Seeds of concern

During the past few decades, worries about environmental threats to human health have centred on the possible induction of cancers. Now risks to the male germ line, both real and potential, are also causing disquiet.

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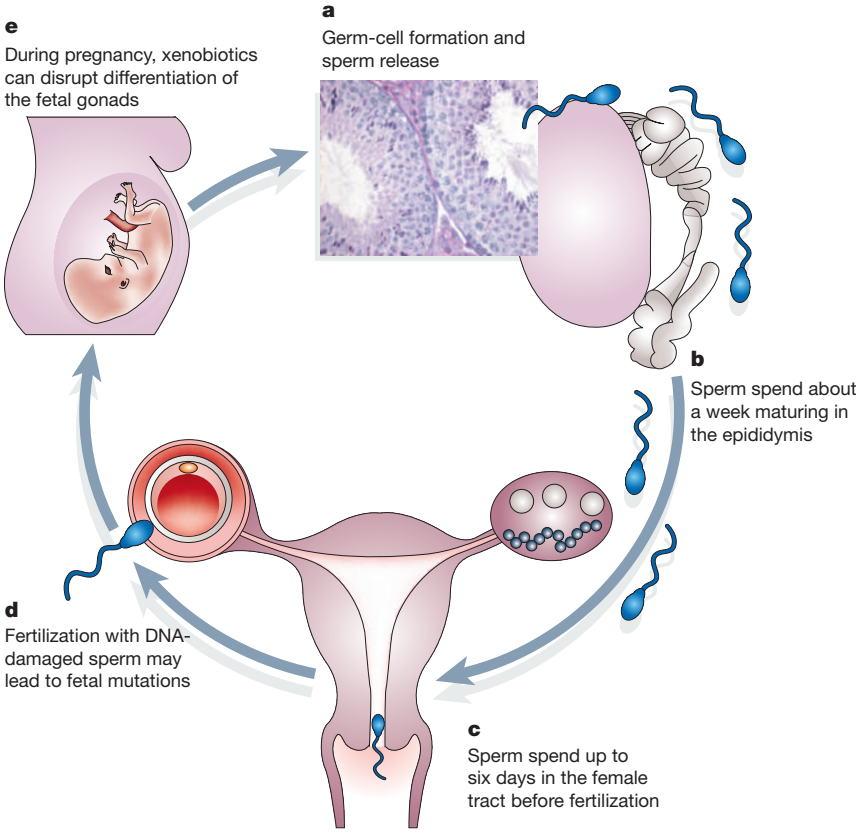

Figure 1 **Points in the life cycle of male germ cells when they are vulnerable to xenobiotics.** a, **During their formation in the germinal epithelium, germ cells lose their capacity to self-destruct in response to injury, or to repair damaged DNA. They also lose the protection afforded by Sertoli cells.** b, c, **Following their release as sperm, they become susceptible to damage and remain so during their perilous journey through the male epididymis and the female reproductive tract.** d, **DNA damage carried into the egg by a fertilizing sperm must be repaired; any deficiencies in this process will create mutations that may affect the wellbeing of the fetus and offspring.** e, **Later, during pregnancy, xenobiotics can disrupt the differentiation of both germ and non-germ (somatic) cells in the fetal gonads.**

What do male alligators in Florida and male industrial workers in California have in common? The answer is that, in the latter part of the twentieth century, both provided landmark case histories showing the severe effects that pesticides can have on fertility. Since then investigations of the adverse influence of 'xenobiotics' — molecules that are foreign to biological systems — on male reproduction have turned up more evidence, of various kinds, that all is not well in the man's world.

During the past 50 years, the rapid expansion of the chemicals industry in both the developed and developing worlds has resulted in the release of a plethora of xenobiotics into the environment[1,2]. These alien molecules have worked their way into our lives in a variety of forms, including pesticides, herbicides, cosmetics, preservatives, cleaning materials, municipal and private waste, pharmaceuticals and industrial by-products. Awareness of the biological risks of chemical toxicity has increased considerably in recent years, but some of these chemicals have long half-lives and have been detected in environmental samples 10–20 years after they were banned for sale or use.

