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MiniReview

Evidence for a Role of Paternal Exposures in Developmental Toxicity

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Abstract: Experimental evidence from radiation exposure, antimitotic drugs or chemicals such as pesticides or metals does suggest the possibility of transmission of paternally mediated developmental effects across generations. The mechanistic framework is growing with suggestion of transmission of epigenetic modifications as a mechanism alternative to germ-line mutagenesis. There is also ample experimental evidence for a specific susceptibility of the male embryo to the action of endocrine disrupters. In parallel, interpretation of epidemiological findings regarding effects of well-characterized paternal exposures, such as ionizing radiation or persistent organic pollutants (dioxins), on intrauterine development remains equivocal. Many epidemiological studies have included paternal exposures as an add-on to existing studies and focused mainly on birth defects, sex ratio, childhood cancers or spontaneous abortions. Functional alterations such as neurobehavioural parameters or reproductive dysfunction resulting from paternal exposure have been barely studied. Improved knowledge on possible consequences of paternal exposures in future generations is needed and has strong implication in terms of regulation, in the workplace for instance. One may expect human studies to be conducted with a particular focus on male-mediated developmental toxicity making use of biological markers pertinent to hypothesized mechanisms. Recognition of early determinants of disease onset has led to the setup of a number of mother–child cohorts across the world and careful assessment of paternal exposures should be included in these studies. These cohorts will also have the power to evaluate the specific impact of *in utero* exposure on a number of endpoints of developmental toxicity in males.

The study of the influence of male exposure on the reproductive process has long been limited to its impact on fertility. Occupational exposures to lead [1] or to the insecticide dibromochloropropane [2] and their consequences on male fertility have raised consciousness about the vulnerability of the male reproductive system to environmental exposures. Establishing the causal role of occupational exposure in these infertility problems was facilitated by the short latency between exposure and effects, and by the severity of the observed effects. It is only later, in the 1990s, that the possible wider impact of male exposures at environmental levels on developmental alterations has been considered, concretized by the setting of international symposia discussing available evidence [3]. This occurred in parallel to the onset of a new hypothesis of a particular susceptibility of the male embryo to the action of so-called endocrine disrupters, possibly leading to transgenerational effects on the reproductive system [4].

The length of the spermatogenic cycle in human beings, undergoing a number of developmental phases (mitosis, meiosis, differentiation and maturation) of various durations, starting with spermatogonial stem cells until the production of differentiated motile spermatozoa, lasts about 74 days [5]. This period of intense cellular transformation is considered to be highly susceptible to environmental insults. The germ-cell stage(s) affected can be determined by examining the time between acute exposure (to a drug for instance) and conception: spermatogonia have been recognized as target cells for the action of ionizing radiation or dibromochloropropane, while some glycol ethers such as 2-methoxyethanol are considered spermatocyte toxins [6]. Except for some ecological disasters, environmental exposure of human populations is essentially chronic, from occupational environment, air pollution, lifestyle including diet, and the mechanisms involved have to be inferred from experimental models. Therefore, much has been learned from some unfortunate quasi-experimental situations in which human beings, both males and females, have been heavily exposed at one point in time to ionizing radiation (Hiroshima, Nagasaki, Chernobyl), dioxins/furans (Seveso, Yucheng) or drugs (diethylstilboestrol).

In human beings, gonadal sex determination and testis development occurs between 8 and 14 weeks of gestation and is hypothesized to be a period particularly sensitive to environmental insult from agents with oestrogenic or anti-androgenic activities [4]. One can then consider two different situations (models) of vulnerability for the male reproductive system with potential consequences on embryonic or child development: (i) exposure of a male adult before mating; (ii) prenatal exposure during embryonic development of the

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Table 1.

Paternal occupational exposure and risk of spontaneous abortions (SA) or birth defects (BD).

Exposure	Outcomes	Strength of the association RR (95% CI)	Reference
Metals			
Lead	SA	Moderate	Savitz et al. [11]
Mercury	SA	Strong	
Anaesthetic gases	SA	Strong	Savitz et al. [11]
Laboratory work	BD	1.30 (0.8–21)	Magnusson et al. [14]
Solvents			
	SA	Moderate	Savitz et al. [11]
		1.30 (0.8–2.1)	Logman et al. [12]
Painters		1.1 (0.4–2.7)	Hooiveld et al. [13]
	BD	1.86 (1.4–2.5)	Logman et al. [12]
		6.2 (1.4–28)	Hooiveld et al. [13]
Pesticides			
	SA	Weak	Savitz et al. [11]

CI, confidence interval; RR, risk ratio.

reproductive system of male conceptus. Endpoints of male-mediated developmental toxicity in subsequent generations that have been considered in both animal and human studies are: embryonic losses, birth defects, (secondary) sex ratio and tumour incidence. Although there is experimental evidence of functional alterations, such as altered reproductive potential or behavioural deficits resulting from paternal exposures, these consequences have been barely studied in human beings [7].

