–Evans rat. J. Toxicol. Environ. Health 10:871–78; 1982.
75. Mann, T.; Lutwak–Mann, C. Passage of chemicals into human and animal semen: Mechanisms and significance. In: Crit. Rev. Toxicol. CRC Press, Inc. Fla. 2:1–14; 1982.
76. McDiarmid, M. A. Occupational exposure to pharmaceuticals: Antineoplastics, anesthetic agents, sex steroid hormones. In: Paul, M., ed. Occupational and environmental reproductive hazards: A guide for clinicians. Baltimore: Williams and Wilkins; 1993: 280–295.
77. McDiarmid, M. A.; Lees, P. S. J.; Agnew, J.; Midzenski, M.; Duffy, R. Reproductive hazards of fire fighting. 11.Chemical hazards. Am. J. Indust. Med. 19:447–472; 1991
78. Miller; R. K., Bellinger; D. Metals. In: Paul, M., ed. Occupational and environmental reproductive hazards: A guide for Clinicians. Baltimore: Williams and Wilkins; 1993:233–252.
79. Molenaar, P. C.; Boomsma, D. D.; Dolan, C. V. A third source of developmental differences. Behav. Genet. 23:519–524; 1993.
80. Nelson, B. K.; Brightwell, W. S.; Mackensie–Taylor, D. R.; Burg, D. R.; Massari, V. J. Neurochemical, but not behavioral, deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol. Neurotoxicol Teratol.10:15–22;1988.
81. Nelson, B. K.; Brightwell, W. S.; Burg, J. R.; Massari, V. J. Behavioral and neurochemical alterations in the offspring of rats after maternal or paternal inhalation exposure to the industrial solvent 2-methoxyethanol. Pharmacol. Biochem. Behav. 20:269–279; 1984.
82. Nomura, T. Role of radiation-induced mutations in multigeneration carcinogenesis. In: Perinatal and Multigeneration Carcinogenesis, IARC Sci Publ. Lyon; International Agency for Research on Cancer. 1988:375-387.
83. Norris, M. L.; Barton, S. C.; Surani, M. A. H. The differential role of parental genomes in mammalian development. Oxford Rev. Exp. Biol. 12:225–244; 1990.
84. O'Leary, L. M.; Hicks, A. M.; Peters, J. M.; London, S. Parental occupational exposures and risk of childhood cancer: A review. Am. J. Ind. Med. 20:17–35; 1991.
85. Olshan, A. F.; Breslow, N. E.; Daling, J. R.; Falletta, J. M.; Grufferman, S. L.; Robison, L. L. Wilms' tumor and paternal occupation. Cancer Res. 50:3212–32; 1990.
86. Olshan, A. F.; Faustman, E. M.; Male-mediated developmental toxicity. Annu. Rev. Publ. Health 14:159–181; 1993.
87. Overstreet, J. W.; Katz, D. F. Sperm transport and selection in the female genital tract. In: Development in mammals. Johnson, M. H. ed. Amsterdam, N.Y.: North Holland Pub Co. 2:31–65; 1977.
88. Parker, L.; Craft, A. W.; Smith, J.; Dickinson, H.;Wakeford, R.; Binks, K. Geographical distribution of preconceptional radiation doses to fathers employed at the Sellafield nuclear installation, West Cumbria. Br Med. J. 307:966–967; 1993.
89. Peters, J. M.; Preston–Martin, S.; Yu, M. C. Brain tumors in children and occupational exposure of parents. Science 213: 235–37; 1981.
90. Pfeiffer, W. D.; MacKinnon, J. R.; Seiser, R. L. Adverse effects of paternal alcohol consumption on offspring in the rat. Bull. Psychon. Soc. 10:246; 1977.
91. Randall, C. L.; Burling, T. A.; Lochry, E. A.; Sutker, P. B. The effect of paternal alcohol consumption on fetal development in mice. Drug Alc. Depend. 9:89–95; 1982.