Analysis of the biological fallout from environmental pollution has generally centred on the risks for induction of certain kinds of cancer. But it is becoming increasingly apparent that another major target of this chemical barrage is the reproductive system, particularly in the male[2]. This was first recognized more than 30 years ago, when male workers exposed to 1,2-dibromo-3-chloropropane, an agricultural control agent used to kill nematodes, exhibited severe disruption of sperm development and infertility[3]. Since then, numerous independent studies[4] have associated occupational exposure to pesticides, herbicides, industrial agents and heavy metals with poor semen quality and impaired fertility. Although male reproduction can be affected by a variety of mechanisms that affect hormone balance and other metabolic systems, the disruption of germ-cell differentiation and sperm quality seems to involve two fundamentally different routes of exposure (Fig. 1a–d, and Fig. 1e).

First, xenobiotics and other environmental factors such as radiation can act directly on male germ cells within the mature testis. The highly effective proofreading and repair of DNA in the stem cells that produce sperm means that the male germ line has one of the lowest spontaneous mutation rates in the body[5]. But as these cells go through meiosis,

the cell-division process that produces reproductive gametes, their capacity for DNA repair is reduced and their ability to respond to such damage by undergoing programmed cell death is progressively lost. Moreover, once they are released from the tissue that produces them, the germinal epithelium, male germ cells can no longer rely on the protection previously afforded by their nurse cells in the testes, the Sertoli cells.

Thus, as soon as sperm are released from the germinal epithelium, they are on their own. Bereft of the cytoplasm that houses protective enzymes such as catalase or superoxide dismutase in somatic (non-germ) cells, sperm are committed to a sojourn of about a week in the epididymis, the duct system in which they are remodelled in preparation for ejaculation. Subsequently, they must spend up to six days swimming around the female reproductive tract searching for an egg. During this long and perilous marathon, sperm are particularly vulnerable to DNA damage by a variety of environmental factors[6]. All in all, the sperm is much more susceptible to damage than the egg because of its prolonged solitary existence and relative lack of protective, repair and self-destruct mechanisms.

The second route by which xenobiotics exert an influence on male reproduction is less direct, through exposure of women during pregnancy and subsequent disruption of reproductive tract development in male embryos (Fig. 1e). Such action is thought to affect both the germ cells and the somatic tissues of the male tract, and the consequences include a complex array of pathological changes collectively known as the testicular dysgenesis syndrome, or TDS, in the offspring. The features of TDS include poor semen quality, hypospadias (defective development of the urinary tract), testicular cancer and cryptorchidism (the failure of one or both testes to descend).

The various symptoms of TDS have common risk factors, such as low birth weight, retained placenta and previous pregnancy history, supporting the idea that they have a common cause involving the perturbation of normal fetal development[7]. The importance of abnormal gonad development in testicular cancer is also supported by analysis of the seemingly unaffected testis of men with this condition. Although the gross testis anatomy is apparently normal, a closer inspection reveals disordered development of all of the major cell types within the testes, including Sertoli cells, the testosterone-secreting Leydig cells and the germ cells themselves. Experimental evidence for the xenobiotic induction of TDS comes from administration of a testicular toxicant (dibutyl phthalate) to pregnant rats, which produces TDS-like tissue abnormalities in the testes of male offspring[8]. If xenobiotics are involved in causing TDS, they must act relatively early in fetal development. Testicular germ-cell tumours (the most common cancer in young men aged 15 to 35 in Western countries) develop from a precursor condition, known as carcinoma *in situ*, that derives from the earliest stages of germ-cell development in the fetal testis.

Box 1 Uncertain science and male fertility

Analysing environmental effects on male reproduction is a highly complex business. For example, measuring male fertility is a tough proposition in itself (see page 38). The correlations between fertility and measures of semen quality such as sperm count are weak, and are complicated by factors that include the relative fertility of the female partner and the inherent variability of human semen (which is influenced by abstinence, stress, disease and even the time of year). Moreover, there are selection biases in obtaining semen samples.

Epidemiological studies are dogged by various problems. We do not understand how chemicals are metabolized within the male reproductive tract, and measuring the type and duration of exposure to xenobiotic agents is fraught with error (self-reporting surveys are a very blunt instrument in this context). There are also great difficulties in establishing relationships between pathological conditions seen in children or young adults and parental exposure to potential toxicants. Long periods of time may have elapsed between parental exposure and the appearance of a condition such as offspring infertility, and distinguishing between maternal and paternal exposures may be impossible where environmental factors are concerned.

