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Environmental and occupational factors affecting fertility and IVF success

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Reproductive function has been shown to be sensitive to changes in the physical, psychosocial and chemical environments. Although reproductive effects of occupational exposure to hazardous chemicals have been well documented in the literature, the potential effects of chemical contaminants at levels representative of contemporary exposures in the general population are much less certain. Evidence for adverse effects of exposure to environmental contaminants is more conclusive among the lower animals than for humans where considerable controversy remains. In addition to potential reproductive hazards of exposure to environmental contaminants, there is also evidence for adverse reproductive effects of the physical and psychosocial environments. In this review we focus on the difficulties involved in linking exposure to putative hazardous substances in environmental and occupational settings to adverse reproductive outcomes, especially success of IVF procedures. We highlight the plausibility of adverse events through animal and cell studies and the application of these results to the interpretation of human data. We consider both the male and female partners since it is essentially their combined contributions of gametes which may be affected by chemicals, which lead to successful outcomes.

Key words: environmental contaminants/IVF/polychlorinated biphenyls/pesticides

Introduction

Although the focus of this review is the success of IVF following exposure to hazardous substances in the workplace, the outcome of any attempt to achieve a live birth depends on many factors, principally the union of putatively normal sperm with oocytes. The Medline and PubMed databases were searched from 1979 to 2004 for papers linking IVF successes and infertility with occupational exposures to hazardous substances. Titles and abstracts were used to select relevant publications which were then retrieved either through PubMed or the E-resources of McMaster University. Links to other publications were scanned from the full papers and bibliographies of relevant studies handsearched. However, there is a paucity of data linking exposure of humans to hazardous substances and IVF success. Therefore we will discuss the available exposure data in relation to reproductive outcomes and describe their relevance to IVF.

Infertility has often been defined as failure to achieve pregnancy within 1 year of unprotected intercourse. Delays in the time it takes to become pregnant or loss of pregnancy before term have been considered as evidence for subfertility. Perception of increased prevalence of infertility together with increased awareness of the toxicity of environmental contaminants on reproductive function in wildlife and experimental animals has led to increased research attention on the aetiology of infertility. Among the factors thought to affect human fertility are the physical environment, behavioural and socioeconomic factors as well as environmental contaminants. Physical, behavioural and socioeconomic factors have been reported to have an effect on fertility. In one of the earlier reviews on the subject, Schull (1984) reported that the evidence is inconclusive as to whether adverse conditions at the workplace pose a risk to human fertility. Nine years later, Baranski (1993) reviewed the then recent literature on the adverse effects of occupational factors on fertility and related reproductive outcomes, concluding that the existing data were not sufficient to support the hypothesis of occupational exposure-induced female infertility. Lindbohm (1999) suggested that psychological job stress is becoming an important factor in infertility. However, research in this area is still considered to be immature (Bonde, 1999). McElgunn (1998) indicated that the critical period prior to conception and during pregnancy are important periods for adverse influence on fertility and pregnancy outcome, and that environmental tobacco smoke, and exposures from video display terminals (VDT) and indoor air quality, are the most common concerns of women in their

places of work. Some of these environmental exposures may affect IVF outcomes.

In addition to physical and psychosocial factors thought to affect human reproductive function and fertility, there is concern that human fertility has been adversely affected by exposure to chemical contaminants. The impetus for expounding the causal relationship between chemicals released in the environment and human health came from Carson's (1962) book *Silent Spring*. She proposed that there is a connection between changes observed in wildlife ecology and human cancers and suggested that these effects were the consequences of the release of countless chemicals into the environment via manufacturing and agricultural processes. It took another two decades before Colborn advanced the environmental endocrine hypothesis which is now widely accepted (Colborn, 1991, 1995; Colborn *et al.*, 1993, Colborn *et al.*, 1996). This hypothesis states that environmental endocrine-disrupting contaminants have more control over the development of the offspring of exposed adults than the genes the offspring inherit, or the training they receive.

Endocrine-disrupting chemicals (EDC) are synthetic and naturally occurring chemicals that cannot be classified by any unique physical or chemical properties but are characterized by their ability to mimic the effects of endogenous hormones. Specifically, endocrine disrupters can: mimic (Soto *et al.*, 1995) and antagonize the actions of endogenous hormones (Kelce *et al.*, 1995, 1997); induce changes in steroidogenic enzyme expression and/or activity (Chapin *et al.*, 1997, Crellin *et al.*, 2001); and alter circulating steroid hormone levels (Lindenau *et al.*, 1994; Chapin *et al.*, 1997; Diawara *et al.*, 1999; You *et al.*, 2001). These characteristics of EDC have led to concerns that exposure to these compounds may be linked to adverse health effects in humans (Damstra *et al.*, 2002). Endocrine disrupters have deleterious effects in wildlife and fish populations (Damstra *et al.*, 2002), though adverse health effects in the human population have not been clearly demonstrated. To date, epidemiological studies fail to support an association between exposure to endocrine disrupters and infertility or decreased fecundity (Foster and Holloway, 2003). However, quantification of endocrine toxicants in human ovarian follicular fluid and their association with IVF outcomes (Younglai *et al.*, 2002), together with observed adverse effects in animals and *in vitro* studies (Gray *et al.*, 2001), support concerns that exposure to endocrine toxicants has the potential to adversely impact human ovarian function.

