

ENVIRONMENTAL FACTORS AND SEMEN QUALITY

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Abstract

Objectives: An increasing number of reports suggest that chemical and physical agents in the environment, introduced and spread by human activity, may affect male fertility in humans. This article aims at evaluating the impact of environmental exposures (pesticides, phthalates, PCBs, air pollution, trihalomethanes (THMs), mobile phones) on semen quality, by reviewing most recent published literature. **Materials and Methods:** Epidemiological studies focusing on exposure to environmental factors and semen quality for the last ten years were identified by a search of the Pubmed, Medline, Ebsco, Agricola and Toxnet literature bases. **Results:** The results from the presented studies suggest that there are strong and rather consistent indications that some pesticides besides DBCP (e.g. DDT/Dichlorodiphenyldichloroethylene [DDE], ethylenedibromide, organophosphates) affects sperm count. PCBs are detrimental to sperm motility. In case of air pollution, studies suggest a link between ambient air pollutants and various semen characteristics. Additional research is needed to corroborate this association and to establish the causal agents. Results of few studies on subfertile men demonstrate associations between phthalate levels commonly experienced by the public and impaired sperm quality (impact on sperm concentration, morphology, motility), but the findings have not been corroborated in studies of men from the general population. Mobile phones might adversely affect the quality of semen by decreasing mostly motility but also the sperm counts, viability and morphology. In spite of their consistent results, most of the studies are rather small. Association between exposure to THMs and poor semen quality was not observed. **Conclusions:** Epidemiological studies suggest awareness of environmental factors which may affect semen quality. In case both of well proven and disputable reproductive and developmental hazards, it is necessary to prevent parental exposure to the agents associated with those hazards.

Key words:

Environmental factors, Semen quality, Environmental exposure

INTRODUCTION

Male reproductive function in the general population has attracted increasing attention due to reports indicating increased occurrence of testicular cancer, cryptorchidism and hypospadias across some time periods in some populations past 50 years [1,2]. Reports indicating declining sperm counts in some regions [3,4] have also greatly

stimulated hypotheses that environmental pollutants may impair male fertility [5].

The discovery in 1977 of the severe spermatotoxic effect of the nematocide dibromochloropropane (DBCP) among workers at a chemical plant [6] initiated several studies of occupational and environmental risks to male reproductive function. Although for several decades semen quality has been used as a marker of male reproductive func-

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tion in case of environmental exposure, the data are still limited [7,8].

An obvious consequence of exposure to reproductive toxicants is infertility. Infertility is defined as inability to conceive after a year of sexual intercourse without the use of contraceptives. A male contributory factor is involved in approximately half of these cases [9], but most of the causes of reduced semen quality and other disturbances of male reproductive function are unknown [10].

Current studies, as reviewed by Sheiner et al. (2003) [11] and Jensen et al. (2006) [12] show that variety of environmental and occupational exposures may impair male fertility. During past years male reproductive function has been addressed in relation to a number of environmental exposures that have only to a very limited extent been investigated or reviewed earlier. These exposures and conditions include air pollution and drinking water pollutants, biopersistent organochlorines, trihalomethanes, phthalates and high frequency electromagnetic radiation related to use of mobile phones. The objective of this paper is to review the literature in order to update current state of the art knowledge on hazards to male reproductive function.

MATERIALS AND METHODS

Epidemiological studies focused on the environmental factors and male fertility were identified by a search of the Pubmed, Medline, Ebsco, Agricola and Toxnet literature databases. Hand search was a second search method used to explore the references of retrieved articles. The combination of key words used were: semen quality, environmental exposure, air pollution, exposure to: phthalates, persistent organochlorine pollutants, tap water, mobile phones and pesticides. From each study, the following information was abstracted: study population, type of outcome (sperm count, volume, concentration, sperm density, semen motility, morphology, aneuploidy, level of sexual hormones), type of exposure and methods used for its assessment (including biomarkers). Finally in this review were included human studies published in English in peer reviewed journals with original information on links

between a given environmental exposure and measures of male reproductive function in terms of semen quality for the last ten years. The period was chosen to reflect findings over the past ten years during which new techniques have emerged for measuring exposures and health effects in reproductive and environmental epidemiology studies.

RESULTS

Air pollution

The major air pollutants in Europe and North America are sulphur dioxide (SO₂), nitrogen oxides (NO_x), particulate matter (PM) and ozone (O₃). Air pollutants can be in the form of solid particles, liquid droplets, or gases. In addition, they may be natural or man-made. Sources of air pollution refer to the various locations, activities or factors which are responsible for the releasing of pollutants into the atmosphere.

Several studies addressing links between ambient air pollution and semen quality have been published past few years in the Czech Republic [13–15] and in USA, Los Angeles [16] (Table 1). Two hundred seventy two young Czech men exposed to high levels of air contaminants in the Teplice region were more likely to have abnormal sperm morphology and sperm chromatin structure than were those who lived in a city (Prachatice) with less air pollution. The authors observed a reduced percentage of sperm with normal morphology and proportionately more sperm with abnormal chromatin in men from Teplice region [13]. The other study conducted in the same region of Czech Republic investigated whether the polluted air in the Teplice district is related to abnormal morphology in males living in this district [14]. More than 300 men living in the Teplice district and in the control district of Prachatice were examined between 1992 and 1994, in autumn and at the end of winter. Significantly increased frequency of sperm with abnormal morphology and reduced motility was observed in men with medium and high exposure to air pollution. More intensively exposed males also had significantly higher frequency of disomy in chromosomes X, XY, and Y [14]. The same young men from Teplice were sampled up to seven times over 2 years, allowing

Table 1. Semen quality and air pollution

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
Czech Republic 215 young men (18 years of age) from Teplice region (industrialized district) and 193 from Prachatice region (rural district)	Prospective cohort	Air pollution data were obtained from air monitoring stations	Semen volume, sperm concentration, total number of sperm per sample, percentage of motile sperm, percentage of sperm with normal morphology and percentage with normal head morphology	Seasonality, age at donation, smoking	Reduced percentage of sperm with normal morphology and proportionately more sperm with abnormal chromatin in men from Teplice region was observed	[13]
Czech Republic 325 males 18-year-old living in the Teplice district (industrialized district) and in the control region of Prachatice (rural district)	Prospective cohort	Air pollution data were obtained from air monitoring stations	Semen volume, pH, motility, number and morphology of spermatozoa, aneuploidy	Seasonality, age at donation, smoking	Significantly increased frequency of sperm with abnormal morphology and reduced motility was observed in men with medium and high exposure for air pollution. More intensively exposed males also had significantly higher frequency of disomy in chromosomes X, XY, and Y	[14]
Czech Republic 36 young men (18 years of age) from Teplice region (industrialized district)	Prospective cohort	Air pollution data were obtained from air monitoring stations	Sperm concentration, sperm count, volume, motility, sperm morphology, aneuploidy	Seasonality, age at donation, smoking	A significant association was found between exposure to periods of high air pollution (at or above the upper limit of US air quality standards) and the percentage of sperm with DNA fragmentation according to sperm chromatin structure assay (SCSA). Other semen measures were not associated with air pollution.	[15]
United States, California 48 semen samples collected from sperm donors	Cross-sectional	Air pollution levels (ozone, nitrogen dioxide, carbon monoxide and particulate matter) were obtained from air monitoring stations	Semen volume, sperm concentration, motility, sperm morphology	Date of birth, seasonality, age at donation	A significant negative correlation between ozone levels at 0–9, 10–14 and 70–90 days before donation and average sperm concentration was observed	[16]

evaluation of semen quality after periods of exposure to both low and high air pollution. Using repeated measures analysis, a significant association was found between exposure to periods of high air pollution (at or above the upper limit of US air quality standards) and the percentage of sperm with DNA fragmentation according to sperm chromatin structure assay (SCSA). Other semen measures were not associated with air pollution [15]

