Infertility and environmental pollutants

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While it has long been known that female fertility is impaired by oestrogen exposure, it is unclear whether environmental pollutants with weak oestrogenic effects are sufficiently potent and prevalent to have biological effects in humans. Male fertility, or sperm concentration at least, appears to have deteriorated, and there is substantial spatial variation at both national and global level, as well as a genetic component. Sperm morphology and motility are implicated too. There is good evidence for an increase in testicular cancer, and possibly in other conditions that certain spatial characteristics plus evidence on heritability suggest are linked to impaired spermatogenesis. A candidate agent would need to have started increasing in the early 20th century. Weak environmental oestrogens are not responsible. Candidates include agents affecting endogenous maternal oestrogen levels, environmental anti-androgens (although these cannot explain the epidemiological findings), and dioxin and related compounds. Genetic damage should be considered as a unifying hypothesis, possibly focused on the Y-chromosome.

Introduction

A review of the literature on the determinants of fertility raises more questions than can be answered in the current state of knowledge. Even so, at least this is an advance on the situation that previously existed, when the topic was largely ignored. This state of affairs is mainly the result of the lack of priority that has been given to research on reproduction in basic biology, as well as in epidemiology and toxicology.

Reproduction and/or development can be affected by exposure to a wide variety of agents, including dioxins, poly-chlorinated biphenyls (PCBs), phytoestrogens such as isoflavones, heavy metals (e.g. organic mercury, lead); chlorination disinfection by-products in water, organic solvents, poly-aromatic hydrocarbons, particulate air pollution, substances emitted from landfill sites and caffeine. Often, effects on the reproductive system and/or infant development have been detected at lower doses of the substance in question than is the case for other endpoints. Most of these agents have featured in recent discussions of official regulatory bodies, which have highlighted the paucity of good quality epidemiological evidence.
In principle, fertility can be studied in humans or in laboratory animals by using biomarkers such as measures of semen quality, and/or using a functional measure of the ability to conceive. In practice, for many exposures, little or no epidemiological research has been carried out with fertility as an endpoint, and the toxicology database is typically seriously incomplete in this respect as well.

The much-publicised concern over the possibility of ‘falling sperm counts’ has altered the position in the past 10 years, but during that time the research initiative has been dominated by just one hypothesis, that endocrine disrupting agents are responsible. As a result, we are in the situation of having the following rather fragmentary information:

(i) the effects of a few known agents on fertility in either sex;
(ii) descriptive epidemiology, which suggests that there may be a problem with male fertility, and that this is likely to be linked to deterioration in related conditions such as testicular cancer; it also indicates when and where such a problem may be at its greatest, so that one can begin to outline the epidemiological characteristics of the responsible agent(s);
(iii) some additional observations that allow preliminary assessment of the type of biological process underlying any such effect, in particular whether endocrine disruption or genetic damage are responsible.

This paper reviews these three areas in turn, concentrating on epidemiology. Evidence from toxicology, endocrinology, genetics and research on wildlife is not presented, but informs the discussion. Priority is given to agents or processes that could affect whole populations, or substantial proportions of whole populations, rather than relatively small groups such as those with occupational exposure. The next section focuses on effects of known agents. As the main evidence for widespread impairment comes from epidemiological observations on males, the following section discusses this in detail. In view of the limited data available on male fertility itself, epidemiological information is also presented on possibly parallel variations in other disorders of the male reproductive system that arise early in life, especially on testicular cancer. Genetics is covered, as well as temporal and spatial variation. The available evidence is that the epidemiological observations are not explicable in terms either of the known agents or of agents that are currently known to have endocrine disrupting effects.

### Effects of known agents

#### Infertility in females

In the 1940s, reduced fertility was noticed in Australian ewes, and it was established that this was due to the clover that they were grazing.
This condition, which came to be known as ‘Clover Disease’, was traced to phytoestrogens—plant compounds that have oestrogenic properties—in the clover. Ewes feeding on Australian clover developed abnormal plasma concentrations of endogenous hormones, with subsequent reduced fertility.

In humans, dietary phytoestrogen consumption is considerable in some populations, and constitutes by far the major route of exposure to exogenous ‘endocrine disruptors’ (Table 1). Probably the most important of these are isoflavones such as genistein, which occur in legumes and are particularly abundant in soybeans and soy-based foodstuffs. The isoflavone

Table 1 Principal exogenous substances that may affect sex hormone function

A. Oestrogenic effects
1. High potency—pharmacological agents
   - DES (diethylstilboestrol)
   - ethinyl oestradiol (component of contraceptive pill)
2. Medium potency—dietary phytoestrogens
   - isoflavones, *e.g.* genistein, daidzein
   - coumestans, *e.g.* coumestrol
   - lignans
3. Low potency—environmental or occupational agents
   - bisphenol A
   - octylphenol and nonylphenol
   - pesticides, including chlordecone, DDT, dieldrin, endosulphan, *p,p*-methoxychlor, toxaphene

B. Anti-androgenic effects
   - *p,p*-DDE (the major breakdown product of DDT)
   - certain phthalates, *e.g.* DBP, DEHP
   - pesticides, including linuran, procymidone, metabolites of vinclozolin
   - hydroxyflutamide

C. Others
   - dioxins, furans and ‘dioxin-like’ PCBs

Adapted with permission from Joffe.