Many reviews over the past few years have focused on environmental factors as possible harmful influences on fertility (Chapin *et al.*, 1996; Daston *et al.*, 1997; Harrison *et al.*, 1997; Van Oostdam *et al.*, 1999; Cocco, 2002; Joffe, 2003), with an emphasis either on the male (Twombly, 1995; De Celis *et al.*, 1996; Spira and Multigner, 1998; Cooper *et al.*, 1999; Fisch *et al.*, 2000; Foster *et al.*, 2001; Guillette and Gunderson, 2001; Oliva *et al.*, 2001; Sharpe, 2001; Damgaard *et al.*, 2002; Myers *et al.*, 2003; Fisher, 2004; Myers, 2004) or female (Feichtinger, 1991; Kamrin *et al.*, 1994; Sharara *et al.*, 1998; Nicolopoulou-Stamati and Pitsos, 2001; Foster and Holloway, 2003). These reviews have dealt with substances such as polychlorinated biphenyls (PCB), phthalates, pesticides, solvents, dioxins, organotins, bisphenol A, as well as lifestyle habits and emotional stress. The majority of the reviews have centred on chemicals thought to disrupt endocrine function. In Table I are listed some of the substances which have been implicated as having adverse

Table I. Examples of common contaminants from several different chemical classes that have been linked with adverse reproductive health effects in humans

Chemical	Sources	Effects	References
Benzo[a]pyrene	PAH in tobacco smoke and diesel exhaust	Meiotic maturation of oocytes and DNA adducts in sperm, oocytes and embryos of IVF patients	Zenzes *et al.*, 1995a
PCB[a]	Oils, electric coolant	Impaired response to ovulation induction, reduced parity, impaired lactation, and potential reduced fecundability	Gerhard and Runnebaum, 1992; Kostyniak *et al.*, 1999; Buck *et al.*, 2000
Dioxins and polychlorinated dibenzofurans (PCDF)[a]	Incineration of plastics, automobile exhaust, and pesticide manufacturing	Potential for change in sex ratio and increased risk of endometriosis	Mocarelli *et al.*, 1996, Mocarelli *et al.*., 2000; Mayani *et al.*, 1997
Hexachlorobenzene[a]	Pesticides, fungicide	Developmental abnormalities of the male reproductive tract; increased risk of spontaneous abortion	Jarrell *et al.*, 1998; Hosie *et al.*, 2000
Pesticides in general	Herbicide used in combination	No apparent effects alone but decreased semen quality and fecundity, spontaneous abortion, pre-term birth, and small for gestational age in mixtures	Gerhard and Runnebaum, 1992; De Cock *et al.*, 1994; Curtis *et al.*, 1997, 1999; Savitz *et al.*, 1997; Arbuckle *et al.*, 1999, Arbuckle *et al.*., 2001; Abell *et al.*, 2000
DDT/DDE[a]		Reduced parity, impaired lactation, decreased semen quality, impaired fertility, and small-for-gestational-age babies	Kostyniak *et al.*, 1999; Longnecker *et al.*, 2001;Younglai *et al.*, 2002
Dibromochloropropane		Decreased sperm counts & infertility	Whorton *et al.*, 1979; Potashnik and Porath, 1995; Slutsky *et al.*, 1999
Lead	Metals	Decreased semen quality, increased time to pregnancy, and spontaneous abortion	Cullen *et al.*, 1984; Assennato *et al.*, 1987; Alexander *et al.*, 1996; El-Zohairy *et al.*, 1996; Lin *et al.*, 1998; Sallmen *et al.*, 2000; Telisman *et al.*, 2000; De Rosa *et al.*, 2003; Tang and Zhu, 2003

PAH = polycyclicaromatic hydrocarbons; PCB = polychlorinated biphenyls; DDT/DDE = dichlorodiphenyltrichloroethane/dichlorobischlorophenylethylene.

effects on the human reproductive system. However, other factors such as gene–environment interactions, where environment is defined as the both the physical and chemical environment, including dietary and lifestyle factors, are thought to be involved in IVF success. We will therefore examine the relationship between environment and fertility. Success of assisted reproductive technology, and the possible mechanisms for adverse effects, will be considered in three main categories:

(1) Physical
(2) Psychosocial
(3) Chemical
  (a) Occupational, e.g. solvents, welding, agriculture
  (b) Lifestyle, e.g. alcohol, caffeine, smoking
  (c) Inadvertent, e.g. air, water, food.

Occupational exposures

Occupational exposure to a vast array of putative hazards and adverse reproductive outcomes or fertility has been described. These hazards include the physical environment such as VDT and noise, psychosocial stress, as well as chemical agents. Some of these may directly or indirectly affect IVF outcomes.

The physical environment

In a case–control study of 1583 pregnant women in Northern California, Goldhaber *et al.* (1988) reported that the odds ratio (OR) for miscarriage was 1.8, 95% confidence interval (CI) 1.2–2.8 for working women using VDT for >20 h per week. In a Finnish study of bank clerks and clerical workers, Lindbohm *et al.* (1992) found that the OR for miscarriages in workers who used a VDT with a high level of extremely low frequency magnetic fields ($>0.9\,\mu$T) was 3.4 (95% CI 1.4–8.6) compared to workers using a VDT with a low level of these magnetic fields ($<0.4\,\mu$T). On the other hand, no association was found between occupationally related categories of magnetic field exposure and male subfertility in an infertile population of 177 men attending the Yale New Haven infertility clinic (Lundsberg *et al.*, 1995) or in 57 Danish couples planning their first pregnancy (Hjollund *et al.*, 1999).

Seasonal variation in fertility has been described (Rojansky *et al.*, 1992; Lam and Miron, 1994). However, the evidence supporting this view was judged to be inconclusive (Bronson, 2004). It was suggested that whereas some humans are photoresponsive, others are not, and that the inconsistencies are due to individual variation. Some seasonality has been observed in IVF success. Weigert *et al.* (2001) reported that the lowest pregnancy rate in 8184 IVF cycles of two European centres was 25.7% in July compared with 35.5% in December. Further analysis showed that the anomalistic moon period was the most significant (Weigert *et al.*, 2002). This is in contrast to British (Fleming *et al.*, 1994) and Canadian (Yie *et al.*, 1995) studies where no significant seasonal variation in IVF success was observed.