The relationship between air pollutant levels and semen quality was also evaluated over 2-year period in Los Angeles, by analysing repeated semen samples collected by sperm donors. Semen analysis data derived from 48 semen samples provided by sperm donors were correlated with air pollution level (ozone, nitrogen dioxide, carbon monoxide, particulate matter). There was a significant negative correlation between ozone levels at 0–9, 10–14 and 70–90 days before donation and average sperm concentration, which was adjusted for donor's birth date, age at donation, temperature and seasonality [16]. However, several occupational studies of TIG (tungsten inert gas) welders with exposure levels to ozone that are several orders of magnitude higher have not revealed reduced sperm counts in exposed men [18].

Summing up, while some studies suggest a link between ambient air pollutants and various semen characteristics like reduced percentage of sperm with normal morphology and proportionately more sperm with abnormal chromatin and reduction of motility, additional research is needed to corroborate this association and to establish the causal agents.

Organochlorine contaminants, dioxins and polychlorinated biphenyls

Organochlorines were widely used worldwide from 1940 through 1970s, but most have been eliminated or restricted in use after recognition of their persistence in the environment, bioaccumulation in animals and humans and toxicity in laboratory animals and wildlife.

Exposure mainly occurs through ingestion of contaminated food, but can also occur through dermal contact and inhalation. A time-related deterioration in male reproductive function caused by exposure to persistent organochlorines

(POCs), has been hypothesized. In animal studies, POCs were found to have adverse effects on male reproductive function. The studies on exposure to organochlorine compounds and semen quality suggested association between polychlorinated biphenyls (PCBs) exposure and poor sperm quality. The findings of the pilot study carried out in the Boston area population were also indicative of an association between PCBs and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and abnormal sperm count, motility and morphology [19]. The total motile sperm counts were inversely proportional to the PCB concentrations and were significantly lower among infertile male than those of the controls [20]. Also, in a study performed by Dallinga et al. among 65 males with fertility problems, sperm count and motility were inversely related to the sum of PCB congeners [21] (Table 2).

A strong and monotonically increasing DNA fragmentation index with increasing serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) was found in a study performed by Spanò et al. (2005) [22]. Sperm motility was also inversely related to the lipid adjusted serum concentration of CB-153 among men in each of four regions (Greenland, Sweden, Warsaw and Ukraina), which is consistent with several earlier findings and results of animal studies [23] (Table 2). Thus, there is fairly strong evidence to suggest that persistent organic pollutants interfere with sperm motility, although the causal agent cannot be identified because of a strong correlation in serum concentration of a wide number of biopersistent pollutants [24].

In a study of young fishermen from the coastal stretches of Sweden prostate-specific antigen (PSA), neutral α -glucosidase (NAG), fructose and zinc levels were analysed. There was a significant linear association between CB-153 and total amount of PSA. With age, abstinence time and smoking included in the model the association became non-significant [25]. The same author performed also study among 176 Swedish fishermen (with low and high consumption of fatty fish). A significantly lower % DNA fragmentation index (DFI) was found in the lowest CB-153 quintile (<113 ng/g lipid) compared with the other quintiles; there was a similar tendency, although not statistically significant, between % DFI and

p,p'-DDE [26]. In the other cross-sectional study in Sweden, negative correlations between CB-153 levels and both the testosterone and sperm motility were found [27] (Table 2).

The results of the presented studies suggest that there is an association between exposure and poor sperm quality, especially an inverse relationship was found between sperm motility and the concentration of PCBs, while other effects (sperm count and morphology) are rather uncertain. Increasing DNA fragmentation index with increasing serum levels of CB-153 was also found. On the other hand, no effects of concentration of CB-153 and p,p'-DDE on sperm concentration and prostate-specific antigen (PSA) were observed.

Trihalomethanes in tap water

Trihalomethanes (THMs) are a byproduct of the water treatment process. They are formed when natural organic material reacts with chlorine used to treat the water. This reaction produces "disinfection by-products" (DBPs) the most common of which are trihalomethanes (THMs). THMs such as chloroform, bromoform, chlorodibromomethane, bromodichloromethane, are the most prevalent and routinely measured class of DBPs found in the water [27]. Routes of exposure to THMs include dermal absorption during hand washing and bathing, inhalation during showering and ingestion of drinking water [28].

Animal studies have consistently demonstrated an association between oral exposure to DBPs including haloacetic acids (HAAs) and trihalomethanes and adverse effects in male reproductive system: acute spermatotoxicity, impaired reproductive competence, sperm quality [29], delayed spermiation and distorted sperm motility and morphology [30], histopathologic changes in testis and epididymis [31], transient subfertility [32], altered sperm production and epididymal tubule changes [33].

Contrary to large amount of evidences on the detrimental effects of DBPs on male reproductive function in animals, two so far completed human studies have not supported these findings [34,35] (Table 3). In one cohort study performed in United States the semen quality was evaluated among 228 fertile men with different profiles of exposure

to DBPs. Exposure to DBPs was evaluated by incorporating data on water consumption, bathing and showering with concentrations measured in tap water. No consistent pattern was found of increased abnormal semen quality (sperm concentration and sperm count) with elevated exposure to trihalomethanes or haloacetic acids [34]. In the second study the relationship between THMs and semen quality was examined in 157 healthy men from couples without known risk factors for infertility. Total THM levels were assigned based on water utility measurements taken during the 90 days preceding semen collection. THM level was not associated with decrements in semen quality. Only for motility, a small decrease for every unit increase in bromodichloromethane exposure level was found [35].

Based on presented results, there seems to be no association between exposure to THMs and poor semen quality (sperm concentration and sperm count). As the number of studies is limited, further studies of the effect of THMs on semen quality are needed.

Pesticides

Pesticides form a large group of heterogeneous chemicals, which are used to kill insects, weed, fungi and rodents. On the one hand these substances bring a significant public health benefit by increasing productivity in the food industry and decreasing the incidence of diseases.