This classification is an over-simplification: it conflates receptor-mediated effects with those due to other mechanisms, *e.g.* interference with hormone synthesis. Moreover, several of the ‘oestrogens’ show considerable affinity for the androgen receptor. In addition, many of these compounds have important biological actions that are not endocrine in mechanism.

For the reasons given in the text, compounds in the ‘Low potency’ category cannot plausibly be considered responsible for the types of impairment of the male reproductive system considered in this paper, and the exposures may also be too low to affect females.
content of soy varies in relation to many factors, including plant species, strain, crop year and geographical location. The concentrations are sufficient to cause biological effects in humans, even after cooking or other processing. Populations that traditionally consume large quantities of soy, notably Chinese and Japanese people, tend to have relatively high phytoestrogen exposure. Less is known about other types of phytoestrogen; exposure to lignans is probably widespread, but the potency is lower and may not have biological significance1.

In rodents, exposure to isoflavones and other phytoestrogens has been shown to alter a number of functions of the female reproductive system, including advancement of puberty, subfertility and irregular oestrus cycling. Perinatal, neonatal or prepubertal exposure appears to produce the most marked effects. It is unclear to what extent these findings are relevant to humans, owing to species differences in sexual development, experimental considerations such as route of administration, and uncertainty over comparability of plasma concentrations1.

A different source of oestrogen exposure was the synthetic compound, diethylstilboestrol (DES), which has oestrogenic potency comparable to that of oestradiol. From the late 1940s onwards, it was widely used during pregnancy, especially in the USA, in the belief that it could prevent miscarriage and a range of pregnancy complications. It is estimated that more than two million women were exposed to this drug. Pharmacological doses were given, often at the stage of pregnancy during which the sexual organs develop.

A randomized controlled trial published in 19532 showed that DES was ineffective for the conditions for which it was being prescribed. However, clinical use of the drug continued until it was banned in 1971, after the discovery that in utero exposure of female fetuses led to a risk of developing clear cell adenocarcinoma some 15 years later3. While this particular risk is fortunately rare, DES-exposed girls have reproductive tract anomalies, and they subsequently have reduced fertility and increased rates of ectopic pregnancy, spontaneous abortion and preterm delivery3.

The sensitivity of the developing female reproductive tract to oestrogens raises the question of whether exposure to environmental chemicals having oestrogenic activity (Table 1) might affect fertility through the female route. There is insufficient evidence to answer this question definitively, as research on these exposures has tended to focus on the male, even though toxicological experiments consistently find stronger effects of oestrogens on females than males1. As their potency combined with exposure concentrations are many orders of magnitude lower than endogenous hormones, or even than phytoestrogen intake in oriental populations, a strong effect seems unlikely.

Aside from the specific question of oestrogens, high maternal but not paternal consumption of sport fish from the heavily polluted Lake Ontario...
has been associated with reduced fertility as measured using TTP (see below), but the findings are inconsistent. This could be due to PCBs, or to other pollutants (including oestrogens)\textsuperscript{4}.

Other specific populations who have high exposures, generally occupational, to particular agents have been identified as having an increased risk of subfertility. Such agents include solvents\textsuperscript{5,6}, inorganic mercury\textsuperscript{7}, nitrous oxide\textsuperscript{8,9} and antineoplastic agents\textsuperscript{10}, but these do not have implications for environmental exposures to the same substances, which are too low.

**Infertility in males**

The nematicide dibromochloropropane (DBCP), used for soil fumigation on fruit plantations, is a potent testicular toxin. This was discovered when the wives of occupationally exposed male workers were discussing the problems they had had in becoming pregnant, and was subsequently confirmed by epidemiological studies\textsuperscript{11}. High exposure causes permanent azoospermia. DBCP was banned in the late 1970s in the USA, although it is still used elsewhere.

The other exposures that are known to affect fertility in human males are also predominantly occupational, and include other pesticides, and heavy metals such as lead and cadmium\textsuperscript{12}. That these risks are not generalizable to the general population (e.g. via pesticide residues) is illustrated by evidence for a threshold for inorganic lead and sperm concentration: no effect was seen below a blood level of about 44 µg/dl\textsuperscript{13}—and even in the occupational context few men have higher exposures than this, in the economically developed world.

**Descriptive epidemiology: conditions affecting the male reproductive system**

**Fertility and semen quality**

Two types of endpoint can be studied: semen quality, and fertility as measured by the time taken to conceive (Time To Pregnancy, or TTP). TTP reflects the probability of conception for couples having unprotected intercourse. It is a functional measure of biological fertility at the level of the couple, and validity studies have shown that it can be studied retrospectively as well as prospectively\textsuperscript{14}. Care in the design and analysis of TTP studies is important, to avoid potential pitfalls.