Frequent changes in time zones in the workplace as experienced by flight attendants seem to have a slightly increased risk of spontaneous abortions (SA) (OR 1.3, CI 0.9–1.8) but this was not consistent between the two time periods studied (Aspholm *et al.*, 1999), since in one time frame there was a positive association but none in another time frame. Male exposure to heat and female exposure to noise were associated with infertility in a Danish population (Rachootin and Olsen, 1983). Work in the electronics industry was significantly associated with low birthweight babies (OR 5.38, CI 1.42–20.46) (Lipscomb *et al.*, 1991). However, these studies were not conclusive and similar adverse effects on IVF outcomes have not been reported. Thus, as with other potential hazards to fertility, the literature is both sparse and inconsistent.

The psychosocial environment (stress)

Psychological stress in the workplace could have an effect on fertility (Negro-Vilar, 1993) and this factor appears to have an effect on IVF outcomes (Eugster and Vingerhoets, 1999).

Females. Psychosocial factors such as ineffective coping strategies, anxiety and/or depression are associated with a lower pregnancy rate in IVF. In a group of 192 patients waiting for IVF, 8% became pregnant (Jarrell *et al.*, 1993a). In a more recent multicentre, randomized controlled trial of 139 couples randomized to receive IVF or no treatment for 90 days, the live birth rate in the IVF group was 29% in the IVF-treated group compared to 1% in the untreated group (Hughes *et al.*, 2004). It was not mentioned whether the untreated group received counselling. In Austria where psychological counselling is legally required before IVF, the cumulative pregnancy rate of 1156 such patients was 56.4% (Poehl *et al.*, 1999). Thus, stress reduction through relaxation training or behavioural treatment improves conception rates even before IVF is undertaken. In a prospective study among seven clinics involving 151 patients, acute and chronic stress did contribute to negative outcomes (Klonoff-Cohen *et al.*, 2001a). The negative outcomes have been attributed to the elevation of activated T cells in prolonged conditions of stress, leading to reduced implantation rate in such women undergoing IVF (Gallinelli *et al.*, 2001). These conclusions were not supported by the data of Milad *et al.* (1998) who found no difference in hormonal markers of stress and adverse pregnancy outcome in 40 patients undergoing IVF. It could be argued that this sample size was too small. However, using spontaneous abortion (SA) as an index of fertility, Fenster *et al.* (1995) found no association with stressful work and IVF success.

Males. The negative outcome of IVF as a result of stress may have arisen from fertilization with poor quality sperm. Bigelow *et al.* (1998) found that there was a significant dose–response relationship between level of perceived job stress and poor sperm quality. Using a questionnaire to assess stress, Hjollund *et al.* (2004a) found no effect on sperm quality and only those men with low sperm concentration and high stress level had moderately decreased fecundability. On the other hand, the emotional burden of unsuccessful repeat IVF procedures could lead to discontinuation of cycles even with subsidization (Hammarberg *et al.*, 2001; Olivius *et al.*, 2004). Thus the question of physical factors leading to adverse IVF outcomes cannot be answered from studies to date.

The chemical environment: occupational

Solvents

Decreased fertility has been linked with occupational exposure to chemical agents in several studies.

Females. Female dental surgeons who are constantly exposed to mercury, chloroform and benzene have no difficulty becoming pregnant (Dahl *et al.*, 1999) but the level of exposure may be too low to cause an effect. With SA as a marker for fertility, it has been demonstrated that maternal exposures to toluene, xylene and formalin (Taskinen *et al.*, 1994), chloroform (Wennborg *et al.*, 2000) and ethylene glycol ethers (Correa *et al.*, 1996) can decrease fertility. There was no increased risk when males were exposed (Correa *et al.*, 1996). In a meta-analysis of research papers from 1966–1994, McMartin *et al.* (1998) concluded that there was a tendency (OR 1.25, CI 0.99–1.58) toward an increased risk for SA with maternal exposure to organic solvents. First trimester solvent exposure was significantly associated (OR 5.38, CI 1.42–20.46) with SA (Lindbohm *et al.*, 1990; Lipscomb *et al.*, 1991). Other workers have confirmed this conclusion (Taskinen *et al.*, 1986; Khattak *et al.*, 1999).

Males. Using the endpoint of TTP (time to pregnancy, length of time to achieve pregnancy), Sallmen *et al.* (1998) found limited support for the hypothesis that paternal exposure to organic solvents might be associated with decreased fertility. A tendency toward increased SA was also seen when only the husbands were exposed to organic solvents (Taskinen *et al.*, 1989; Lindbohm *et al.*, 1991). However, the NTP-CERHR (National Toxicology Program- Center for the Evaluation of Risks to Human Reproduction) expert panel recently concluded that there was negligible risk to human reproduction of exposure to ethylene glycol or propylene glycol, two solvents that are ubiquitously released into the atmosphere (NTP-CERHR, 2004a,b).

Metals

Males. Workers in the metal industry are exposed to a number of hazardous substances including lead and steel. In a study of 400 Danish battery workers (Bonde and Kolstad, 1997), blood levels of lead were in the region of 35.9 μg/dl but no reduction in the birth rate (OR 0.983, CI 0.88–1.1.3) was observed over the time period studied. Time to pregnancy is also not affected by occupational lead exposure (Joffe *et al.*, 2003). Welders are constantly exposed to metals and solder vapours and their sperm quality has been equated with fertility. These men had a greater risk for poor sperm quality (Mortensen, 1988) and reduced fertility (OR 0.89, CI 0.83–0.97) (Bonde *et al.*, 1990). The poor sperm quality was confirmed in a Canadian study (Bigelow *et al.*, 1998) but sperm quality was not associated with decreased fertility. With SA as a marker for fertility, Hjollund *et al.* (1995) reported that in 2520 pregnancies from 1715 married metal workers, the proportion of SA was not increased for pregnancies at risk from stainless steel welding. However, the same group later reported (Hjollund *et al.*, 2000) that welding of stainless steel was associated with an increased risk of SA. It is not known whether embryos conceived with sperm from welders by IVF suffer the same fate. However, the opposing conclusions from the same group demonstrate the difficulty in interpreting data from such studies.