Pesticides in general may directly damage spermatozoa, alter Steroli cell or Laydig cell function, or disrupt the endocrine function in any stage of hormone regulation (hormone synthesis, release, storage, transport and clearance, receptor recognition and binding) [36]. Clear effects on male fertility have been demonstrated for some pesticides: dibromochloropropane [4], ethylene dibromide [37], organophosphorus [38,39], alochlor, metochlor, 2,4-D, atrazine [40], fenvalerate [41], carbaryl, chlorpyrifos [42].

The results of the recent studies performed in Denmark, China and Mexico indicated that exposure to pesticides (however not confirmed by exposure measurements) increased the risk of specific morphological abnormalities of the sperm and decreased sperm count per ejaculate and the percentage of viable sperm. No effects of pesticide

Table 2. Exposure to organochlorine contaminants, dioxins, polychlorinated biphenyls and semen quality

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
United States 29 subjects from Massachusetts General Hospital/Andrology Laboratory including 18 subjects with normal spermatozoa	Cross-sectional pilot study	Blood serum samples were analysed for PCB, p,p'-DDE and HCB	Sperm concentration, sperm count, volume, motility, sperm morphology	Age, smoking, abstinence time	An association between PCBs and p,p'-DDE and abnormal sperm count, motility and morphology was detected	[19]
India 21 infertile men with "unexplained male factor" and 32 fertile controls were examined	Cross-sectional	PCBs were estimated in seminal plasma	Total motile sperm count	Age, smoking, abstinence time, diet	PCBs were detected in seminal plasma of infertile men but absent from controls. Sperm quantity and quality were significantly lower in infertile men compared to controls. The highest average PCB concen- trations were found in fish-eating urban dwellers, followed in suc- cession by fish-eating rural dwellers, non fish-eating urban dwellers and non fish-eating rural dwellers. The total motile sperm counts were inversely proportional to the PCB concentrations and were signifi- cantly lower than those of the respective controls	[20]
Netherlands A total of 65 male counterparts of couples visiting the Maastricht University Hospital for fertility treatment were selected for this study. 31 men with normal sperm quality and 34 with very poor semen quality	Cross-sectional	Blood samples were investigated with regard to organochlorine compounds: hexachlorobenzene (HCB), p,p'-DDE:1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene p,p'-DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethylene, 2,3',4,4',5-pentachlorobiphe- nyl (PCB-118), 2,2',4,4',5,5'-hexachlo- robiphenyl (PCB-153), 2,2',3,4,4',5-hexachlorobiphenyl (PCB-138) and 2,2',3,4,4',5,5'-hepta- chlorobiphenyl (PCB-180)	Sperm volume, sperm concentration (sperm count), overall and pro- gressive motility and mor- phology	Age, smoking, abstinence time, diet,	Focusing on the subgroup of men with normal semen quality showed that sperm count and sperm pro- gressive motility were inversely re- lated to the concentrations of PCB metabolites within that group	[21]

Greenland, Sweden, Poland, Ukraine 707 adult males (193 Inuits from Greenland, 178 Swedish fishermen, 141 men from Warsaw, Poland, and 195 men from Kharkiv, Ukraine)	Cross-sectional	Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), as a proxy of the total PCB burden, and of p,p'-DDE were determined	Sperm chromatin structure assay (SCSA) was used to assess sperm DNA/chromatin integrity	Lifestyle, diet, abstinence	[22] Increasing DNA fragmentation index with increasing serum levels of CB-153 among European but not Inuit men, reaching a 60% higher average level in the highest exposure group. No significant associations were found between SCSA-derived parameters and p,p'-DDE serum concentrations
Greenland, Sweden, Poland, Ukraine 763 men from all regions in Greenland (n = 194), fishermen from Sweden (n = 185), inhabitants of the city of Kharkiv, Ukraine (n = 195), and inhabitants of the city of Warsaw, Poland (n = 189)	Cross-sectional	Serum concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE) were examined	Sperm chromatin structure assay (SCSA) was used to assess sperm DNA/chromatin integrity	Lifestyle, diet, abstinence	[23] Sperm motility was inversely related to CB-153 concentration in Greenland and the Swedish fishermen population. Across all 4 regions, the sperm motility decreased on average by 3.6% (95% confidence interval = 1.7% to 5.6%) per one unit increase in the log of blood CB-153 (ng/g lipid). The concentration of p,p'-DDE was negatively associated with sperm motility in the Greenlandic population and in the compiled dataset
Sweden 157 fishermen from the coastal stretches of Sweden, aged 27-67 years		Serum levels of CB-153 and p,p'-DDE were determined.	Prostate-specific antigen (PSA), neutral α -glucosidase (NAG), fructose and zinc levels	Age, abstinence time, smoking	[25] There was a significant linear association between CB-153 and total amount of PSA. With age, abstinence time and smoking included in the model the association became non-significant
Sweden 176 Swedish fishermen (with low and high consumption of fatty fish)		Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and p,p'-DDE were examined	Sperm chromatin structure assay (SCSA) was used to assess sperm DNA/chromatin integrity	Lifestyle, diet, abstinence	[26] A significantly lower %DFI was found in the lowest CB-153 quintile (< 113 ng/g lipid) compared with the other quintiles; there was a similar tendency, although not statistically significant, between %DFI and p,p'-DDE

Table 2. Exposure to organochlorine contaminants, dioxins, polychlorinated biphenyls and semen quality — cont.

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
Sweden 305 young Swedish men 18-21 years old from the general population		Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153)	Sperm concentration, total sperm count, sperm motility assessed manually and with a computer-aided sperm analyser (CASA), and serum levels of follicle-stimulating hormone, inhibin b, testosterone, sexual hormone-binding globulin (SHBG), luteinizing hormone, and estradiol	Lifestyle, diet, abstinence	Negative correlations between CB-153 levels, testosterone and sperm motility	[27]

Table 3. Exposure to trihalomethane (THM) and semen quality

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
United States 228 fertile men	Cohort study	Water was sampled at frequent intervals (weekly or biweekly) at a representative location and analysed for THM4, HAA9 and TOX concentrations that were indeed representative of the entire system on that day	Sperm concentration, count, morphology, DNA integrity, chromatin maturity	Age, smoking, alcohol drinking, education level, season, vitamin intake, coffee drinking, race	No consistent pattern was found of increased abnormal semen quality (sperm concentration and sperm count) with elevated exposure to trihalomethanes	[34]
United States 157 healthy men from couples without known risk factor for infertility	Cross-sectional	Total THM (THM) levels were assigned based on water utility measurements taken during the 90 days preceding semen collection	Sperm concentration, sperm count, volume, motility, sperm morphology	Age, smoking, alcohol drinking, education level, season, coffee drinking	THM level was not associated with decrements in semen quality. Only for motility, a small decrease for every unit increase in bromodichloromethane exposure level was found	[35]

exposure on sexual hormones were observed [39,43,44] (Table 4). However, this approach rarely provided any indications of cause-effect relationship.