Methodological issues also affect the interpretation of studies of semen quality, which is usually taken to include sperm concentration, motility and morphology. All are subject to large degrees of within-person biological
variation and/or measurement error that varies between centres and very likely over time. In addition, representative samples of the general population, which are so important for descriptive epidemiology, are unachievable as participation rates are too low. The best evidence is from candidates for semen donation and for vasectomy; data from men in contact with medical services for a fertility-related problem, or from those accepted for semen donation, are too unreliable to use.

**Trends**

For various reasons, long-term trends in fertility and semen quality are difficult to confirm. One study has found that fertility as measured by TTP increased (not decreased, as was hypothesized) in the period 1961–93, based on a representative sample of the British population\(^{15}\). However, firm conclusions cannot be drawn from a single report. Another study, from Sweden, that reported a decline in clinical subfertility in 1983–93\(^{16}\) was shown to be likely to have resulted from truncation bias that had not been allowed for\(^{17}\).

The ‘time trend’ debate regarding semen quality has focused mainly on sperm concentration. A much-cited paper published in 1992 reviewed the world literature, relating this variable to the date of publication\(^{18}\)—a crude exercise in terms both of the methodology and of the hypothesis, which treated location as irrelevant. Its claim of a 50\% decline in mean concentration over 50 years, from 113 to 66 million/ml, should be treated with great caution. An attempt at a more rigorous analysis along the same lines, but dividing the world into three, found the decline in sperm density to be much steeper in Europe than in America; studies from elsewhere were too sparse and diverse to draw confident conclusions\(^{19}\).

However, the 1992 paper did stimulate several centres to analyse their semen quality data, which had been continuously collected for some two decades. Those data are less likely to have been distorted by possible changes in the method of semen examination and/or in selection processes affecting the populations studied. The principal conclusions that emerge are that: (i) declines in semen quality have occurred in some places (e.g. Paris, Edinburgh, Gent) but not in others (Toulouse, Finland and the five US cities with published data)\(^{20}\); (ii) at most, the available data go back to the early 1970s; and (iii) where concentration has deteriorated, so usually have sperm motility and morphology.

Where a decline has occurred, the findings are compatible not only with a period effect but also with a birth cohort effect, men born in the 1940s having better quality semen than those born in the 1960s. As the observed decline, with either method of analysis, is already visible in the earliest available data in all affected centres, it is impossible to locate the year when the decline started or what the pre-decline values were. As semen quality is inferior in humans compared with other mammalian species, it
is possible that deterioration from a ‘natural’ level has a much longer history than we have the data to substantiate\textsuperscript{20}.

While it is difficult to be confident about drawing conclusions from this literature, it is likely that semen quality deteriorated in some parts of Europe for two decades after the early 1970s as a period effect, or the mid-1940s as a birth cohort effect. This deterioration involved not only sperm concentration, but also morphology and motility. No evidence is available on earlier periods, so that a decline may possibly have begun earlier. The evidence for a similar trend in America is unconvincing\textsuperscript{20}.

**Spatial variation**

Substantial spatial variation in sperm concentration has been demonstrated, within both Europe and America\textsuperscript{20}. Based on the available evidence, concentrations appear to be relatively high in New York and Finland and low in California and north-western Europe including Denmark and Britain. Couple fertility assessed by TTP is high in parts of southern Europe compared with the north\textsuperscript{21}, with the exception that it is also high in Finland\textsuperscript{22}. The congruence of the findings for Finland suggests that the higher levels of sperm concentration observed there are not the result of differences in methodology or to longer abstinence (less frequent intercourse).

**Genetic factors**

Male fertility problems tend to aggregate in families\textsuperscript{23,24}, infertile men have relatively few siblings\textsuperscript{24}, and their brothers have inferior semen quality\textsuperscript{25}. However, most fertility-affecting genetic aberrations cannot be detected using current clinical laboratory methods\textsuperscript{24}. A recent report based on a twin study reported the heritability of sperm concentration, uncorrected for biological variation and measurement error (and therefore an underestimate), as 20\%. The heritability of sperm morphology was 41\%, and that of chromatin stability was 68\%. Certain paternal lineages, identified through their Y chromosomes, are predisposed to low sperm counts\textsuperscript{27,28}. There is also evidence for heritability of TTP, probably by nonadditive polygenic inheritance\textsuperscript{29}.

**Testicular cancer**

Epidemiological information on cancer of the testis is very reliable. As a disease of relatively young men that has unmistakable features, it is likely to be rarely missed or misdiagnosed, so that only an efficient collating system is required to produce high quality ascertainment. Good incidence data have been available from cancer registries in developed countries for some decades. Mortality data are also available for certain countries going back a hundred years, and since the disease is invariably
fatal if untreated, these are reliable for the early 20th century, although not more recently as cure rates are now high.

Testicular cancer is strongly and consistently associated with subfertility, and this has been shown to be present before the cancer appears\textsuperscript{30}.

**Trends**
This disease has shown an increasing trend in recent decades throughout the developed world, typically with rates being trebled or more. An important and often overlooked question is when this began. In England and Wales, mortality started rising around 1920, having been stable before World War I\textsuperscript{31}. In Denmark, a continuous rise in age-standardized incidence is observable since cancer registration began in 1943\textsuperscript{32}.