Pharmaceuticals

Occupational exposure to antineoplastic agents may affect gametes as well as the developing fetus. In a study of 7094 pregnancies among 2976 pharmacy and nursing staff (Valanis *et al.*, 1999), exposure of the mother to, or handling of, antineoplastic agents during pregnancy was associated with a significant increased risk of SA (OR 1.5, CI 1.2–1.8). Similar risks to fertility are seen for workers in biomedical research laboratories (Wennborg *et al.*, 2002).

Agriculture

Males. Farmers and agricultural workers are exposed to a variety of potentially harmful chemicals. No association was previously found between exposure to chemicals and infertility (Gerber *et al.*, 1988). By contrast, occupational exposure to pesticides in fruit growers in The Netherlands (De Cock *et al.*, 1994) was found to have an adverse effect on TTT (OR 0.46, CI 0.28–0.77) and male agricultural workers in Austria seeking IVF were found to have a higher prevalence of male factor infertility compared to normal controls (Strohmer *et al.*, 1993). In addition, impaired fertility has been documented in agricultural workers with exposure to pesticides (De Cock *et al.*, 1995; Fuortes *et al.*, 1997; Tielemans *et al.*, 1999b). By contrast, no association was found between male exposure to pesticides and fertility among French and Danish men (Larsen *et al.*, 1998; Thonneau *et al.*, 1999a). However, Arbuckle and Sever (1998) have emphasized that many epidemiological studies suffer from methodological problems, and, although the reviewed literature suggested an increased risk of fetal death associated with pesticides in general and maternal employment in the agricultural industry, they cautioned that research must focus on specific products and improved exposure assessment.

Depending on the endpoint used, conclusions can differ, e.g. when TTP was assessed in the Ontario Farm Family Health Study (OFFHS) using questionnaires, no pattern was found with exposure to pesticides (Curtis *et al.*, 1999) but when SA at <12 weeks was used as an index, there was an increased risk (Arbuckle *et al.*, 2001). Similarly, Thonneau *et al.* (1999b) found no association between TTP and male exposure to pesticides in France and Denmark. However, with SA as the endpoint, exposure to phenoxy herbicides was found to be hazardous to pregnancy (OR 2.5, CI 1.0–6.4) (Arbuckle *et al.*, 1999).

With the IVF population the picture is more confusing. Using data from a generic questionnaire and the laboratory results from inseminating oocytes *in vitro*, Tielemans *et al.* (1999a) found a significantly lower fertilization rate among 16 Dutch couples where the male partner had potential pesticide exposure; OR ranged from 0.22 to 0.54 depending on the alleged severity of exposure. However, within the same study group, the seven fruit farmers with high exposure to pesticides had an increased implantation rate (Tielemans *et al.*, 2000). This is in contrast to a Danish study where 128 couples, from a pool of 5879 women who had IVF treatment, had partners with known exposure to pesticides and growth retardants (Hjollund *et al.*, 2004b). tment, had partenrs where 128 couples from a pool of osure to pesticides had an increased implantation rate (TiNo increased risk of SA was found. Interestingly, there appeared to be less SA in the exposed group of 192 pregnancies (19.7–22.2%) compared to the control group of 2925 pregnancies (28.4%). It is therefore not possible to conclude that pesticide exposure can lead to adverse IVF outcomes.

The chemical environment: lifestyle exposures

Infections, diet and obesity

Pasquali *et al.* (2003) have reviewed the effects of diet and obesity on infertility. The general consensus is that in women, a body mass index (BMI) $>25\,kg/m^2$ was associated with a significant reduction in fecundity (Hassan and Killick, 2004). In men, a BMI <20 or $>25\,kg/m^2$ was associated with reduced sperm quality (Jensen *et al.*, 2004a). The reduction in female fecundity seems to be related to the relatively high increase in leptin levels in serum and follicular fluid (Butzow *et al.*, 1999). On the other hand, Brannian *et al.* (2001) showed that there was no correlation between BMI and IVF outcomes, although the leptin:BMI ratio was predictive of IVF success, with low leptin:BMI ratio associated with successful pregnancies. More recently, it was demonstrated that high, but not low, BMI was associated with poor IVF outcomes (Fedorcsák *et al.*, 2004). No studies have looked at the effects of diet on IVF outcomes. Although infections may be acquired through environmental conditions, they are not associated with occupational exposures. However, it may be mentioned that viral infections such as human immunodeficiency virus, hepatitis B or C virus are not contraindicated in IVF (Steyaert *et al.*, 2000; Baker *et al.*, 2003; Cleary-Goldman *et al.*, 2003).

Caffeine and alcohol consumption

The consumptionof three or more cups of decaffeinated coffee was associated with an increased risk for SA whereas drinking caffeinated beverages had no effect (Fenster *et al.*, 1997). Tolstrup *et al.* (2003a) also reported that high intake of caffeine prior to pregnancy seemed to be associated with increased risk of SA. However, Signorello and McLaughlin (2004) reviewed 15 epidemiological studies on caffeine consumption during pregnancy and the risk of SA. They concluded that the evidence for such a link was inconclusive based on all studies which did not have IVF cases. They mentioned one study on IVF (Klonoff-Cohen *et al.*, 2002) which showed a significant association between female but not male caffeine consumption and live births (OR 3.1, CI 1.1–9.7). Using fecundability (the monthly probability of conception) as an endpoint, Curtis *et al.* (1997) showed that a decrease was present in women who were coffee drinkers and men who were heavy tea drinkers. TTP was also significantly extended in females when coffee or tea intake was >6 cups per day or when the male partner consumed >20 alcohol units per week (Hassan and Killick, 2004), suggesting a dose-dependent effect. Klonoff-Cohen *et al.* (2003) also found an association between alcohol consumption among partners and adverse IVF outcomes. Alcohol intake has been associated with female infertility (Juhl *et al.*, 2003; Tolstrup *et al.*, 2003b; Eggert *et al.*, 2004). However, no such association was found among 2607 planned pregnancies and alcohol use over a 30 year period (Curtis *et al.*, 1997). Further work is needed to confirm or dispute these associations in light of the recent review (Signorello and McLaughlin, 2004).