Finally, estimating DNA damage in male germ cells is an inexact science. Commonly used techniques (such as the sperm chromatin structure assay and the so-called Comet test) give a general indication of damage, but are only semi-quantitative and provide no information on the exact nature or cause of the damage observed.

All in all, the evidence for and against toxic environmental substances as key factors in male infertility and testicular cancer is nowhere near as clear-cut as we would like. Nonetheless, such evidence as we can trust indicates that here is a serious public health issue requiring closer scrutiny. ■

Trends in male reproduction

Whatever the route of exposure, environmental factors can clearly affect the development and function of the male reproductive tract. This was first recognized in animal species, a famous example being the disordered reproductive development of male alligators in Lake Apopka, central Florida, following an insecticide spill in the early 1980s[9,10]. The alligators had abnormal differentiation of male reproductive organs and lowered levels of testosterone, the steroid hormone that is central to male reproductive biology. Similar findings have been reported for fish, initially in England and more recently in the rivers of other Northern European countries. A mixture of compounds originating from the environmental degradation of certain industrial and household detergents, as well as the urinary excretion of metabolites originating from the female oral contraceptive, seem to be responsible for these emasculating effects on aquatic species[11].

In addition, there have been several claims of a deterioration of semen quality in the human male. This issue took wing in 1992, with the publication[12] of a meta-analysis of the biomedical literature that reported on sperm counts. Within this data set, the authors detected an approximate halving in the concentration of sperm in human ejaculates between 1940 and 1990. Analysis of larger data sets supported these general trends and suggested rates of decline in Europe and Australia (3% per yr) that were higher than those observed in either the United States (1.5% per yr) or non-Western countries, where no decline was seen —

albeit on the basis of limited data[13]. Additional studies from Edinburgh[14] indicated that the secular trend in semen quality is a 'birth cohort' effect — that is, the age of the subject when semen analysis is performed does not matter; rather it is the date of birth that is important. According to these data, men born before 1959 have significantly higher motile sperm counts than men born after 1970. So it is not just wisdom and experience that distinguishes the lecturer from his students.

The situation seems to be particularly severe in Denmark, where low fertility rates have been linked with poor semen quality: 25% of 19-year-old Danish men currently exhibit sperm counts in the subfertile range (less than 20 million per ml)[15]. But not everyone is convinced by these data on falling sperm counts, and several studies have failed to confirm these trends[16]. Certainly, the difficulties in securing reliable data in this area are considerable (Box 1).

If semen analysis were the only method we had of measuring male reproductive potential, the possibility of falling sperm counts would be more a cause for curiosity than concern. However, global trends in testicular cancer bear out the view that the male reproductive system is under attack: the incidence of such cancer has increased in Caucasian men in all developed countries; the current lifetime risk is 0.3–0.8%[17]. This contrasts with cancers of the female reproductive tract, with risks that remained largely unchanged or actually declined during the same period (Fig. 2). The increase in testicular cancer cannot be accounted for by the fact that we are living longer or have better methods of detection. Testicular cancer is a disease of young men and is easily detected, but its rising incidence is certainly a cause for concern, even if it is still a relatively rare condition.

Indications of a possible link between low sperm counts and testicular cancer come from the differences in male reproductive pathology between men from Denmark and those from Finland. Not only do Danish men have the lowest sperm counts in Europe, but they also exhibit high incidences of testicular cancer and malformations of the genital tract such as hypospadias. By contrast, the incidence of testicular cancer in Finland is nearly three times lower, genital malformations are rare and mean sperm counts are among the highest in the world[18]. We have no idea why the reproductive fate of men in these two countries is so different. One recent publication, however, has highlighted the powerful correlation between the incidence of maternal smoking during pregnancy and the relative incidence of testicular cancer across four Nordic countries (Sweden, Denmark, Norway and Finland)[19].