Among Danish greenhouse workers exposed to pesticides, the median values of sperm concentration and proportion of normal spermatozoa were lower by 60% and 14%, respectively, in the high- and low-level exposure groups. The median sperm concentration was lower by 40% for men with over 10 years' work experience in a greenhouse than for those with experience below 5 years [43]. Also significantly lower sperm count was found in pesticide plant workers exposed to fenvalerate compared with non-exposed controls in the study performed in China [41] (Table 4).

Some studies assessed the levels of reproductive hormones. Farmers exposed to pesticides in Argentina had higher serum oestradiol concentrations and lower luteinizing hormone (LH) concentrations than non-exposed men [45]. In Japan, pesticide sprayers had the serum testosterone concentration in winter higher than the controls ($p < 0.05$), though luteinizing hormone and follicle stimulating hormone concentrations were not significantly different. The sperm counts and vitality were comparable between the groups, but detailed sperm motility analysis in summer revealed that the percentages of slow progressive and non-progressive motile sperm were twice as high in the sprayers ($p < 0.05$), and that of rapid progressive sperm tended to be lower ($p = 0.06$). Such differences were not observed in winter [46]. Semen quality and reproductive hormones across a spraying season were examined among Danish farmers (using and not using pesticides) and controls (non farmers) that were asked to give two semen samples [44]. The median sperm concentration declined significantly from the first to the second sample in both groups, but there was no statistical difference in the decline between the two groups. It was concluded that semen quality did not change across a spraying season as a result of pesticide exposure. Sprayers and non-sprayers had an equal decline in sperm concentration from the first to the second semen sample [44] (Table 4).

In China and Mexico, studies of the prevalence of sperm aneuploidy [38,39] in agricultural workers exposed to organophosphorous pesticides like ethyl parathion,

methamidophos or endosulfan were conducted. In China, male workers at a large pesticide-manufacturing plant had an excess risk of aneuploidy and the risk of specific chromosome abnormalities: disomy for chromosome 18 and the three different types of sex chromosome disomy (XX, XY, YY) [39]. In the Mexico study, aneuploidies were found in 0.67% of total sperm nuclei. The authors concluded that exposure to organophosphates could interfere with sperm chromosome segregation and increase the risk of Turner's syndrome [38]. The next study performed in Mexico showed the poorest semen quality among the subjects with the highest OP exposure and the highest urinary OP levels. Seasonal variations in sperm concentration and sperm count were registered. The results showed a significant decrease in total sperm count among subjects with the highest exposure to OP [47] (Table 4).

The first study which demonstrates links between specific biomarkers of environmental exposure to pesticides and biomarkers of male reproduction in humans was performed in United States [40]. Men with high exposure level of alachlor ($> 0.15 \mu\text{g/g}$ creatinine) had poorer semen parameters (concentration, percentage sperm with normal morphology, percentage motile sperm) than those less exposed. The exposure to herbicide 2,4-D, metolachlor and atrazine was associated with poorer sperm quality as well [40]. The next study where the exposure to carbaryl and chlorpyrifos was analysed by detecting the urinary metabolites showed an associated between exposure and lower sperm concentration and motility among men attending an infertility clinic [42] (Table 4).

Yucra et al. [48] reported a significant reduction of semen volume and an increase in semen pH in men with organophosphate metabolites in urine in the study performed in Peru [48]. The poorest semen quality was found among farmers with the highest OP exposure and the highest urinary OP levels. The results showed a significant decrease in total sperm count among subjects with the highest exposure to OP [48] (Table 4). In summary, there are several indications that some pesticides may impair semen quality in humans, but weak exposure assessment in most studies precludes proper identification of responsible agents and evaluation of exposure-response relations.

Table 4. Exposure to pesticides and semen quality

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
Hawaii 46 men employed in the papaya fumigation industry, exposed to ethylene dibromide 43 non-exposed men from sugar refinery	Cross-sectional	Based on job title 'workers working in production of pesticides'. No biomarker	Sperm count, concentration, viability, motility, morphology	Lifestyle: smoking, drinking	Exposure to pesticides significantly decreased sperm count per ejaculate, the percentage of viable and motile sperm. Increase in the proportion of sperm with specific morphological abnormalities (tailed heads, absent heads and abnormal tails) was observed among exposed men compared with controls. No effect of exposure to ethylene dibromide on sperm velocity, the overall proportion of sperm with normal morphology was observed	[37]
Mexico agricultural workers exposed to pesticides	Cross-sectional	Metabolites of organophosphate pesticides in urine were determined.	Sperm concentration, aneuploidy	Lifestyle: smoking, drinking	Exposure to organophosphate pesticides was associated with sperm hyperploidy/polyploidy. There was a significant association between the concentration of organophosphate metabolites and increased frequency of sperm aneuploidies	[38]
China 32 workers from pesticide-manufacturing plant 43 workers from a nearby textile factory free of pesticides	Cross-sectional	Based on job title 'workers manufacturing pesticides'. No biomarker	Aneuploidy, motility, sperm concentration, proportion of sperm with normal morphology	Age, smoking, alcohol drinking, education level, season, coffee drinking	Exposure to organophosphate pesticides (parathion, methamidophos) increased the prevalence of sperm aneuploidy giving the ratio of 1.56 (95% CI: 1.06–2.31). The specific chromosome abnormalities were disomy for chromosome 18 and the three different types of sex chromosome disomy (XX, XY, YY). Median semen parameters for the exposed (and non-exposed) men were as follows: — proportion of sperm with normal motility — 50.5% (61.3%) — proportion of sperm with normal morphology — 59% (61.5%) — sperm concentration was lower in the exposed group compared with the non-exposed one	[39]

<p>United States</p> <p>50 men in whom all semen parameters (concentration, percentage sperm with normal morphology, and percentage motile sperm) were low (cases) and 36 men in whom all semen parameters were within normal limits (controls) within Missouri and Minnesota</p>	<p>Cross-sectional</p> <p>Metabolites of eight current-use pesticides in urine: alachlor, 2,4-D, metalochlor, atrazine, diazinon [2-isopropoxy-4-methyl-pyrimidinol (IMPY)], malathion, acetochlor, DEET- (<i>N,N</i>-diethyl-<i>m</i>-toluamide) were determined</p>	<p>Cases: all semen parameters (concentration, percentage sperm with normal morphology, and percentage motile sperm) were low controls: all semen parameters were within normal limits</p>	<p>Education level, season, coffee drinking</p>	<p>Pesticide metabolite levels were elevated in Missouri cases, compared with controls, for the herbicides alachlor and atrazine and for the insecticide diazinon [2-isopropoxy-4-methyl-pyrimidinol (IMPY)]. Men from Missouri with high levels of alachlor or IMPY were significantly more likely to be cases than were men with low levels. The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and metolachlor were also associated with poor semen quality in some analyses, whereas acetochlor levels were lower in cases than in controls ($p = 0.04$). No significant associations were seen for any pesticides within Minnesota, where levels of agricultural pesticides were low, or for the insect repellent DEET (<i>N,N</i>-diethyl-<i>m</i>-toluamide) or the malathion metabolite (malathion dicarboxylic acid)</p>	<p>[40]</p>
<p>China</p> <p>32 male workers who were exposed to fenvalerate and 46 male administrators in the office in the same pesticide factory</p>	<p>Cross-sectional</p> <p>The amount of fenvalerate in individual sampling and dermal contamination was evaluated</p>	<p>Sperm concentration, motility, morphology, sperm progression, beat cross frequency</p>	<p>Age, education, smoking</p>	<p>Sperm motion parameters through routine semen analysis in the exposure group were decreased significantly, and the abnormality rate of viscosity and coagulation was increased significantly as compared with the internal and the external control groups. Furthermore, sperm progression and beat cross frequency (BCF) in the exposure group were also significantly lower than those in the external control group</p>	<p>[41]</p>