Smoking

The adverse effects of cigarette smoking on human health have been well documented and will therefore be covered in more depth. Cigarette smoking during pregnancy is associated with an increased risk of a number of adverse obstetric and fetal outcomes including spontaneous miscarriage, placenta previa, premature rupture of the membranes, preterm birth and low birthweight (Ness *et al.*, 1999; Shiverick and Salafia, 1999; Andres and Day, 2000). Furthermore there is a significant association between smoking and reduced fertility among female smokers (Hughes and Brennan, 1996; Curtis *et al.*, 1997; Augood *et al.*, 1998; Hull *et al.*, 2000; Lindbohm *et al.*, 2002; Greenlee *et al.*, 2003). Therefore it is generally accepted that women who smoke should be counselled to quit prior to attempting to become pregnant. Smoking by men and passive and active smoking by women were associated with a longer TTP (Hull *et al.*, 2000). These authors reported that smoking >20 cigarettes per day by either partner was associated with longer TTP. Little evidence of any trend was seen with smoking from one to 14 cigarettes per day. A similar result was found by Hassan and Killick (2004) who noted that TTP was significantly longer if either the woman or partner smoked >15 cigarettes per day. However, these results must be tempered by the observation that when cotinine levels were measured to assess exposure, no differences could be detected in pregnancy rates in women with cotinine levels ≤ 20 ng/ml (non-smokers), compared to 20–50 ng/ml (passive smokers) and >50 ng/ml (active smokers) (Sterzik *et al.*, 1996).

Smoking and assisted reproductive technology

Females. Although the impact of smoking on IVF success has been less well documented, there have been reports of an association between smoking and decreases in the success rates of IVF. Meta-analyses that have examined the effects of smoking on the outcome of pregnancies achieved via IVF have suggested that smokers require approximately twice as many IVF cycles to conceive as non-smokers (Feichtinger *et al.*, 1997; Augood *et al.*, 1998; Klonoff-Cohen *et al.*, 2001b). In an earlier meta-analysis, Hughes and Brennan (1996) reported that in 13 studies of natural conception there was a small but clinically significant detrimental effect of female smoking but not male smoking on IVF success. This effect in the female may be a result of a combination of decreased fertilization rates, reduced number of oocytes retrieved, decreased pregnancy rates, and/or increased miscarriage rates in women who smoke (Harrison *et al.*, 1990; Zenzes *et al.*, 1995a; Van Voorhis *et al.*, 1996; Ness *et al.*, 1999; Klonoff-Cohen *et al.*, 2001b; Neal *et al.*, 2003).

Decreased success rates with assisted reproductive technology in couples who smoke may be a result of adverse effects of the constituents of cigarette smoke on the follicular microenvironment. Cotinine, the metabolite of nicotine, and cadmium, a heavy metal in cigarette smoke, have been detected in the follicular fluid of women who smoke (Zenzes *et al.*, 1995b; Drbohlav *et al.*, 1998; Younglai *et al.*, 2002), demonstrating that the chemicals present in cigarette smoke have access to the developing follicle. It was also demonstrated in IVF patients that estradiol concentrations in the follicular fluid (Paszkowski, 2001) or serum (Sterzik *et al.*, 1996) were significantly lower in women who smoked compared to non-smokers. Taken together these data suggest that components of cigarette smoke which have been detected in follicular fluid from IVF patients may impair follicular steroidogenesis. The estradiol:testosterone ratio in follicles is predictive of the maturity and quality of

the oocytes retrieved for IVF (Xia and Younglai, 2000). Therefore reduced estradiol production in the follicles from women who smoke could adversely affect oocyte quality and maturity and ultimately decrease the chance of the success of assisted reproduction treatment. Although impaired follicular steroidogenesis in women who smoke may significantly reduce the success of assisted reproduction treatment, it has also been suggested that cigarette smoking can alter folliculogenesis via other pathways as well. Paszkowski *et al.* (2002) have demonstrated that active smoking alters the pro-oxidant/antioxidant balance in follicular fluid such that there is oxidative stress in the growing follicle. Oxidative stress may in turn lead to cell damage and cytotoxicity in the oocyte and granulosa cells.

It has also been suggested that cigarette smoking can adversely affect gamete quality (Van Voorhis *et al.*, 1996; Weigert *et al.*, 1999; Zenzes, 2000) and follicle reserve (El-Nemr *et al.*, 1998). It has been reported that, of the oocytes that fail to fertilize after *in vitro* insemination, ~25% have chromosomal abnormalities (Plachot, 2001). Zenzes *et al.* (1995a) have reported that women who smoke have an increased frequency of oocytes with a diploid chromosome complement compared to non-smokers. They suggested that this difference occurred due to the prevention of first polar body extrusion, suggesting that there is an effect of maternal cigarette smoking on oocyte maturation (Zenzes *et al.*, 1995a). Similarly, Neal *et al.* (2003) have shown that the percentage of oocytes that were meiotically mature as determined by the presence of the first polar body at the time of ICSI was lower in female patients who were smoking at the time of assisted reproduction treatment. This effect on oocyte maturity was more pronounced in smoking women aged >30 years (Zenzes *et al.*, 1997). In men, Shi *et al.* (2001) have demonstrated that cigarette smoking is associated with an increased risk of aneuploidy in sperm, but that this effect appears to be chromosome specific. Cigarette smoking may also increase the risk for DNA damage in ovarian cells and sperm. Benzo-[a]-pyrene, a potent mutagen and carcinogen found in cigarettes, has been shown to form DNA adducts in granulosa–lutein and sperm cells which may increase the risk for DNA damage in these cells (Zenzes *et al.*, 1998, 1999a). The higher level of DNA adducts in the embryo following fertilization with such sperm (Zenzes *et al.*, 1999b) may contribute to failed implantation following fertilization.