Clinical research strongly suggests that the predisposition to testicular cancer is present from an early age, probably \textit{in utero}\textsuperscript{33}, so that the possible influence of environmental agents needs to be evaluated in relation to time of birth rather than of diagnosis or death. Accordingly, if these trends are examined in terms of birth cohorts, mortality started rising among men born before 1900 in England and Wales\textsuperscript{31}, and incidence in Denmark, Norway and Sweden started rising among men born around 1905\textsuperscript{34}. In these latter three countries, rates stabilized or fell for men born during 1935–45, whereas the rise was rapid and inexorable among men born from 1920 until at least 1960 in East Germany, Finland and Poland\textsuperscript{34}. Recent data indicate that the rates may be stabilizing for Danish men born since about 1960\textsuperscript{35}, but the 1965 birth cohort shows a continuing rise in other countries\textsuperscript{34}.

**Spatial and ethnic variation**
There is considerable spatial and ethnic variation. Denmark has the highest incidence in the world, the lifetime risk now being almost 1\%. However, the Nordic countries do not have a uniformly high risk, as Finnish men have comparatively low rates, with Norway and Sweden in intermediate positions\textsuperscript{32,36}. The spatial pattern for testicular cancer in the Nordic countries does not resemble that of other hormone-sensitive carcinomas such as those of the prostate or female breast, but is similar to that of colo-rectal cancer in both sexes\textsuperscript{36}.

Other high-risk populations include Switzerland and New Zealand (including Maoris), whereas the Baltic states and African-Americans have comparatively low rates\textsuperscript{20}. The tumour is rare among Chinese and Japanese men\textsuperscript{20}.

**Genetic factors**
Whereas the rapid trends in testicular cancer indicate the importance of environment in the broadest sense, migrant studies suggest a genetic component as well: for example, a high risk among European immigrants to Israel was still present, albeit reduced, in the next generation\textsuperscript{37}. This is confirmed by family\textsuperscript{38} and case-control studies\textsuperscript{39}. However, brothers have
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a far higher risk than father-son pairs, suggesting the importance of shared maternal characteristics as well as shared genes; dizygous twins have a particularly high risk, which may indicate that endogenous maternal oestrogen levels play a part, although twins also share a time-specific maternal (and paternal) environment.

**Anomalies of the male genitalia**

Hypospadias and cryptorchidism have been grouped with male infertility and cancer of the testis into the ‘testicular dysgenesis syndrome’, on the grounds that they occur together more often than expected by chance, and that they all probably originate early in life. Hence, it is argued, they probably share at least some risk factors.

Both anomalies are likely to be unreliably ascertained at birth, particularly in mild cases, and the study of cryptorchidism is further complicated by the difficulty of distinguishing testes that have not descended from those that readily but reversibly retract back into the abdominal cavity in early infancy. The consequence is that published data from congenital malformation registries cannot be relied on to reflect real variations: reported time trends and differences between registries may both merely reflect differences in ascertainment and reporting. Self-reported data (by mothers) are similarly unreliable.

**Trends and spatial/ethnic variation**

For hypospadias, the apparent increase in many countries may well be because of variations in the registry system rather than a real change, apart from a step increase between 1982 and 1985 in the severe form in Atlanta, Georgia. Recent studies in Denmark and Finland using strict criteria have shown a higher rate in Denmark. In the case of cryptorchidism, a study was carried out in Oxford during the 1950s, using strict diagnostic criteria and examination of the baby boys at 3 months when the diagnosis is more reliable. A subsequent study in southern England using the same criteria found almost double the proportion of boys having cryptorchidism. Recent studies in New York and in Finland using the same criteria found a similar proportion to the original (lower) Oxford estimate, whereas in Denmark it was close to the later English value. Unlike for testicular cancer, African-Americans do not appear to have a lower risk.

**Genetic factors**

Both hypospadias and cryptorchidism show familial association, with different modes of inheritance having been suggested, probably reflecting the importance of several different genes. They tend to
occur together more often than would be expected by chance, in individuals though not in families. Hypospadias is also associated with parental subfertility and with impaired paternal sperm motility and morphology.

**Sex ratio**

Although the proportion of births that are male, conventionally but inaccurately called the sex ratio, is not included in the definition of the testicular dysgenesis syndrome, it is prudent to consider what is known about its temporal and spatial variation in this discussion.

**Trends**

A reduction in the sex ratio has been observed in several countries, including Denmark and other Nordic countries, the Netherlands, the USA and Canada (but not Australia). For those countries with data going back that far, this began around 1950 and continued at least into the 1990s. The changes are small—typically the decline is in the order of 2 per 1000 births—but they may nevertheless indicate a biological process that is important for other reasons. Before 1950, an increasing trend was observed, which has been attributed to a fall in the proportion of stillbirths (a disproportionate number of whom are male) and therefore discounted as the mirror image of the more recent trend. However, an alternative possibility is that long-term cyclical fluctuations occur, possibly as a result of an adjustment process.