Paternal preconceptional exposure: some animal evidence

Extensive research has been conducted on the impact of male exposure to the anticancer drug *cyclophosphamide* on progeny. Effects on embryonic loss, malformations, tumour incidence and behavioural abnormalities in several generations have been observed at doses similar to those used in clinical regimens [5]. Subsequent studies failed to replicate initial findings by Nomura [8] showing that paternal *irradiation* can induce germ-line mutations leading to increased tumour risk in subsequent generations [9]. A number of experiments have also suggested enhanced susceptibility to cancer following exposure to carcinogens such as urethane or methylnitrosourea after subchronic paternal irradiation [8]. Male pre-conceptional exposure to *ethylnitrosourea* or *urethane* have been shown to induce malformations and tumours in several generations, while the industrial compound *1,3-butadiene* has been associated with embryonic loss [3,7,9]. Male exposure to low levels of a non-mutagenic compound such as *lead* has been shown, in the absence of testicular damage, to impair learning behaviour and mating behaviour in the F_1 generation of rodents [10].

Paternal preconceptional exposure: human evidence

Spontaneous abortions or birth defects.

Evidence regarding the association between paternal occupational exposures and the risk of spontaneous abortions or birth defects in subsequent pregnancies has been summarized in 1994 by Savitz [11] (table 1). Evidence for an association with the risk of spontaneous abortions was considered strong for metallic mercury and anaesthetic gases in use at that time, moderate for lead and weak for pesticides. The likelihood of a causal association between paternal exposure to solvents and spontaneous abortions was considered moderate. Since then, two studies among workers exposed to solvents including painters have not reinforced the strength of evidence for an impact on spontaneous abortions but showed increased risks of birth defects in offspring of fathers exposed to solvents [12,13]. A recent study of laboratory workers reported a moderately increased risk of birth defects [14].

Childhood cancer.

A possible association between childhood cancer and paternal occupational exposure has been the subject of a large number of investigations since the early publication by Fabia and Thuy in 1974 [15] suggesting the impact of paternal exposure to hydrocarbons on the risk of childhood brain tumours. Many of these investigations were registry-based case–control studies using occupation of the father as a proxy of exposure, sometimes completed by indirect exposure assessment by means of job-exposure matrices or expert evaluation. Overall, recent studies do not confirm the association of leukaemia risk with paternal exposure to solvents or to exhaust hydrocarbons suggested in earlier studies as reviewed by Colt and Blair [16] (table 2). Increased risk of brain tumours has been reported among children of men exposed to paints and pigments in a number of studies (table 3), while findings are inconsistent regarding the association with paternal exposure to hydrocarbons or exhaust fumes. Electromagnetic fields have been postulated to be responsible for an increased risk of brain tumours explaining excess risks repeatedly reported among children of electricians, electronic workers or power linemen [16]. This association does not appear to be confirmed in recent studies [19–21].

Table 2.

Paternal occupational exposure to solvents and exhaust fumes and risk of child leukaemia/lymphoma.

Main exposure	Results	OR (95% CI)	Reference
Solvents	5 studies (1989–1992)	OR > 2	Colt and Blair [16]
		0.8–1.3 (ns)	Shu et al. [17]
		1.0	Schüz et al. [18]
		1.25 (0.8–2.0)	Feychting et al. [19]
		1.07 (0.8–1.4)	McKinney [20]
Exhaust fumes	12 studies (1974–1993)	OR > 1(s) in 6	Colt and Blair [16]
		0.7 (0.5–1.1)	Shu et al. [17]
		0.8 (0.5–1.6)	Feychting et al. [19]
		1.3 (1.1–1.6)	McKinney et al. [20]

CI, confidence interval; ns, not statistically significant; OR, odds ratio; s, statistically significant.

The interpretation of these studies is weakened by the imprecision in exposure assessment (both in terms of identification of compounds and timing of exposure) and the potential simultaneous exposure of the mother. This is particularly true when trying to interpret studies relative to pesticide exposure (table 4).

Childhood cancer and paternal radiation exposure.