Males. Interestingly, although most of the epidemiological evidence linking infertility and cigarette smoking has focused on the female partner, Neal *et al.* (2003) have reported that compared to women who do not smoke, fertilization rates and pregnancy rates in an assisted reproduction treatment clinic were decreased not only in women who smoked but in women who were exposed to cigarette smoke because their partner actively smoked at home. Similar results were obtained by Zitzmann *et al.* (2003) who found a significantly decreased pregnancy rate (22%), in women with smoking male partners compared to 38% with non-smoking partners for both IVF and ICSI. This observation has now been confirmed in an animal model where Kapawa *et al.* (2004) have demonstrated that paternal exposure to cigarette smoke significantly reduced the number of live offspring in relation to the number of transferred oocytes following IVF and ISCI techniques, suggesting that paternal cigarette smoke affects implantation. Taken together these data demonstrate that cigarette smoking may be deleterious to the outcome of assisted reproductive technology.

Cigarette smoking has been associated with a significant decrease in sperm density, total sperm count, and the total number of motile sperm (Pacifici *et al.*, 1993; Vine *et al.*, 1996; Kunzle *et al.*, 2003). Normal sperm morphology is also decreased significantly in active smokers (Ratcliffe *et al.*, 1992). Reduced sperm quality has been reported for males born to mothers who smoked during pregnancy (Jensen *et al.*, 2004b) with the threshold being >10 cigarettes per day (Storgaard *et al.*, 2003). Experimental studies have shown that whole animal exposure to cigarette smoke or exposure of human sperm to cotinine, the metabolite of nicotine, *in vitro* can decrease the percentage of motile sperm, the percentage of viable sperm and the ability of sperm to penetrate the oocyte (Gandini *et al.*, 1997; Sofikitis *et al.*, 2000; Kapawa *et al.*, 2004). Furthermore, Wong *et al.* (2000) have reported that cotinine concentrations in seminal plasma are positively associated with abnormal sperm morphology. When combined with alcohol, the effects of smoking on sperm parameters were magnified (Martini *et al.*, 2004). These data suggest that paternal smoking may also have a significant impact on gamete quality and therefore the success of assisted reproduction treatment. Therefore, both partners should be counselled to stop smoking prior to undertaking infertility treatment. In spite of the large body of data available, the mechanism of cigarette smoke effects on fertility are unknown and identification of the chemical agents responsible for these effects are similarly unknown.

Whereas effects of environmental contaminants on human reproduction remain controversial, the evidence for cigarette smoke-induced changes in reproductive function is more certain. However, the contaminants in cigarette smoke responsible and the mechanisms of action have not been defined. Of the thousands of chemical contaminants present in cigarette smoke, the majority of research has focused on nicotine.

The chemical environment: inadvertent exposure to environmental contaminants

Humans are continually exposed to pollutants in the air, food and water. In recent years the role of EDC in adverse reproductive outcomes has received attention because of their persistence and ability to mimic natural hormones. These compounds are capable of long distance transport through the air and food chain. A comprehensive list of chemicals thought to be EDC can be found in Myers (2004). Chemicals on this list include a wide variety of xenoestrogens, alkylphenolic chemicals, phthalates and pesticides which possess estrogenic activity and could have biological effects. These chemicals are ubiquitous in the environment and everyone experiences some degree of exposure. While routes of exposure vary, the majority of human exposure is inadvertent and through our food (Schecter *et al.*, 1994, 2001, Schecter *et al.*, 2003). Exposure to environmental contaminants has been suggested to play a role in the pathobiology of adverse reproductive health effects, including decreased semen quality, subfertility, change in birth sex ratio, and an increase in the prevalence of developmental abnormalities of the male reproductive tract (Carlsen *et al.*, 1992; Colborn *et al.*, 1993; Swan *et al.*, 1997, 2000; Marcus *et al.*, 1998; Allen *et al.*, 1997; Hosie *et al.*,

2000; Toft *et al.*, 2004). Adverse effects on human fertility are suggested from several distinct lines of evidence. In a retrospective study of the Hutterite population in Pennsylvania, total fertility rates were calculated for each 5 year period beginning with 1901–1905 and ending with 1981–1985, and a decrease in age-specific fertility rates was demonstrated (Nonaka *et al.*, 1994). These data suggest a tendency towards decreasing fertility rates in this population that appears to correspond with the rise of chemical production and use in North America. Furthermore, TTP was found to be longer in couples where the female partner was exposed to EDC (Gerhard *et al.*, 1999; Toft *et al.*, 2004). Consumption of sport-caught fish from the Great Lakes has been linked with an increased TTP (Courval *et al.*, 1999; Buck *et al.*, 2000). Overall these reports provide evidence of a tendency towards lower fertility and fecundity rates that may be associated with exposure to environmental chemicals.

Regional differences in infertility rates also suggest that environmental toxicants may be contributing factors (Juul *et al.*, 1999; Karmaus and Juul, 1999; Jensen *et al.*, 2001; Carpenter *et al.*, 2001). Analysis of health trend data such as infertility rates are frequently used as an indicator of potential adverse health effects of environmental toxicants including EDC, but these studies have not provided consistent results. For example, in the USA, the pregnancy rate in 1996 was 9% lower than that in 1990 (Ventura *et al.*, 2000) whereas in Sweden, an analysis of birth registries has shown that the number of infertile couples (failure to conceive after 1 year of unprotected intercourse) decreased from 12.7% in 1983 to 8.3% in 1993 (Akre *et al.*, 1999). Analysis of fertility rates on a population basis has the weakness of missing potential regional differences. For example, several studies conducted in a number of European cities have documented regional differences in TTP (Juul *et al.*, 1999; Karmaus *et al.*, 1999; Jensen *et al.*, 2001). The greatest incidence of subfecundity was found in northern Italy, Germany and Denmark (Juul *et al.*, 1999; Karmaus *et al.*, 1999), whereas the highest fecundity was observed in southern Italy and northern Sweden (Juul *et al.*, 1999). In another study (Carpenter *et al.*, 2001), the incidence of infertility was greater in couples residing in heavily polluted areas (Superfund sites) compared to several reference populations who resided in relatively unpolluted areas in the rest of New York State. These studies therefore support the notion that subfecundity may be linked to exposure to environmental toxicants.