**Spatial and other variation**

While a higher proportion of male births has been observed within southern Europe compared to the north, spatial variation is not a major feature of the literature on sex ratio. However, certain chemical exposures have been associated with altered sex ratio, always in the direction of reducing the proportion of males, which may reflect the greater vulnerability of males at all ages from conception onwards. The best documented is of men occupationally exposed to DBCP (see above): those who retained or regained some degree of fertility fathered predominantly daughters, and the impairment of sex ratio was correlated with the degree of oligospermia. Dioxin exposure following the Seveso incident was also followed by a reduction in male births.

The sex ratio can be affected either by an endocrine process, which may be the case for dioxin, or by genetic damage, which is more likely for DBCP as it is mutagenic. These are discussed further below.


Epidemiological characteristics of the responsible agent(s)

The question of linkage

A key question is, to what extent (if at all) these five conditions are linked by shared aetiological factors, as indicated by parallelism in their epidemiological characteristics. Clearly, for the congenital anomalies or sex ratio to be included, the exposure would need to be acting prenatally. As testicular cancer epidemiology is by far the most robust, a useful starting point is to consider its main features:

(i) The incidence has increased at least three-fold in all developed countries, suggesting the importance of one or more environmental risk factors that have changed over the same period.

(ii) The increasing trend started around 1900 in at least some countries, assessed as a birth cohort effect, and continued until at least the 1960s.

(iii) The rise was interrupted for some years around 1940 in Scandinavia, but not in nearby countries that were more severely affected by World War II.

(iv) Marked variations exist between different nationalities (e.g. Denmark versus Finland), and between ethnic groups.

(v) Migrant, family and case-control studies suggest some degree of heritability. This may be related to international and inter-ethnic differences, but cannot explain the rapid trends.

The first three of these refer to trends, so the question is, to what extent the other endpoints have parallel trends, making due allowance for latent periods, etc.: linked trends in congenital anomalies or sex ratio and the adult-life endpoints will tend to be a few decades apart, or to put it another way, comparison would have to be by the year of birth. Even if parallel variations were found, this would be unreliable evidence for linkage, as so many factors vary with time. Whereas recognition of discrepancies would, in principle, be good evidence against the hypothesis of linkage, so that the latter could be tested in a negative way (i.e. how well it survives attempts at refutation), the paucity and low quality of the available data place severe limits on this course of action.

The timing for each observed trend does not appear to be exactly parallel: in particular, the beginning of the fall in the sex ratio (for which the evidence is robust) was later than that of the rise in testicular cancer incidence. Also, the trends in sex ratio and possibly in semen quality have continued later than the early 1960s, which may mark the end of the trend in testicular cancer. However, the latter observation is currently tentative, and even if true may not represent a permanent interruption; also, it applies just to Denmark. More generally, if one allows for the possibility that trends can be interrupted or even temporarily reversed, and that all these features are likely to vary across populations and
sub-populations, the available data on the timing of trends cannot be confidently regarded as evidence for or against linkage.

The spatial location of trends also does not correspond precisely for the different endpoints: whereas those for testicular cancer and the sex ratio are apparently widespread, at least in Europe and North America, semen quality does not seem to have deteriorated in America.

The pattern of spatial variation for the four endpoints of the testicular dysgenesis syndrome, notably the sharp and consistent contrast between Finland and Denmark, suggests possible linkage. The observations on cryptorchidism, while sparse, also show some resonance with data on other endpoints.

Overall, there is enough parallelism to suggest that at least some of the endpoints share one or more risk factors, but that there must also be some additional harmful and/or protective factors, which is perhaps unsurprising. A ‘linked’ risk factor would need to have started increasing (or decreasing, in the case of a protective factor) in its exposure level during the early 20th century in developed countries, which rules out chemicals introduced since the mid-20th century. Possible explanations include environmental pollution, and dietary changes involving macro- or micro-nutrients or contaminants, both natural and man-made. While the epidemiological pattern is compatible with something that increased with rising prosperity, such as increasing meat consumption, there is no direct evidence for this.

In the case of semen quality, a possible ‘unlinked’ factor could include an increasingly sedentary way of life, possibly together with tight clothing, since raising the intra-testicular temperature strongly affects the quality as well as the quantity of sperm—sufficient to cause delayed conception in men with sedentary occupations such as driving—and on the viability of the offspring. This would be a period effect not a birth cohort effect.

Heritability
All four conditions of the testicular dysgenesis syndrome show some degree of heritability. This is compatible with the idea that they are linked.

Heritability of subfertility may at first sight appear to be impossible: a ‘gene for infertility’ is surely impossible, on evolutionary grounds, as it would quickly be eliminated. (Theoretically, this would not apply if the heterozygous state of a recessive gene carried some advantage, but evidence from a Hutterite population is that inbreeding is unrelated to male fertility.) An alternative explanation is, however, possible. In a steady state, a balance would exist between selection against polymorphisms that impair fertility and their de novo creation as a result of genetic damage. As there is nothing to constrain these two processes to be equal,
new damage could occur at a rate greater than elimination, leading to an increase in incidence. The same argument applies, but with less strong elimination, to testicular cancer, hypospadias and cryptorchidism, which are associated with subfertility, and which show evidence of heritability.