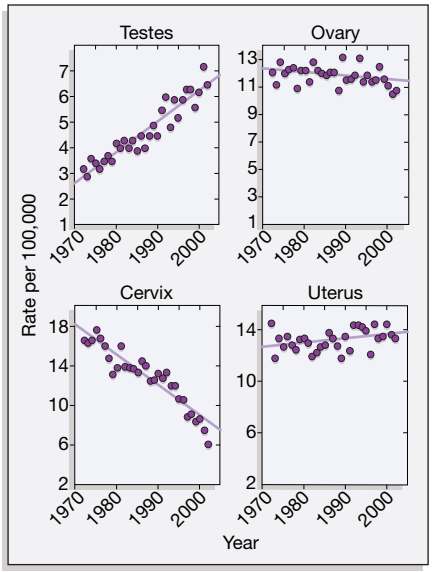

Figure 2 **Trends in reproductive-tract cancers. As exemplified by these data from the New South Wales Central Cancer Registry[31], during the past 30 years the incidence of testicular cancer has continued to rise whereas incidence of cancers of the female reproductive tract has remained constant (ovary, uterus) or has decreased (cervix). Similar trends have been seen in all developed countries where data are available.**

> "Environmental factors, whatever the route of exposure, can clearly affect the development and function of the male reproductive tract."

Another facet of investigations into male reproduction is the possibility that damage to a father's sperm — either genetic, affecting the DNA sequence itself, or otherwise perturbing DNA function through so-called epigenetic mechanisms — can be responsible for diseases in his offspring, as well as being itself a cause of infertility or early loss of pregnancy. A frequently quoted example is the link between heavy paternal smoking and increased rates of childhood cancer[6], thought to be mediated by oxidative damage to the DNA in the father's sperm. Oxidative injury results from an overexposure to 'reactive oxygen species' — excited oxygen-containing molecules, generated as a by-product of cell metabolism and the intracellular processing of xenobiotics, that can attack and damage DNA.

With the traditional emphasis on the impact of cigarette smoke on somatic tissues (for instance in heart disease and lung cancer), we tend to forget that smoking can also induce oxidative damage to the DNA in sperm, and thereby affect the health and wellbeing of the ensuing children. Moreover, because such damage is in the germ line, it can be transmitted to future generations. We are probably all carrying around in our genes the consequences of our great grandfather's pipe-smoking habit.

DNA damage in human sperm has also been associated with a reduction in overall pregnancy rates following natural conception. Moreover, such damage has been linked with impaired fertilization, disrupted development of the early embryo, and loss of pregnancy in assisted reproduction programmes[6].

Xenobiotics and reproduction

What can be causing all of these male reproductive problems? Long-term impacts on human fertility are associated with our evolutionary heritage, in that poor sperm morphology is a burden we share with some of our close primate relatives, such as the gorilla. Also, there is a lack of selection for 'high-fertility' genes in countries that have gone through the demographic transition — that is, the transition from high birth and death rates, to low birth and death rates. The increasing availability of assisted conception clinics will further dilute selection for high-fertility genes and, in this context, the slow drift towards increased infertility in developed countries is inexorable.

But these gradual trends are being accelerated by environmental factors that seem to be having a particular impact on the male germ line: Fig. 3 provides examples of some of the main groups of xenobiotics. One of the most intensively researched groups is the environmental oestrogens: these phenolic compounds, found in plants but also in man-made products, competitively interact with the body's receptors for the natural oestrogen, a steroid hormone. Oestrogen is generally thought of as a female hormone. But normal male development and function also depend on it. One explanation for reduced sperm counts involves the capacity of environmental oestrogens to suppress production of a hormone (follicle-stimulating hormone) by the fetal pituitary gland. As this hormone stimulates the growth of Sertoli cells in the developing testes, the number of these cells is consequently decreased[17]. Sertoli cells rarely, if ever, replicate, and each cell can only support the