Drinking water subjected to chlorination procedures contains by-products such as trihalomethanes and trichloroethylene which appear to have a moderate association with SA, one of the endpoints used for assessing fertility (Bove *et al.*, 2002). Using a utility-wide average total trihalomethane exposure assessment method, together with variance-based weights and subsets (Waller *et al.*, 2001), demonstrated that a dose response is present between SA and exposure to drinking water pollutants. However, an international workshop on assessing exposure to disinfection by-products in drinking water concluded that more collaboration and further work are needed before the risks can be properly assessed (Arbuckle *et al.*, 2002). In addition, a newly emerging concern is the impact of pharmaceuticals being released into the aquatic environment from widespread use (Daston *et al.*, 2003) or the rapidly expanding field of nanotechnology where nanoparticles could affect cell membranes (Service, 2004).

Females. Actual toxicant exposure data in relation to fertility are limited; however, persistent organochlorine chemicals with documented endocrine-disrupting activity have been measured in ovarian follicular fluid of women undergoing IVF (Trapp *et al.*, 1984; Baukloh *et al.*, 1985; Schlebusch *et al.*, 1989; Jarrell *et al.*, 1993b; Foster *et al.*, 1996; Younglai *et al.*, 2002). In one study (Jarrell *et al.*, 1993b), levels of persistent organochlorine contaminants in ovarian follicular fluid were determined in women attending fertility clinics in three Canadian cities. Although some geographical differences in body burdens were observed, there was no association between exposure and adverse outcomes. In a larger study in Germany (Gerhard *et al.*, 1999), elevated concentrations of chlorinated organic compounds with endocrine-disrupting characteristics, including pentachlorophenol, polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB), were found in infertile women. In the Trapp *et al.* (1984) study, oocyte recovery and embryo cleavage rates were inversely related to chlorinated hydrocarbon concentrations. In a similar study from IVF centres in six Canadian cities (Foster *et al.*, 1996), there were regional differences in the amounts of pollutants found but several chemicals were detected in the majority of subjects: hexachloroethane, 1,2,4-trichlorobenzene, dichlorobischlorophenylethylene (*p*,*p*'-DDE), the organochlorine pesticide mirex, and the PCB 138, 153, and 180. Some of these pesticides persist in maternal adipose tissue and may be transported to the fetus (Foster *et al.*, 2000). Of the contaminants measured in serum by Foster *et al.* (1996) and adverse outcomes studied (number of oocytes recovered, fertilized, cleavage rates), only endosulfan levels were positively associated with irregular menstrual cycles. In the three Canadian cities study by Jarrell *et al.* (1993b), no adverse outcome was observed in association with contaminant levels.

Males. In men, a pilot study by Hauser *et al.* (2002) suggested there may be a correlation between PCB and DDE in serum and sperm quality. However, the results of a more detailed study failed to support this association (Hauser *et al.*, 2003). The Ministry of Public Health in Mexico in collaboration with Laval University (Ayotte *et al.*, 2001) found that there was an association between high serum levels of DDE and abnormal sperm. DDE is a metabolite of DDT which was used for the eradication of mosquitoes and banned in the USA since 1972, but is still being used sporadically in Mexico. The Mexican men examined by Ayotte *et al.* (2001) had DDE serum levels of 77.9 mg/kg lipids, a value some 350-fold higher than that documented by the same laboratory in Canadians exposed to background environmental levels. The lack of correlation between DDE and PCB 153 levels in serum and sperm quality was also demonstrated in 195 Sweden fishermen (Rignell-Hydbom *et al.*, 2004). Younglai *et al.* (2002) found limited amounts of contaminants in seminal plasma from 21 men. In a more recent study (Swan *et al.*, 2003) high levels of a number of pesticides were associated with poor semen quality. A similar association was found in men with high phthalates (Duty *et al.*, 2003). This study by Duty *et al.* (2003) and that of Storgaard *et al.* (2003) prompted Swan (2003) to suggest that the hypothesis that environmental agents do affect semen quality must be taken seriously. Taken together these reports demonstrate that there are regional differences in fertility rates and that EDC reach the ovary and can be

measured in seminal plasma and ovarian follicular fluid, raising concern that these chemicals may be toxic to gametes.

Biological plausibility: animal studies

While there is still controversy (Joffe, 2001) regarding the effects of environmental contaminants on fertility, occupational exposure to chemicals together with experimental evidence support possible mechanisms of action and biological plausibility for the association between exposure and effect on fertility. Although environmental chemicals and environmental estrogens in particular have been suggested as causes of adverse reproductive outcomes (Sharpe and Skakkebaek, 1993; Sharara *et al.*, 1998), attempts to establish a link between adverse reproductive outcomes and environmental chemicals are inconclusive due largely to the failure to document exposure. However, the biological plausibility of the hypothesis that environmental contaminants are linked to impaired semen quality has been shown in animal studies. Sharpe *et al.* (1995) have demonstrated that gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production, illustrating a possible mechanism by which reduced sperm density in men could occur. However, Feichtinger (1991) noted that although pollutants such as hexachlorobenzene (HCB) and PCB were found in seminal plasma, no correlation was observed with semen quality. Oliva *et al.* (2001) have also concluded that although men may be exposed to many environmental toxicants, the number of substances which have been proven to be deleterious to human spermatogenesis is very small. Guo *et al.* (2000) found that children exposed in the womb to PCB had increased abnormal sperm and motility, further highlighting the fact that contaminants could have different actions depending on the window of exposure and the stage of development of the individual.