The selection process can also vary in its intensity. Czeizel has pointed out that as family size decreased markedly during the 20th century, births to the biologically most fertile couples became a less dominant proportion of births at the population level\textsuperscript{61}. Secondly, towards the end of the century, assisted reproduction meant that the proportion of births to clinically subfertile couples increased. These two tendencies would have the effect of decreasing the rate of removal from the population of polymorphisms that reduce fertility and that might also predispose to testicular cancer or one of the other endpoints.

The first of these at least may well be important to consider. However, it is unlikely that on its own it would be strong enough to bring about, for example, a three-fold rise in testicular cancer incidence. Furthermore, this hypothesis depends on the existence of polymorphisms that decrease fertility, raising the question, why had they not already been eliminated from the population, even before 1900. New generation of such polymorphisms is required to complete the picture, and once this is accepted it becomes important to consider:

(i) the nature of the defects and their location in the genome,

(ii) the rate of their appearance,

(iii) the identification of agents that could affect their generation, and

(iv) the possible other effects that this process could have.

Before considering genetic mechanisms, it is necessary to review other possibilities.

**The type of underlying biological process**

*Endocrine disruption*

**Oestrogens**

The original version of the endocrine disruption hypothesis was concerned with exposure to oestrogens. As we have seen, effects on female fertility are well established, although it is unlikely that current environmental exposure levels are sufficient, except possibly in the case of dietary phytoestrogens.

In 1979, Henderson et al\textsuperscript{62} observed that factors such as high maternal weight and excessive vomiting during pregnancy, which are associated with high levels of endogenous maternal oestrogens such as oestradiol,
increased the risk of testicular cancer. Cryptorchidism is also implicated. This invites the question, whether trends or spatial variations in these or related conditions could be due to variations in maternal oestrogens, as a result of exposure to environmental, nutritional or other factors, but there has been insufficient research in this area.

The idea was subsequently extended to exogenous substances with oestrogenic activity. The criticism that the exposure levels and the potency of such substances are too low by several orders of magnitude, compared with oestradiol, was refuted by the argument that endogenous oestrogen does not reach the fetus (e.g. because of protein binding)—although this postulates a near-absolute barrier that seems implausible, and is directly contradicted by the evidence just cited for Henderson’s hypothesis.

Nevertheless, the ‘oestrogen hypothesis’ became influential, both scientifically and in society at large, where public concern about ‘gender benders’ ensued. While it is superficially plausible that oestrogens ‘demasculinize’ the developing male, this is biologically naïve because mammals are adapted to starting life inside their mothers, whose internal environment is oestrogen-rich (even before the early pregnancy surge). In contrast to other vertebrates, the mammalian default sex is female, and masculinization of the gonads and central nervous system depends on the presence of androgens.

In contrast to the marked impact on girls, boys exposed to DES show relatively minor effects. They tend to have genital abnormalities such as cysts and urethral stenosis, but among the features of the testicular dysgenesis syndrome only cryptorchidism is clearly and strongly affected, despite the high exposures. The risk of testicular cancer may be raised, but by less than the trend observed throughout the developed world, and the position for sperm concentration is similar; hypospadias has only been implicated because of a propagated error in the literature.

Although the DES disaster has often been cited in favour of the hypothesis that exogenous oestrogens are responsible for the observed deterioration in the health of the male reproductive system, it is rather strong evidence against. This is reinforced by the low incidence of testicular cancer among Chinese and Japanese men who are exposed to high levels of phytoestrogens in soy. In relation to the ‘environmental oestrogens’ listed in Table 1, which are orders of magnitude less potent, it is now accepted that their uniformly weak oestrogenicity excludes the possibility that they could induce these disorders.

**Anti-androgens and other types of endocrine disruption**

Interference with either the synthesis or the action of androgens could prevent the normal masculinization of the male fetus, and could also affect male infants postnatally. There is toxicological evidence that
p,p′-DDE, the stable breakdown product of DDT, can block the androgen receptor, as can certain other pesticides, and that some phthalates inhibit testosterone synthesis (see Table 1)\textsuperscript{66}. More nuanced hypotheses that relate, for example, to the balance between oestrogens and androgens, or to their interconversion \textit{via} aromatase\textsuperscript{66}, are interesting, but lack candidate substances that could explain the epidemiological findings.

It is therefore plausible that exposure to anti-androgens can affect male fertility, as well as other related endpoints, and this is supported by toxicological evidence\textsuperscript{66}. Could pollution with DDE, phthalates or other anti-androgens explain any of the epidemiological findings presented above?

One obvious objection is that the rising trend, at least in testicular cancer, started before any of the known anti-androgens were introduced. Secondly, the striking contrast between Denmark and Finland cannot be explained by exposure to DDE, which has been monitored in human breast milk, and the concentrations were similar in all the Nordic countries\textsuperscript{67}. Thirdly, high levels of exposure to DDE in developing countries, in the course of attempts at malaria control, have not resulted in an epidemic of testicular cancer\textsuperscript{20}.

The answer, then, is no; the idea that disorders of the male reproductive system are due to chemicals that interfere with the sex hormone system, in any of its variants, cannot explain any of the main features of the epidemiological evidence.