Table 4. Exposure to pesticides and semen quality — cont.

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
United States 272 males were recruited through Massachusetts infertility clinic.		Urinary concentrations of 1-naphthol (1N), a metabolite of carbaryl and naphthalene, and 3,5,6-trichloro-2-pyridinol (TCPY), a metabolite of chlorpyrifos and chlorpyrifos-methyl were determined	Sperm concentration, percent motile sperm, and percent sperm with normal morphology, along with sperm motion parameters	Education, seasonality	For increasing 1N tertiles, adjusted odds ratios (ORs) were significantly elevated for low-reference sperm concentration (OR for low, medium, and high tertiles = 1.0, 4.2, 4.2, respectively; p-value for trend = 0.01) and percent motile sperm (1.0, 2.5, 2.4; p-value for trend = 0.01). The sperm motion parameter most strongly associated with 1N was straight-line velocity. There were suggestive, borderline-significant associations for TCPY with sperm concentration and motility, whereas sperm morphology was weakly and nonsignificantly associated with both TCPY and 1N	[42]
Denmark 122 greenhouse workers exposed to pesticide: 44 with low exposure 65 with medium exposure 13 with high exposure	Cross-sectional	Based on job title 'greenhouse workers exposed to pesticides' with: low, medium and high exposure No biomarker	Sperm concentration, proportion of normal spermatozoa, viability, velocity, sexual hormones	Age, education, smoking	The median values of sperm concentration and the concentration and proportion of normal spermatozoa were 60% and 14% in the high- and low-level groups, respectively. No differences were observed for the viability and velocity of sperm and sexual hormones. The median sperm concentration was 40% lower for the men with > 10 years' period of work in a greenhouse than for those with < 5 years' period	[43]
Denmark 161 agricultural workers exposed to pesticides 87 agricultural workers not using pesticides	Cross-sectional	Based on job title 'agricultural workers using pesticides' No biomarker	Sperm morphology, vitality, motility, sperm chromatin denaturation (SCSA) and reproductive hormones	Age, education, smoking	The median sperm concentration declined significantly from the first to the second sample for the men spraying pesticides and the men not spraying pesticides, but there was no statistical difference in the decline between the two groups. Only minor changes were found in the sperm morphology, vitality, motility, sperm chromatin denaturation (SCSA) and reproductive hormones	[44]

Argentina 225 male partners from consecutively recruited couples, who had their first infertility consultation between 1995 and 1998	Cross-sectional	Based on job title "farmers" No biomarker	Sperm concentration, sperm motility, morphology, reproductive hormones	Education, seasonality	Men exposed to pesticides had higher serum oestradiol concentrations, and those exposed to solvents had lower LH concentrations than non-exposed men	[45]
Japan 18 male pesticide sprayers out of 54 working for 9 companies in central Japan and 18 age-matched students	Cross-sectional	Based on job title "pesticide sprayer" No biomarker	Sperm concentration, sperm motility, morphology, reproductive hormones	Age, smoking, alcohol drinking, education level	Serum testosterone concentration in winter in the sprayers was higher than in the controls ($p < 0.05$), though luteinizing hormone and follicle stimulating hormone concentrations were not significantly different. Sperm counts and vitality were comparable between the groups, but detailed sperm motility analysis in summer revealed that the percentages of slow progressive and nonprogressive motile sperm were twice as high in the sprayers ($p < 0.05$), and that of rapid progressive sperm tended to be lower ($p = 0.06$). Such differences were not observed in winter	[46]
Mexico 52 volunteers	Longitudinal follow-up study	Urinary organophosphate metabolites		Education level, season	The results revealed that the poorest semen quality was found among the subjects with the highest OP exposure and the highest urinary OP levels. Seasonal variations in sperm concentration and sperm count were registered. The results showed a significant decrease in total sperm count among subjects with the highest exposure to OP	[47]
Peru 31 pesticide applicators exposed to organophosphate (OP) pesticides and 31 non- exposed were recruited (age, 20–60 years)	Cross-sectional	Urinary levels of OP me- tabolites (dimethyl and diethyl phosphates and thiophosphates)	Sperm concentration, percentage of sperm motil- ity, percentage of normal morphology, semen leuco- cytes and concentrations of fructose and zinc	Age, smoking, alcohol drinking, education level	Semen analysis revealed a significant re- duction of semen volume and an increase in semen pH in men with OP metabolites. Multiple regression analysis showed that both occupational exposure to pesticides and the time of exposure to pesticides were more closely related to alterations in semen quality parameters than the single mea- surement of OP metabolites in urine	[48]

Table 5. Exposure to phthalates and semen quality

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
United States 168 men who were part of subfertile couples and who presented to Massachusetts General Hospital andrology laboratory for semen analysis between January 2000 and April 2001	Cross-sectional	Eight phthalate metabolites were measured	Sperm concentration, motility, morphology	Age, abstinence time, and smoking status	A dose-response relation between tertiles of mono-butyl phthalate and sperm motility and sperm concentration was observed. In addition, there was a dose-response relation between tertiles of mono-benzyl phthalate and sperm concentration	[53]
United States 295 male partners of subfertile couples who presented for semen analysis to Massachusetts General Hospital between 1999 and 2003	Cross-sectional	Selected phthalate metabolites were measured in urine	Sexual hormones	Educational level, age, abstinence time, smoking status	An interquartile range (IQR) change in monobenzyl phthalate (MBzP) exposure was significantly associated with a 10% decrease in FSH concentration. Additionally, an IQR change in monobutyl phthalate (MBP) exposure was associated with a 4.8% increase in inhibin B but this was of borderline significance	[54]
United States 463 males in subfertile couples who presented for semen analysis to Massachusetts General Hospital between January 2000 and May 2004	Cross-sectional	Eight phthalate metabolites were measured	Sperm concentration, motility, morphology	Age, abstinence time, smoking status	There were dose-response relationships of mono-butyl phthalate (MBP) with low sperm concentration and motility. There was suggestive evidence of an association between the highest mono-benzyl phthalate (MBzP) quartile and low sperm concentration	[55]
Sweden 234 young Swedish men	Cross-sectional	Urinary concentrations of phthalate metabolites were determined	Semen volume, sperm concentration and motility were measured, together with sperm chromatin integrity (sperm chromatin structure assay) and biochemical markers of epididymal and prostatic function, reproductive hormones in serum	Education level, age, abstinence time, smoking status	Subjects within the highest quartile for monoethyl phthalate (MEP) had fewer motile sperm, more immotile sperms, and lower luteinizing hormone values, but there was no suggestion of harmful effects for most other endpoints	[56]