92. Reik, W.; Surani, M. A. H. Genomic imprinting and embryonal tumours. Nature. 338:112–113; 1989.
93. Robaire, B; Trasler, J. M.; Hales, B. F. Consequences to the progeny of paternal drug exposure. In: Male fertility and its regulation. Lobl, J. T.; Hafez, E. S. E. eds. MTP Press, London. Reproductive Health Series 7:225–243; 1985
94. Roeleveld, N.; Vingerhoets, E.; Zielhuis, G. A.; Gabreels, F. Mental retardation associated with parental smoking and alcohol consumption before, during, and after pregnancy. Prevent. Med. 21:110–119; 1992.
95. Rom, W. N. Effects of lead on the female and reproduction: A review. J. Mt. Sinai Sch. Med. 43:542–552; 1976.
96. Rosett, H. L.; Weiner, L. Alcohol and the fetus: A clinical perspective. Oxford Univ. Press, New York; 1984.
97. Savitz, D. A.; Chen, J. Parental occupation and childhood cancer: Review of epidemiologic studies. Environ. Health Perspect. 88:325–337;1990.
98. Savitz, D. A.; Zhang, J.; Schwingl, P.; John, E. M. Association of paternal alcohol use with gestational age and birth weight. Teratol. 46:465–471; 1992.
99. Savitz, D. A.; Sonnenfeld, N. L.; Olshan, A. F. Review of epidemiologic studies of paternal occupational exposure and spontaneous abortion. Am. J. Ind. Med. 25:361–363; 1994.
100. Schrader, S. M.; Kesner, J. S. Male reproductive toxicology. In: Paul, M., ed. Occupational and environmental reproductive hazards: A guide for clinicians. Baltimore: Williams and Wilkins; 1993:3–17.
101. Schrag, S.; Dixon, R. L. Occupational exposures associated with male reproductive dysfunction. Ann. Rev. Pharmacol. Toxicol. 25:567–592; 1985.
102. Schull, W. J.; Otake, M.; Neel, J. V. Genetic effects of the atomic bombs: A reappraisal. Science 213:1220–1227; 1981.
103. Sever, L. E.; Congenital malformations related to occupational reproductive hazards. Occupat. Med.9:471–494; 1994.
104. Sever, L. E. Parental radiation exposure and children's health: Are there effects on the second generation? Occupat. Med. 6:613–627; 1991.
105. Sever, L. E.; Gilbert, E. S.; Hessol, N. A.;McIntyre, J. M. A case control study of congenital malformations and occupational exposure to low–level ionizing radiation. Am. J. Epid. 127:226–242; 1988.
106. Silbergeld, E. K.; Akkerman, M.; Fowler, B. A.; Albuquerque, E. X.; Alkondon, M. Lead: Male–mediated effects on reproduction and neurodevelopment. Toxicol. 11:81:1991.
107. Sins of the father. In: Science and Technology; The Economist (London); February 23; 1991; page 87.
108. Soyka, L. F.; Peterson, J. M.; Joffee, J. M. Lethal and sublethal effects on the progeny of male rats treated with methadone. Toxicol. Appl. Pharmacol. 45:797–807; 1978.
109. Spergel, G.; Levy, L. J.; Goldner, M. G. Glucose intolerance in the progeny of rats treated with a single subdiabetogenic dose of alloxan. Metabolism 20:401–13; 1971.
110. Spitz, M. R.; Johnson, C. C.; Neuroblastoma and paternal occupation. Am J Epid. 121:924–929; 1985.
111. Stellman, S. D.; Stellman, J. M.; Sommer, J. F. Health and reproductive outcome among American Legionnaires in relation to combat and herbicide exposure in Vietnam, Environ. Res. 47:150–174; 1988.
112. Stockard, C. R. The effect on the offspring of intoxicating the

male parent and the transmission of the defects to subsequent generations. Am. Nat. 47:641–682; 1913.
113. Stowe, H. D.; Goyer, R. A. The reproductive ability and progeny of F1 lead-toxic rats. Fert Steril. 22:755–760; 1971.
114. Surani, M. A. H.; Allen, N. D.; Barton, S. C. Developmental consequences of imprinting of parental chromosomes by DNA methylation. Phil. Trans. R. Soc. B. 326:313–327; 1990.