The expected spectrum of effects

It is far from clear that endocrine disruption would affect non-quantitative aspects of semen quality, especially morphology. However, it is necessary to go further. In addition to focusing on the various endpoints and asking ‘could this be due to endocrine disruption?’, it is important also to turn the question around and ask: ‘if an endocrine-disrupting substance were responsible, what spectrum of effects would be predicted?’ One plausible expectation is of a coherent pattern in hormone-sensitive cancers, but this is not observed\textsuperscript{20}.

A second is that endocrine agents would be expected to influence growth and development, secondary sexual characters and the timing of puberty. No such change has been reported among boys, either in Europe or America\textsuperscript{20}.

As mentioned earlier in the section ‘Infertility in females’, such effects have, however, been found in female rodents fed with phytoestrogens. Precocious puberty has been reported to be widespread among girls in the USA, especially African-American girls\textsuperscript{68}, and this raises the question of possible excessive oestrogenic stimulation. The potential effect on their subsequent fertility is unknown.
Dioxin

‘Dioxin’ (2,3,7,8-tetrachlorodibenzo-p-dioxin) is the most potent of a group of chemicals, dioxins and furans; dioxin-like activity is also displayed by some PCBs. While it is sometimes called an endocrine disruptor, as it has some effects on the endocrine system such as anti-oestrogenicity, it mainly acts through a distinct receptor, the aryl hydrocarbon receptor (AhR)\(^69\). Toxicological evidence, albeit with some inconsistencies, shows that sperm production and morphology are adversely affected even by very low doses given during pregnancy and lactation\(^69,70\).

There is little information on infertility and related conditions based on human exposure. In men, a case–control study has suggested lowered serum testosterone and raised follicle stimulating hormone and luteinizing hormone levels with occupational dioxin exposure\(^71\). Vietnam veterans tended to have lower sperm concentrations and fewer morphologically ‘normal’ cells than non-Vietnam veterans, but few of the former group were greatly exposed to Agent Orange, a pesticide that was heavily contaminated with dioxin\(^72\). In US military working dogs, an excess of testicular cancer (seminoma) was found among those that had worked in Vietnam, and possibly exposed to Agent Orange\(^73\). All these findings would relate to a period, not a birth cohort, effect, following exposure of adult males.

For exposure of women, we have already noted the suggestive findings of longer TTP associated with high consumption of sport fish from Lake Ontario\(^4\), where the pollution includes PCBs, and the low sex ratio in births to women with relatively high dioxin exposure following the Seveso incident\(^58\).

Dioxin is persistent, in the environment and in the body: in humans, it has a half-life of 6–11 years. The intake estimates for the UK in 1997 are below the levels thought necessary to affect the reproductive system, but this may not be true for earlier periods; for example, in 1982, intakes were four times as high as in 1997\(^69\). Interpretation of the possible role of dioxin and related compounds in reproductive health requires information on the spatial and temporal variation of exposure to be considered alongside the descriptive epidemiological findings discussed above.

Genetic damage

The hypothesis

A possible unifying hypothesis for impairment of the male reproductive system is that genetic damage in the germ line is responsible. If so, the health implications could extend beyond the conditions discussed in this paper, and could include chromosomal abnormalities and other genetic anomalies, malformations and cancer in the offspring and in future
generations (Fig. 1)\textsuperscript{14,74}. With such heterogeneous outcomes, each being uncommon, it is possible that increasing trends have escaped detection.

The evidence that these endpoints have both genetic and environmental determinants could lead discussion in the direction of trying to apportion causation between the two, and/or to consideration of gene–environment interaction\textsuperscript{75}. But another possibility is more interesting: the routine distinction between environmental and genetic factors breaks down when we consider germ-line genetic damage. Unless such damage fails to be passed on, for example due to lethality or sterility, there is a heritable element—but the origin of the defect lies in an environmental cause (Fig. 2). If this is true, then it would no longer make sense to refer to ‘environmental or genetic influences’, nor to equate ‘genetic’ with ‘inherited’, as is commonly done. (Obviously the three possibilities are not mutually exclusive.)

This hypothesis would accord with the observations outlined previously, on inheritance of the elements of the testicular dysgenesis syndrome. Exposure to a genotoxic agent would lead to some form of mutation; its survival in subsequent generations would depend on:

(i) the degree to which it affects health (including lethality at one extreme), at all stages of life from conception to the end of reproductive life;

(ii) the extent to which it affects biological fertility, the probability of achieving a fertilized ovum, given unprotected intercourse;

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**Fig. 1** Possible consequences of germ line genetic damage (reproduced with permission from Joffe\textsuperscript{14}).
(iii) additional factors that involve volition and control, as well as biology: contraception and achieved family size, and the use of artificial reproductive technology.

The epidemiological implication would be to introduce a degree of inertia into the time trend: whereas an increase in the health outcome would still directly follow an increase in the causal agent (allowing for latency), there would be a gap of some generations between their respective disappearance. At the individual level (e.g. in a cohort study), the relevant exposure would not necessarily be to a parent, as it could well be to a grandparent or earlier ancestors.