<p>United States Males (N = 45) of subfertile couples presenting to Michigan infertility clinic</p>	<p>Cross-sectional pilot study</p>	<p>Urinary concentrations of phthalate metabolites were measured</p>	<p>Motility, concentration, morphology</p>	<p>Education level, age, abstinence time</p>	<p>Low sperm concentration was significantly associated with elevated median concentrations of monoethyl phthalate (MEP) and low morphology with elevated median concentrations of mono-3-carboxypropyl phthalate. Increased odds for low concentration and elevated median concentrations of metabolites of di(2-ethylhexyl) phthalate (DEHP) (OR = 5.4, 95% CI: 0.9–30.8) and low morphology and elevated median concentrations of MEP (OR = 3.4, 95% CI: 0.9–13.8) were also found</p>	<p>[57]</p>
<p>India The study was conducted in the urban/rural population of Lucknow visiting Obstetrics and Gynecology Department</p>	<p>Cross-sectional</p>	<p>Urinary concentrations of phthalate metabolites were measured</p>	<p>Motility, concentration, morphology</p>	<p>Age, abstinence time, smoking status</p>	<p>Infertile men showed statistically significant ($p < 0.05$) higher levels of pollutants in the semen than fertile men. A negative correlation between semen phthalate level viz DEHP and sperm quality and positive association with depolarised mitochondria, elevation in ROS production and LPO, DNA fragmentation was established</p>	<p>[58]</p>
<p>China Participants residing in Shanghai were surveyed in 2002. Semen species of 52 men (aged from 23 to 48 years) were collected from outpatients of Shanghai Institute of Planned Parenthood Research</p>	<p>Cross-sectional</p>	<p>Concentrations of three kinds of commonly used phthalates (di-ethyl phthalate, DEP, di-n-butyl phthalate, DBP; di-2-ethylhexyl phthalate, DEHP)</p>	<p>Sperm density, viability, concentration</p>		<p>There was a significant positive association between liquefied time of semen and phthalate concentrations of semen. There was no significant difference between phthalate concentrations of semen and sperm density or viability</p>	<p>[59]</p>

Phthalates

Phthalates are among the most widely used man-made chemicals released to the environment over the last several decades [49]. Phthalates are used as plasticizers to increase the flexibility of PCV products like: toys, vinyl flooring and electricity cables or medical devices. The main plasticizer used in PVC based medical devices is di(2-ethylhexyl) phthalate (DEHP). Phthalates are also used as solvents or fixing agents in perfumes, body lotions and other cosmetics. Humans are mainly exposed to these compounds through the diet, medical devices and consumer products. In spite of short half times in the organism, the compounds or their metabolites have been detected in urine in more than 95% of men and women that have been investigated [50].

There are numerous animal studies on the risk of infertility related to phthalates exposure. Laboratory experiments with rodents have shown repeatedly that phthalate at very high doses (g/kg in gavage range) can adversely affect sperm quality. Ge et al. (2007) [51] found that at lower doses phthalates increase testosterone production either by increasing Leyding cell numbers or by directly stimulating testosterone production [51]. However, inhibition of testosterone was noticed in both foetal and adult Leyding cells when rats were exposed to higher doses [51]. Other studies in animals showed reduction of protein levels in foetal Leyding cells and deregulation of cholesterol transport and steroid synthesis [52].

Several recent epidemiological studies have addressed the male reproductive toxicity of phthalates [53–59] (Table 5). Duty et al. (2003) [53] compared levels of eight phthalate metabolites in urine among men with normal and abnormal semen quality recruited from 168 subfertile couples. They observed an inverse dose-response relationship between 2 phthalate metabolites (monobutyl- and monobenzyl phthalate (MBP, MbzP)) on the one side and sperm concentration and motility on the other. These findings were corroborated in a subsequent study of 463 infertile men using same study design [55] and to some extent by a smaller study including 45 infertility clients from the Great Lakes Region in US [57]. Also in a study performed in India, a negative correlation between semen phthalate

level (DEHP) and sperm concentration, motility, % of abnormal sperm was found in a population of men that visited an infertility clinic [58].

Another study performed by Duty and co-workers [54] to explore the association between environmental levels of phthalates and altered reproductive hormone levels in adult men showed that an interquartile range (IQR) change in monobenzyl phthalate (MBzP) exposure was significantly associated with a 10% decrease in follicle-stimulating hormone (FSH) concentration. Additionally, an IQR change in monobutyl phthalate (MBP) exposure was associated with a 4.8% increase in inhibin B, but this was of borderline significance [54].

On other hand, in a Swedish study, Jonsson et al. (2005) [56] reported that only subjects within the highest quartile for monoethyl phthalate (MEP) had fewer motile sperms, more immotile sperms, and lower luteinizing hormone values, but there was no suggestion of harmful effects for most other endpoints. In the study in China there was no significant difference between phthalate concentrations of semen and sperm density and liveability, while there was a significant positive association between liquefied time of semen and phthalate concentrations of semen [59].

Summing up, results of few studies of subfertile men demonstrate associations between phthalate levels commonly experienced by the public and impaired sperm quality (impact on sperm concentration, morphology, motility), but findings have not been corroborated in studies of men from the general population. Effects of phthalates in sexually mature rodents have only been seen at much higher exposures levels but perhaps the human male is more susceptible to the class of chemicals. It remains to be investigated if maternal exposures to phthalates have bearings as to semen quality in the offspring.

Mobile phones

There has been a tremendous increase in the use of mobile phones in the past decade and concerns are growing about the possible detrimental effects of high-frequency electromagnetic fields (EMF) emitted by these devices on

human health. Although many studies have recently been published on this topic, the effects of the EMF emitted by cell phones on living cells and organs are still unclear. Here we review the few studies addressing possible effects of cellular phone usage on human semen quality.

In a small prospective study involving 13 men with normal sperm, GSM phone usage in 5 days for 6 hours per day decreased the rapid progressive motility of sperm [60] (Table 6). Interesting results were obtained in a pilot study performed in Australia; men who carried their mobile phone in their hip pocket or on their belt had lower sperm motility than men who did not carry a mobile phone or who carried their mobile phone elsewhere on the body [61].