Considerable work has been generated in order to prove the hypothesis of a link between radiation exposure of the fathers and the risk of cancer in their children after the publication by Gardner et al. [28] of a cluster of childhood leukaemias and lymphomas near a nuclear power plant in Sellafield in the UK. This original report was not confirmed by five subsequent studies among nuclear plant workers in the UK and Canada conducted between 1991 and 1996 and in a large case–control study on childhood leukaemia conducted in Germany [29]. Recently, records of 34,538 childhood cancer cases diagnosed in the UK between 1952 and 1986 were linked with the registry of radiation workers (161 fathers retrieved) using updated dosimetric data. The analysis showed a large association between the risk of leukaemia and non-Hodgkin's lymphoma in the offspring with paternal employment as a radiation worker at the time of conception, but not with preconception radiation dose [30]. This finding is interpreted by the authors as an argument in favour of the infectious aetiology hypothesis to explain clusters of childhood leukaemia observed around nuclear plants, arising from population mixing. Scepticism about this alleged association was also reinforced by the absence of an increased risk of leukaemia/non-Hodgkin's lymphoma (RR = 0.84; 95% CI: 0.3–2.4) among children of fathers exposed in Hiroshima and Nagasaki (mean dose estimated at 400 mSv) [31]. Children of parents exposed to radiation around Chernobyl, however, show an elevated level of minisatellite mutations arising from the germ line of exposed fathers, but not from the germ line of exposed mothers [32]. These observations in human populations suggest differential effects according to the rate of exposure (acute, chronic or subchronic) that is in accordance with evidence from experimental studies.

Secondary sex ratio.

Another debate originated from the publication by Mocarelli et al. [33] of a decreased sex ratio at birth (secondary sex ratio – SSR) (% of male births lower than expected) following paternal exposure to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in Seveso, Italy (table 5). Paternal serum TCDD levels were above 15 ppt. No impact of maternal exposure on SSR was observed. Other epidemiological studies have later investigated this issue with mixed results. American

Table 3.

Paternal occupational exposure to solvents, hydrocarbons and electromagnetic fields and risk of childhood brain cancer.

Main exposure	Results	OR (95% CI)	Reference
Paints and pigments	5 studies (1981–1992)	4 with OR > 2	Colt and Blair [16]
Occupation		1.2 (0.8–2.0)	Cordier et al. [21]
Occupation		3.7 (1.7–7.8)	Feychting et al. [19]
Exposure to solvents		1.2 (0.7–1.9)	
Hydrocarbons	8 studies	4 with OR > 1	Colt and Blair [16]
Oil products		2.5 (1.2–5.1)	Smulevich et al. [22]
PAH		0.96 (0.5–1.8)	Feychting et al. [19]
Exhaust fumes		1.1 (0.8–1.5)	McKinney et al. [20]
PAH		1.4 (1.1–1.7)	Cordier et al. [23]
Electromagnetic fields	6 studies	OR > 1	Colt and Blair [16]
	Estimated exposure >0.3 μT	0.5 (0.3–1.0)	Feychting et al. [19]
	Electrical workers	1.1 (0.9–1.5)	Cordier et al. [21]
		1.04 (0.7–1.5)	McKinney et al. [20]

CI, confidence interval; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons.

Table 4.

Paternal occupational exposure to pesticides and risk of childhood cancer.

Main exposure	Results	OR (95% CI)	Reference
Leukaemia			
Pesticides	12 studies (1978–1996)	OR > 1	Zahm and Ward [24]
		1.7 ns	Buckley et al. [25]
Chlorophenate fongicides		1.0 (0.5–1.8)	Heacock et al.[26]
		0.9 (0.4–2.2)	Feychting et al. [19]
		1.6 (1.1–2.3)	Meinert et al. [27]
		0.8 (0.6–1.2)	McKinney et al. [20]
Brain cancer			
Pesticides	7 studies (1974–1996)	4 with OR > 1	Zahm and Ward [24]
Chlorophenate fongicides		1.3 (0.6–2.5)	Heacock et al. [26]
		1.3 (1.0–1.8)	Cordier et al. [21]
		2.4 (1.3–4.4)	Feychting et al. [19]
		0.8 (0.4–1.4)	McKinney et al. [20]

CI, confidence interval; OR, odds ratio.

veterans exposed to Agent Orange during defoliant sprayings in Vietnam (Operation Ranch Hand) with a level of exposure to TCDD of the same order of magnitude as in Seveso, Italy, had slightly more boys than girls [34]. Two recent studies of pesticide producers (2,4,5-trichlorophenoxy acetic acid or trichlorophenol) in the USA [35] or in Russia [36] with much higher exposure levels showed discordant findings. Other persistent organochlorine compounds such as polychlorobiphenyls (PCB) have also been studied in particular in the populations highly exposed to contaminated rice oil in Yusho and Yucheng. A decreased SSR following fathers' exposure before the age of 20 was observed in Yucheng [37] but no effect was seen in Yusho [38]. A study conducted among Michigan fish eaters exposed to PCBs showed an increased rate of male births following paternal exposure >8 μg/l [39]. Several possible mechanisms could explain an association between paternal exposure and SSR in their offspring: one is a pre-conceptional impact of toxicants on the proportion of Y-bearing spermatozoa; another would be a particular sensitivity of male embryos leading to differential pregnancy loss. A recent study conducted in four groups of males from Sweden, Greenland, Poland and Ukraine with very different blood levels of PCB-153 and p,p′-DDE showed a positive correlation between the proportion of Y-bearing chromosomes and PCB-153 and p,p′-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] levels in Sweden, but a negative correlation with PCB-153 level in Poland. This study suggests an impact of exposure to persistent pollutants on the proportion of ejaculated Y-bearing spermatozoa, but with varying direction [40]. This is in accordance with experimental evidence showing until now, inconsistent findings on sex ratio of the offspring of male rats exposed to TCDD *in utero* [41].