115. Symposium: The father and the fetus: Facts and fallacies. Annual meeting of American Association for the Advancement of Science. 41; Abstracts; 1991.
116. Tannenbaum, T. N.; Goldberg, R. J. Exposure to anesthetic gases and reproductive outcome: Aa review of the epidemiologic literature. J. Occupat. Med. 27:659–668; 1985.
117. Toner, J. P.; Adler, N. T. The pre–ejaculatory behavior of male and female rats affects the number of sperm in the vagina and uterus. Physiol. Behav. 36:363–67; 1986.
118. Torkelson, T. R.; Sadek, S. E.; Rowe, V. K. Toxicologic investigations of 1,2 dibromo-3-chloropropane. Toxicol. Appl. Pharmacol. 3:545–549; 1961.
119. Trasler, J. M.; Hales, B. F.; Robaire, B. Chronic low dose cyclophosphamide treatment of adult male rats: Effect on fertility. pregnancy outcome and progeny. Biol. Reprod. 34:275–83; 1986.
120. Trasler, J. M.; Hales, B. F.; Robaire, B. Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting fertility. Nature 316:144–146; 1985.
121. Trasler; J. M., Hales, B. F.; Robaire, B. A time-course study of chronic paternal cyclophosphamide treatment in rats: Effects on pregnancy outcome and the male reproductive and hematologic system. Biol. Reprod. 37:317– 326; 1987.
122. Tycko; B. Genomic imprinting: Mechanism and role in human pathology. Am. J. Pathol. 144:431–433; 1994.
123. VanThiel, D. H.; Gavaler, J. S. The adverse effects of ethanol upon hypothalamic-pituitary-gonadal function in males and females. Alc. Clin. Exp. Res. 6:179–85; 1982.
124. Vimy; M. Maternal–fetal distribution of mercury (203-Hg) released from dental amalgam fillings. Am. J. Physiol. 258:R939–945; 1990.
125. Volkers, N. Father's lifestyle can have fetal consequences. J. Natl. Cancer Inst. 83:1611–1612; 1991.
126. Warner, R. H.; Rosett, H. L. The effects of drinking on offspring. An historical survey of the American and British literature. J. Studies Alc. 36:1395–1420; 1975.
127. Whorton, D.; Krauss, K. M.; Marshall, S.; Milby, T. H. Infertility in male pesticide workers. Lancet 2:1259–61; 1977.
128. Wilcox, A. J.; Weinberg, C. R.; O'Conner, J. F.; Baird, D. D.; Schllatter, J. P.; Canfield, R. E.; Armstrong, E. G.; Nisula, B. C. Incidence of early pregnancy loss. N. Engl. J. Med. 319:189–194; 1988.
129. Wozniak, D. F.; Cicero, T. J.; Kettinger, L.; Meyer, E. R. Paternal alcohol consumption impairs learning performance in male offspring. Psychopharm. 105:289–302; 1991.
130. Wyrobek, A. J. Male biomarkers of abnormal reproductive outcome. Health Environ. Digest. 4:1–4; 1990.
131. Wyrobek, A. J. Methods and concepts in detecting abnormal reproductive outcomes of paternal origin. Reprod. Toxicol. 7 Suppl 1:3–16; 1993.
132. Wyrobek, A. J.; Gordon, L. A.; Burkhart, J. G. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. Mutation Res. 15:73–148; 1983.
133. Yazigi, R. A.; Odem, R. R.; Polakoski, K. L. Demonstration of specific binding of cocaine to human spermatozoa. JAMA 266:1956–1959; 1991.
134. Zenick, H.; Clegg. E. D.; Perrault, S. D.; Klinefelter, G. R.; Gray, L.E. Assessment of male reproductive toxicity: A risk assessment approach. In: Hayes, A. W.ed. Principles and methods of toxicology, 3rd ed. New York: Raven Press, Ltd; 1994:937–988.
135. Zhang, J.; Savitz. D. A.; Schwingl, P. A case control study of paternal smoking and birth defects. Internat. J. Epid. 21:273–278; 1992.