There are also two small studies where semen sample was exposed to cellular phone radiation [62,63] (Table 6). The first study was performed in Turkey where statistically significant changes were observed in the rapid progressive, slow progressive and no-motility categories of sperm movement. Electromagnetic radiation (EMR) exposure caused a subtle decrease in the rapid progressive and slow progressive sperm movement. It also caused an increase in the no-motility category of sperm movement. There was no statistically significant difference in the sperm concentration between two groups [62]. The second study was performed in United States where samples exposed to radiofrequency electromagnetic waves (RF-EMW) showed a significant decrease in sperm motility and viability, increase in reactive oxygen species (ROS) level, and decrease in ROS-TAC (Total Antioxidant Capacity) score. Levels of TAC and DNA damage showed no significant differences from the non-exposed group [63].

A recent study involving 361 men attending an infertility clinic suggested that use of cell phones for longer durations adversely affected the quality of semen by decreasing the sperm counts, motility, viability and morphology [64]. In Hungary a study was performed among 371 male patients of infertility clinics. The duration of mobile phone use was correlated negatively with the proportion of rapid progressive motile sperm, and positively with the proportion of slow progressive motile sperm [65] (Table 6).

In the study performed in Poland, 304 males attending an infertility clinic were divided into three groups: 99 patients who did not use mobile phones, 157 males who had used GSM equipment sporadically for the period of 1–2 years, 48 patients who had been regularly using mobile phones for more than 2 years. In the analysis of the effect of GSM equipment on the semen, an increase was noted in the percentage of sperm cells of abnormal morphology with the duration of exposure to GSM phone [66] (Table 6).

The results of the presented studies provide limited support to the hypothesis that use of mobile phones may adversely affect semen quality, but most of the presented studies are small [60–63] only three [64–66] were conducted on the sample of about 300 men. So there is a need for future studies in this area.

DISCUSSION

The results of the reviewed studies suggest that exposure to environmental factors (air pollution, pesticides, phthalates, PCB and the use of mobile phones) may affect semen quality. However, when reviewing the epidemiological studies on the influence of environmental hazards on semen quality, it is important to take into account the current limitations of these studies resulting from inadequacies in semen analysis, exposure evaluation and design of studies. The studies conducted in the general population are very rare mainly because of the difficulties in collecting the semen samples. The fact that most of the studies are drawn from infertility clinics may introduce a selection bias. Some of the subjects are classified as fertile; they belong to a population of men seeking medical care, which is probably different from the general population in terms of other exposure factors, because these men are more health-concerned and therefore less exposed to other agents such as alcohol or drugs. In addition, subjects may underreport their lifestyle habits, especially for smoking and drinking, thus biasing the results. Another possible variable that needs to be considered is the simultaneous presence of other exposure factors. In reviewed studies authors collected

Table 6. Mobile phones and semen quality

Study population	Type of study	Definition of exposure	Semen analysis	Confounders	Results	References
United States 361 men undergoing infertility evaluation	Cross-sectional	Four groups according to their active cell phone use: group A: no use; group B: < 2 h/day; group C: 2-4 h/day; group D: > 4 h/day	Sperm parameters (volume, liquefaction time, pH, viscosity, sperm count, motility, viability, and morphology)	Controlling for lifestyle factors, education level	The comparisons of mean sperm count, motility, viability, and normal morphology among four different cell phone user groups showed statistically significant differences. Mean sperm motility, viability, and normal morphology were significantly different in cell phone user groups within two sperm count groups. The laboratory values of the above four sperm parameters decreased in all four cell phone user groups as the duration of daily exposure to cell phones increased	[64]
Hungary 371 males from infertility clinic	Cross-sectional	Group 1 was subdivided into those who used a cell phone for less than 15 min/day (low transmitters) and those who used it for over 60 min/day (high transmitters). Group 2 was subdivided into those patients who kept their cell phone in the standby position within a distance of 50 cm for less than 1 h daily (short-standby group) and those who kept their cell phone in the standby position within a distance of 50 cm for more than 20 h daily (long-standby group)	Sperm concentration, motility, morphology, volume, count	Controlling for lifestyle factors	The duration of possession and the daily transmission time correlated negatively with the proportion of rapid progressive motile sperm and positively with the proportion of slow progressive motile sperm. The low and high transmitter groups also differed in the proportion of rapid progressive motile sperm. The prolonged use of cell phones may have adverse effects on the sperm motility characteristics	[65]
Germany 13 men with normal sperm	Prospective study	GSM phone usage 5 days/week, 6 h/day	Sperm concentration, motility, morphology, volume, count	Controlling for lifestyle factors	A reduction in the rapid progressive motility of sperm	[60]

<p>Australia 52 men between the ages of 18 and 35 years from the campus of the University of Western Australia</p>	<p>Percentage of motile sperm and the number of sperm per ml of ejaculate</p> <p>Controlling for lifestyle factors</p>	<p>Interestingly, men who carried their mobile phone in their hip pocket or on their belt had lower sperm motility than men who did not carry a mobile phone or who carried their mobile phone elsewhere on the body</p>	<p>[61]</p>
<p>Turkey 27 males</p>	<p>Sperm concentration, motility, morphology, volume, count</p> <p>Controlling for lifestyle factors</p>	<p>Statistically significant changes were observed in the rapid progressive, slow progressive and no-motility categories of sperm movement. EMR exposure caused a subtle decrease in the rapid progressive and slow progressive sperm movement. It also caused an increase in the no-motility category of sperm movement. There was no statistically significant difference in sperm concentration between two groups.</p>	<p>[62]</p>
<p>United States healthy donors (n = 23) and infertile patients (n = 9)</p>	<p>Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage</p> <p>Controlling for lifestyle factors</p>	<p>After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (non-exposed) served as the control sample under identical conditions</p>	<p>[63]</p>
<p>Poland 304 males</p>	<p>Sperm concentration, motility, morphology, volume, count</p> <p>Controlling for lifestyle factors, age, place of residence</p>	<p>Group A: 99 patients who did not use mobile phones, Group B: 157 males who have used GSM equipment sporadically for the period of 1–2 years, and Group C: 48 people who have been regularly using mobile phone for more than 2 years</p>	<p>[66]</p>

information on age, alcohol and coffee drinking, tobacco smoking, occupational exposure, and conducted a multivariate analysis.

Sometimes, like in case of trihalomethanes [34,35] or mobile phones [63–66] there are just a few studies so it is difficult to reach the conclusion. The next problem is also the size of study population. In case of PCBs there are lots of small pilot studies [19–21] but the bigger, well designed studies [22,23,25–27] confirmed the findings derived from those studies.

It is a challenge for future studies to overcome the experienced limitations but also to focus more on mechanistic aspects of reproductive toxicity.

Semen analysis

Presented studies usually have used classical human semen parameters (concentration, motility and morphology), other markers have been used to get a more comprehensive evaluation of spermatogenesis and the function of accessory sex organs (hormone levels, aneuploidy).