Compound	Source	Biological action
1,2-dibromo-3-chloropropane	Used as a pesticide, nematocide and soil fumigant. Exposure by ingestion of contaminated water or food, or breathing air near contaminated sites. Occupational exposure in the chemicals industry.	Disrupts sperm production and causes male sterility. Some evidence that paternal exposure increases the incidence of abortion but results are inconsistent across studies. In animal studies, mating to exposed males increases subsequent pregnancy loss.
Nonylphenol	Industrial surfactant and antioxidant released by degradation of ethoxylated nonylphenol derivatives. Common contaminant of aquatic environments.	Environmental oestrogen responsible for feminization of aquatic species, including fish and oysters. Both fetal and adult exposure causes testicular damage in rats. Can induce DNA damage in human sperm.
Genistein	Plant oestrogen, present in soybean products. High daily exposure (1–30 mg per day) particularly in Asian populations.	No convincing evidence that developmental exposure impairs male reproduction. Acute exposure does not affect human semen quality. But this and related plant oestrogens do induce DNA damage in human sperm.
Polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene	Constituents of cigarette smoke.	Smoking induces modest reductions in semen quality. Heavy smoking results in oxidative base damage in sperm and can affect offspring (for instance by inducing childhood cancer). Animal studies indicate effects on sperm maturation and ability of sperm to establish a viable pregnancy.
Acrylamide	Intermediate in the synthesis of polyacrylamide, with widespread use in water treatment, paper making, ore processing and the manufacture of diverse products. Contaminant of fried food.	Disrupts male reproduction by many mechanisms, including impairment of sperm development and motility. Male exposure leads to pregnancy loss in mated females, and increased incidence of developmental defects and hereditable chromosome disorders.
Dioxins, for example 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	By-products of combustion and various industrial processes. Dietary exposure particularly through foods containing animal fats. Minor exposure from absorption or ingestion of contaminated dust.	Hormone disruptor that impairs sperm development and sexual maturation. Increased risk of testicular cancer and birth defects, but data are inconsistent. Induction of oxidative stress in sperm.

Figure 3 **Xenobiotics under suspicion. Examples of chemicals implicated in adversely affecting the male reproductive system[1].**

differentiation of a finite number of sperm. So a reduction in the size of this cell population should have an irreversible impact on male germ-cell development.

An alternative possibility is that environmental oestrogens impair Leydig cell development or function, thereby affecting the generation of testosterone by the testes. These environmental oestrogens might also inhibit the action of the molecular receptors for testosterone and other male hormones, or suppress the expression of growth factors (such as insulin-like growth factor-3) within the fetal testes[17]. The long list of weakly oestrogenic factors that can act as hormone disruptors includes nonylphenol, plant-derived oestrogens such as genistein, and dioxins (Fig. 3), as well as DDT, furans and the insecticides dieldrin and aldrin. Some of the more toxic compounds (DDT, dieldrin and aldrin) have been banned from most industrialized countries since the 1970s. But the bans are not universal, and these compounds continue to accumulate in the global environment through food imports and contaminated air or water.

The hormone-disruptor hypothesis is certainly plausible, but there are several difficulties with the argument that have yet to be addressed adequately. First, environmental oestrogens exhibit only weak biological activity; DDT for example has a bioactivity level that is 100,000 to 1,000,000 times less than the human oestrogen oestradiol-17β. Potency calculations suggest that the daily birth-control pill involves exposure to about ten billion times more oestrogenic activity than the dietary intake of organochlorine pesticides, such as DDT or dioxin-like compounds[20]. In addition, the male fetus is exposed to very high levels of potent placental oestrogens during the course of normal pregnancy and yet develops normally. Finally, the male offspring of women exposed during pregnancy to a powerful synthetic oestrogen, diethylstilbestrol, failed to exhibit a statistically significant change in the incidence of testicular cancer or infertility. The observed effects (small testes, cryptorchidism, epididymal cysts) are much less frequent than would have been expected if the oestrogenicity of the compound were the only determinant of its developmental toxicity. Other mechanisms must be involved.

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One possibility is that some of these environmental oestrogens can be metabolized further to molecules (quinones) that can cause cellular damage by either binding to DNA or generating reactive oxygen species. The latter are created by a ping-pong type of activity termed 'redox cycling', whereby a given compound alternates between oxidized and reduced states. During this process, electrons are transferred to oxygen to produce the superoxide anion. The latter can then create a state of oxidative stress through a complex series of secondary reactions that result in damage to both the sperm and its DNA[6]. Exposure of human sperm to oestrogenic compounds such as diethylstilbestrol, phytoestrogens (equol, genistein and daidzein), industrial surfactants (nonylphenol) and natural oestrogens (oestradiol-17β) can induce significant DNA damage through mechanisms that seem to involve oxidative stress[21]. Most DNA damage in the sperm of infertile males also seems to have been caused by oxidative damage, resulting in high levels of the oxidized DNA product, 8-hydroxy-2′-deoxyguanosine[22].

At present, we know very little about the nature of the xenobiotic-metabolizing enzymes in the male germ line, and thus the potential that different groups of compounds have for inducing genetic damage by oxidative, or other, mechanisms is uncertain. Experimentally, we know that a state of oxidative stress can be induced in the testes by exposure to common xenobiotics such as nonylphenol or dioxin[23]. But the biochemical mechanisms underpinning this activity remain unclear.