**Mechanism and genomic localization**

Is genetic damage a plausible mechanism? Cytogenetic abnormalities are more frequent among infertile men than in the general population\(^76\). More specifically, severe infertility is a consequence of micro-deletions in three non-overlapping regions of the Y chromosome AZF a-b-c\(^77\); they may arise by a common *de novo* event\(^78\). The generally poor semen quality in Denmark is not attributable to micro-deletions\(^79\), but more minor impairments, for example single-nucleotide polymorphisms (SNPs), could well lead to lesser degrees of impairment of semen quality, not only sperm concentration but also *e.g.* morphology\(^74\).

The Y chromosome is a likely target for genetic damage. The probability of mutation is increased by the rapid division of the germ cells, both in fetal and in adult life. In the former case the exposure would be to the pregnant mother, in the latter to the father before conception; either would result in a birth cohort effect. The Y chromosome is not shielded from a mutagenic environment, as are the other chromosomes, by long inert periods in the ovum\(^77\). The far higher number of cell divisions in
spermatogenesis compared with oogenesis has led to the hypothesis that evolution is ‘male driven’\textsuperscript{80}, and these provide extra opportunities for error. Furthermore, the Y chromosome may be unable to undergo DNA repair, as this depends on having an opposite number to pair with during cell division. Finally, as all its genes are haploid, defects in a single gene are likely to have effects\textsuperscript{77}—although this is complicated by the presence of multi-copy genes on the Y chromosome.

Genotoxicity can readily explain carcinogenesis, but more work is required to identify the particular gene(s) involved; a possible candidate on the Y chromosome is TSPY\textsuperscript{81}, or another nearby locus in the gonadoblastoma region\textsuperscript{82}. A deficit in male births could result from a selective effect on Y-chromosome-carrying spermatozoa, selective loss of male embryos or fetuses, or mutation of the sex-determining gene SRY on the Y chromosome\textsuperscript{54}.

Most cases of cryptorchidism and hypospadias are likely to have an endocrine rather than a genetic mechanism. However, this does not contradict the suggestion of a genetic aetiology, as the impairment may originate upstream, in the gene(s) that control(s) the more distal endocrine processes. The same two-stage (genetic-endocrine) principle could also play a part in the other manifestations of the testicular dysgenesis syndrome, including the hormone-sensitive processes underlying fertility.

The cluster of endpoints, all of them concerned with the male reproductive system, is not a coincidence. The gene determining male sex, and those controlling spermatogenesis and other male-reproduction-related functions, have migrated to the human Y chromosome in the course of evolution\textsuperscript{83}.

A possible causal agent would need to have exposure characteristics that correspond to the epidemiological observations—at least in part, as multi-factorial causation is almost certain to apply. It would need to be genotoxic, to be absorbed, and to localize in the testis. If female (\textit{in utero}) exposure were responsible, it would also have to cross the placenta. Male exposure could in principle affect the stem cells (a permanent effect), or the gamete during spermato- or spermio-genesis (a transient effect) in the weeks before fertilization; maximal damage would be seen after post-meiotic exposure, as elongating spermatids and spermatozoa no longer have the ability to undergo DNA repair or to initiate apoptosis\textsuperscript{84}.

The sperm chromatin stability assay (SCSA) has demonstrated DNA damage following chemical exposures, as well as heat stress\textsuperscript{85}, and this test is associated with fertility\textsuperscript{86}. Oxidative damage has been suggested as a mechanism\textsuperscript{87}, but it is unclear what agents could be responsible. Paternal smoking is one possibility, and has been linked with childhood cancer\textsuperscript{88}, but the epidemiological evidence does not suggest that it is implicated in impaired fertility\textsuperscript{89}.  

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One candidate agent is the heterocyclic amine PhIP, which occurs in meat or fish cooked at high temperatures, a powerful mutagen that has been implicated in colorectal\textsuperscript{90} and other cancers\textsuperscript{91}, and fulfils these criteria. Localization in the testis would occur because it binds to the oestrogen receptor\textsuperscript{92}. As meat consumption increased in the early decades of the 20th century with rising prosperity, it could be that in cultures whose cooking methods generate heterocyclic amines, and/or who lack protective exposures, PhIP-induced damage has increased.

**Conclusions**

There is little epidemiological information on trends or spatial variation in female infertility. Certain occupational exposures have been shown to impair female fertility, but the agents are not sufficiently widespread in the general environment to have any effect on the general population. As females are relatively sensitive to oestrogens, agents with oestrogenic activity should be considered in relation to disturbances in female reproductive function, for example precocious puberty. One reassuring finding is that couple fertility has increased in recent decades, but so far this is based on only one report.

While the observed deterioration in semen quality, and in other possibly-linked conditions affecting the male reproductive tract, have been widely discussed in relation to the ‘oestrogen hypothesis’, pollution with weak environmental oestrogens cannot plausibly be responsible. The anti-androgen variant of the endocrine disruption hypothesis, or androgen/oestrogen balance, may be important, but cannot explain the existing epidemiological findings. A hypothesis that deserves more detailed consideration is the role of dioxin and dioxin-like effects.

Several lines of evidence point towards genetic damage as an explanation of various types of impairment of the male reproductive system. In principle, this could arise through male or female exposure. Possibly the Y chromosome is especially important as a target for mutation. A genetic aetiology raises the possibility that additional health endpoints are also affected.

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