A significant factor impeding the interpretation of studies concerning semen quality is great variation in the same semen variables — in particular, ejaculate volume, sperm density and motility. The fluctuations across time within a man are nearly as great as the variation between men and can only partly be explained by the duration of the period of abstinence [8]. The concentration and probably also the sperm motility seem to be subject to seasonal variation, the best quality in the northern hemisphere being registered during the winter and spring [67]. Seasonal variation accounts only for a part of total variation which, thus, remains largely unknown [8]. Experiments with rhesus monkeys suggest that the seasonal changes in semen quality may be driven by an inherent circannual biological clock reset annually by seasonal changes in the length of daylight [67].

Although there are some guidelines under WHO to introduce standardization and quality control in sperm laboratory work [68], the assessment of semen quality — even within the same laboratory — may be subject to variation between technicians and related to time [69].

Exposure assessment

Exposure assessment is a crucial part of any area of environmental epidemiology. The best assessment of exposure usually provide measurements of contaminants in ambient air samples, or in blood, urine, semen or other biological specimens that might be appropriate.

Exposure in several of the presented studies was based on specific biomarkers of exposure or the assessment of exposure was performed. Phthalates were assessed in urine, air pollution measurement was also taken. However, in the studies on pesticide exposure and semen quality the exposure was based in almost all studies on the job titles. Only Swan et al. and Yucra et al. [40,48] used specific biomarkers of pesticide exposure [40,48]. It is well known that misclassification of exposure may occur if the type of exposure is based solely on subject's memory.

Biological assessment of exposure is the most precise indicator, but sometimes it can be limited by the cost and the large number of suspected chemicals that individuals were exposed to, what usually is the case in relation to pesticides exposure.

It is not only the type of exposure but also the timing in relation to the development of the reproductive system that is important for the assessment of type and magnitude of the harmful effect. The period between the 8th and the 10th gestational week seems to be critical for the development of male gonads [70]. It has been hypothesised that even relatively insignificant exposures acting at this period of prenatal life may have serious consequences for the future reproductive capability of men [5]. So the studies of environmental effects on male reproduction should not only focus on exposures during the reproductive life, but should also include the foetal periods of male reproductive system development.

Type of study design

With few exceptions, all studies comparing the distributions of seminal variables in an exposed populations with those of a non-exposed reference population were cross-sectional. The participation rate was about 40–60% and there was tendency towards lower participation rates

among controls. So, the possibility that the men available for the study may not truly reflect the source population is also the matter of concern.

The cross-sectional approach is most often associated with considerable differential dropout and uncertainty about the comparability of exposed and non-exposed groups. It may be possible to overcome some of the major limitations of studies by choosing a longitudinal design with sampling before and during exposure and with a follow-up of intraindividual changes.

Epidemiological models seem to have played increasing role in the risk assessment of reproductive health effects due to exposure to environmental factors. To be more informative, epidemiological studies should focus on exposure to single factors which were known from animal studies that they might bring health consequences. The factors which may influence the sperm count and explain part of the variability comprise the characteristics of the men included in the study, the methodologies used to analyse the semen or external factors influencing sperm production.

Mechanisms

Exposure to environmental toxicants that disrupt sperm production or the function of reproductive hormones or sperm may increase the risk of male infertility. On the other hand, chronic exposures to reproductive toxins are not well documented and mechanisms of toxicity are either poorly understood or unknown. Some evidences from animal studies suggest that some pesticides and PCBs belong to the substances known as endocrine disrupters.

PCBs and DDT are well known chemicals with estrogen-like characteristics and are referred to as estrogen disrupters. Animal studies suggest that these chemicals readily penetrate the blood-testis barrier and can directly affect spermatogenesis. It was also documented that PCB metabolites bind to estrogen receptors. Jansen has hypothesized that adverse reproductive effects of PCBs may result from PCB congeners increasing gonadotropin-releasing hormone or affecting the production and release of luteinizing hormone from the pituitary [71]. Kelce et al.

(1995) [72] showed that p,p'-DDE had an antiandrogenic activity.

Some pesticides are now suspected of being endocrine disrupting chemicals (EDCs). These chemicals might cause an adverse effect by interfering in some way with the body's hormones or chemical messengers. Many of these endocrine disrupters have been linked to adverse effects on either embryonic development or reproductive function in humans and wildlife [73–75].

Challenges for future studies

It is necessary to conduct longitudinal studies. These studies should take into account other factors which may interfere with male reproductive health and include other sperm characteristics. Future studies should have sufficient power and homogenous groups with an appropriate non-exposed control group.

When the influence of confounding factors is possible, these should be taken into account during the stage of study design and data collection and finally in the statistical analysis. Further evaluation of male reproductive toxicity of commonly used substances or those that are likely to be in contact with human population is highly recommended.

The longitudinal design option is a rational answer to several of the main limitations in the cross sectional approach. Imprecision of measurement and variation between observers can be reduced by implementing good laboratory practice and by computerised methods for objective assessment of sperm motility.

Also exposure assessment based on biomarkers of exposure should be used. As little is known about the basic mechanisms by which environmental exposures exert their effect, current studies should focus more on the explanation of the biological mechanism.

CONCLUSIONS

The results from the presented studies suggest that there are strong and rather consistent indications that some pesticides besides DBCP (as DDT/DDE, ethylenedibromide, organophosphates) affect sperm count. PCBs are

detrimental to sperm motility; those findings are derived from small pilot studies, but are confirmed also by bigger studies. In case of air pollution, studies suggest a link between ambient air pollutants and various semen characteristics. Additional research is needed to corroborate this association and to establish the causal agents. Results of few studies of subfertile men demonstrate associations between phthalate levels commonly experienced by the public and impaired sperm quality (impact on sperm concentration, morphology, motility), but findings have not been corroborated in studies of men from the general population. Mobile phones might adversely affect the quality of semen by decreasing mostly motility but also the sperm counts, viability and morphology. In spite of their consistent results, most of the studies are rather small; only two big studies were performed in this area.

No association between exposure to THMs and poor semen quality was observed.

Presented studies usually used classical human semen parameters (concentration, motility and morphology). The assessment of exposure was performed in most of the studies (PCBs, THMs, air pollution), and only in a few of the pesticide and phthalate exposure studies. The studies conducted in the general population are very rare, mainly because of the difficulties in collecting the semen samples. This has caused also that some works are small pilot studies. The fact that most of the subjects are drawn from infertility clinics may introduce a selection bias. In addition, the most commonly used study design is cross-sectional, which does not allow to make inferences on cause-effect mechanisms. Also chronic exposures to reproductive toxins are not well documented and mechanisms of toxicity are either poorly understood or unknown. So it is necessary to conduct longitudinal studies with exposure assessment that should focus more on the explanation of the biological mechanisms.

In spite of their limitations, epidemiological studies suggest awareness of environmental factors which may affect semen quality, and both in case of well proven and disputable hazards it is necessary to reduce the exposure of parents (including future ones) to those factors.

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