Epidemiological clues

The epidemiology literature offers some clues about the germ-cell toxins that can damage the offspring of affected males. As well as the link between paternal cigarette-smoking and childhood cancer, the chance of contracting testicular cancer can depend on paternal occupation. If a man works in the wood-processing industry, then the risk of his son developing testicular cancer is more than ten times that for the average male; for the sons of men working in the metal industry, the risk is about six times the average[24]. There are also data linking childhood cancer with paternal exposure to hydrocarbons in various forms (benzene, paint, methyl ethyl ketone, plastic and resin fumes, and different types of solvents)[25]. In addition, an increased risk of contracting leukaemia has been reported in children whose fathers are automobile, truck or aircraft mechanics.

These associations suggest that there is a chain that links paternal exposure to xenobiotics with genetic or epigenetic DNA damage to the father's sperm, and with adverse consequences for his offspring. Although the epidemiology literature is not always consistent (Box 1), these links have been observed in animal models where paternal exposure to a wide variety of potential toxicants (including acrylamide, cyclophosphamide and urethane) is significantly associated with increased incidences of spontaneous abortion and birth defects in the offspring[26].

Clinical studies in this area would be much improved if we had a better understanding of the way in which xenobiotics are metabolized in the male germ line and the kinds of DNA damage induced. Metabolism of organic xenobiotics involves the initial biochemical modification of a given compound (phase 1) followed by a conjugation reaction that links the modified compound to a carrier molecule in preparation for excretion (phase 2). The ability of individual males to metabolize and link xenobiotics in this manner depends heavily on genetically determined variations in the enzymes responsible for the biotransformation reactions. Knowing more about those enzymes, and the relative sensitivities of subjects with specific genotypic profiles, will help epidemiologists untangle the relationships between toxicant exposure and adverse reproductive outcomes. This could be the dawning of the age of 'reproductive pharmacogenomics', in which a male's reproductive susceptibility to a xenobiotic can be predicted from his profile for key enzymes such as cytochrome P450 (phase 1) and glutathione-*S*-transferase (phase 2). There is already evidence to suggest that variation in cytochrome P450 enzymes affects male fertility[27].

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Age and mobile phones

Xenobiotics are not the only factors that can induce oxidative DNA damage in the male germ line. Age is another: as a man ages, his sperm count may not change significantly but the amount of DNA damage in his sperm increases dramatically: the amount of DNA damage in sperm of men aged 36–57 is three times that of men below the age of 35 (ref. 28). This age-dependent increase in DNA damage may contribute to the incidence of childhood diseases that increase with paternal age, including complex conditions such as schizophrenia, and genetic disorders such as the achondroplasia caused by defective bone growth[29].

Although the risk to the individual is low, such associations could become more significant if assisted-conception protocols that do not exclude DNA-damaged sperm, such as ICSI (intracytoplasmic sperm injection), continue to be used to address age-related declines in human fertility — in older men starting second families, for example.

Radiofrequency electromagnetic radiation may be another cause of damage to the male germ line, given preliminary reports of DNA damage in the sperm of mice exposed to mobile-phone radiation[30]. Such results are bound to generate widespread publicity, but the data are still much too limited to draw any conclusions.

The future

So we are faced with a situation where semen quality is apparently declining and pathologies of the male reproductive tract are rising; moreover, 3–6% of the population in most developed countries is now produced by assisted conception. To some extent the slide towards lower fertility is a consequence of lifestyle choices (more young adults deliberately delaying parenthood or choosing a child-free future) and a lack of selection pressure on high-fertility genes, which we are powerless to prevent. But some of the reproductive pathologies we are seeing are a biological response to factors in the environment that are affecting every aspect of human reproduction from fertilization of the egg, through fetal development of the reproductive system, to child health.

The developmental consequences of environmentally mediated DNA damage to sperm include impaired embryonic development, abortion and the induction of abnormalities in the offspring such as childhood or testicular cancer. But although we know that environmental factors can induce severe damage in male germ cells, we know little more than that. The questions of the kinds of molecular structure that induce such damage, the nature of the damage induced, and the mechanisms by which such damage affects embryonic development all require urgent attention. ■

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