Table 5.

Paternal exposure to persistent organic pollutants and proportion of male births.

Seveso (1977)		
Fathers' serum TCDD > 15 ppt	M ↓	P = 0.03
No diminution in sex ratio with high maternal exposure	Mocarelli et al. [33]	
Vietnam (Operation Ranch Hand)		
Fathers' serum TCDD > 10 ppt	M ↑	ns
	Michalek et al. [34]	
US pesticide producers		
Fathers' median serum TCDD = 250 ppt	M ↔	
	Schnorr et al. [35]	
Russian pesticide producers		
Fathers' median serum TCDD > 240 ppt	M ↓	P < 0.05
	Ryan et al. [36]	
Yucheng (1979)		
Father's exposed to PCBs before age 20	M ↓	P = 0.02
No association with maternal exposure	Del Rio Gomez et al. [37]	
Yusho (1968)	M ↔	ns
	Yoshimura et al. [38]	
Michigan fish eaters		
Fathers exposed to PCBs > 8 μg/l	M ↑	P < 0.05
No association with DDE	Karmaus et al. [39]	

DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; M, proportion of male births; ns, not statistically significant; P, degree of significance; PCB, polychlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzodioxin.

Prenatal exposure during the embryonic development of the male reproductive system.

In 2005, Anway et al. [42] published a startling report showing that consequences of exposure of pregnant rats to relatively high levels of the insecticide methoxychlor and the fungicide vinclozolin lasted for four subsequent generations with the same magnitude. It affected mostly males showing an impact on fertility, prostate and kidney disease and immune abnormalities. No major effect was noticed among females [43]. A recent report from the same team presented the results of exposure of pregnant rats to vinclozolin on mating preference three generations later: F3 females preferred males with no history of exposure while no preference was exhibited by F3 males [44]. These experiments, if confirmed, suggest transgenerational transmission of epigenetic changes affecting especially males.

There are not many human counterparts of the observations described above. Lessons can therefore be drawn from

the study of health effects induced by *in utero* exposure to diethylstilboestrol (DES). A large number of pregnant women from the late 1940s to the 1970s received prescriptions of DES, a synthetic non-steroidal compound with oestrogenic activity thought to prevent miscarriage and other complications of pregnancy. This exposure was soon related to an increased risk of a very rare form of cancer of the reproductive tract (vaginal adenocarcinoma) among daughters exposed *in utero* [45]. Studies in human beings and results of DES-exposure experimental models concur to observe effects, on both males and females, such as gonadal dysfunction, reproductive tract defects and tumours of the reproductive tract. Recent epidemiological reports have suggested an increased risk of hypospadias among sons of women exposed to DES *in utero* [46,47]. Apart from these last observations, to be confirmed, there is no indication of a specific vulnerability, transmissible to subsequent generations, of males exposed *in utero* to DES.

Conclusion

There is ample evidence of male-mediated developmental toxicity in experimental models, and some evidence of transgenerational effects, showing a particular sensitivity of the male germ line to these transmissible effects. Putative mechanisms have been proposed including direct toxic action such as contamination through the seminal fluid, and both genetic and epigenetic pathways including germ-cell mutation, induction of germ-line genomic instability, suppression of germ-cell apoptosis or interference with genomic imprinting [5,10]. Human evidence on the other hand is still not convincing. Most studies have focused on birth defects, sex ratio, childhood cancers or spontaneous abortions in association with occupational paternal exposures. Functional alterations such as neurobehavioural parameters or reproductive dysfunction have been barely studied.

Thus, there is a need for innovative study designs especially aimed at studying the role of paternal exposures on child development and going beyond the study of fertility. An example of such design is the Danish First Pregnancy Planner Study following couples starting before conception until pregnancy onset, detecting early embryonic losses [48]. An ideal design should follow several generations. Recognition of early determinants of disease has led to the setup of a number of mother–child cohorts across the world and careful assessment of pre-conceptional paternal exposures should be included in these studies. These cohorts will also have the power to evaluate the specific impact of intrauterine exposure on a number of endpoints of developmental toxicity in males.

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