Destruction of growing follicles and oocytes has been observed in several animal models following treatment with endocrine disrupting chemicals. Dicofol, an estrogenic organochlorine pesticide induced a significant decrease in healthy follicles and the number of estrous cycles (Jadaramkunti and Kaliwal, 1999). Follicle destruction has also been reported for PCB-exposed rhesus monkeys (Muller *et al.*, 1978). Specific PCB congeners, 153 (estrogenic) and 126 (dioxin-like), were shown to alter oocyte maturation and blastocyst development in bovine oocytes in culture (Krogenaes *et al.*, 1998). Although PCB 153 did not affect oocyte maturation, the highest concentration reduced the number of oocytes that cleaved. By contrast, PCB 126 had an adverse effect on oocyte maturation at the highest concentration, as well as on blastocyst development at all concentrati ons tested. The organochlorine pesticides, methoxychlor, lindane and dieldrin have been shown to alter oocyte maturation in the mouse (Picard *et al.*, 2003). In a review of organochlorine chemicals known as persistent organic pollutants, because of their persistence in the environment, Pocar *et al.* (2003) concluded that they disrupt mammalian oocyte maturation in every mammalian species studied to date. These data provide insight into the potential mechanisms of contaminant action, but are of limited value, since the oocyte develops in a complex mixture of hormones, growth factors and environmental agents. Campagna *et al.* (2001) evaluated the effect of a mixture of environmental contaminants and demonstrated a dose-related decrease in the quality of cumulus expansion, a decrease in the viability of cumulus cells, and an increase in the number of incompletely matured oocytes. It has also been reported that there are adverse effects of Aroclor-1254 (Kholkute *et al.*, 1994a), polybrominated biphenyls and polychlorinated terphenyls (Kholkute *et al.*, 1994b) on IVF in the mouse. Embryotoxicity has also been demonstrated for Aroclor-1260 in the rabbit (Lindenau and Fischer, 1996) and the organochlorine pesticides methocychlor, DDT and hexachlorocyclohexane in the mouse (Alm *et al.*, 1996). Taken together, these data suggest that environmental toxicants may impair human fertility via altered gamete quality and/or embryo development.

Problems interpreting exposure studies

In reviewing progress over the last decade, several authors have concluded that although much has been accomplished, much more needs to be done (Damstra, 2003; Daston *et al.*, 2003; Nelson, 2003). Some of the more pressing issues are highlighted in the review by Daston *et al.* (2003) of the nine questions posed as data gaps in the US Environmental Protection Agency's research strategy, and they include: the magnitude of the endocrine disrupter problem; the development of standard validated screening tests for the $>70\,000$ chemicals regulated by the Toxic Substances Control Act; resolution of non-monotonic dose–response curves; effects of exposure to multiple EDC; and new potential hazards such as pharmaceuticals in the aquatic environment. Despite the advances made in this area, the application of the precautionary principle should be mandatory (Cairns, 1999). The difficulty of implementing the precautionary principle and where to set limits remain.

The need for sensitive and convenient screening tools in conducting epidemiological research related to adverse reproductive outcomes was raised in the 1980s by Levin (1983) and Baird *et al.* (1986). Some of these problems include inadequate databases, selection and reporting bias, and outcomes chosen for observation. Savitz and Harlow (1991) suggested that when selecting endpoints, several characteristics must be considered: severity (with a proper balance between clinical severity and statistical or biological sensitivity); the relative sensitivity of outcomes and their interrelationships; baseline frequency of adverse outcomes; evidence from reproductive toxicology and specificity of effects. They also indicated that practical considerations such as frequency of occurrences, statistical power, amenability to retrospective analysis, and burden of measurement on the population being studied are necessary. In studies based on questionnaires, there is a high potential for miscalculation of occupational exposure (Blatter *et al.*, 1997). For example, while 72% of pregnant subjects reported no exposure to tobacco smoke, almost all had detectable levels of cotinine (DeLorenze *et al.*, 2002). Confirmation of exposure from another data source such as medical records could increase the power of a study (Hemminki *et al.*, 1995). Observations that suggest freedom from risk are often insufficient to conclude they are not due to chance and observed clinical effects often suffer from small sample size (Schull, 1984). Furthermore, the effects of exposure to environmental contaminants may be completely different depending on the age of the individual. Therefore, the lack of effects of environmental chemicals present in human follicular

fluid or seminal plasma on IVF outcome does not necessarily mean no effect, because effects on the genome will not be manifest in such a short time. On the other hand, adverse IVF outcomes despite apparently morphologically normal oocytes and sperm may be the result of longstanding exposure.

In classical pharmacological studies adverse effects are measured in terms of dose–response and sometimes immediate changes. However, in studying the effects of environmental contaminants and particularly endocrine disrupters, the classical dose–response is not always applicable since effects may be observed at extremely low concentrations and none at higher concentrations (Krimsky, 2000). Moreover, the toxicological notion that a threshold value exists, below which no effect can be seen, is being challenged in the contemporary literature. Exposure to low levels over extended periods of time is another complicating factor. Timing and duration of exposure, as well as dose, dictates the kinds of effects that can be induced and the severity of the lesions (Gray *et al.*, 2001).

There is often a window of susceptibility when exposure can have effects which are manifested later in development. For example, the seminal papers by Vom Saal (Vom Saal and Bronson, 1980; Vom Saal *et al.*, 1983) demonstrated that the 'positioning effect' of fetal mice, a male mouse positioned between two females or a female between two males *in utero*, profoundly affected their physiology and behaviour as adults. This intergenerational effect became obvious with the discovery that when the xenobiotic estrogen diethylstilbestrol (DES) was given to pregnant mothers, their daughters developed clear cell adenocarcinoma of the vagina (Herbst and Scully, 1970; Herbst *et al.*, 1971) and the sons had abnormalities in their sex organs and sperm quality (Herbst *et al.*, 1971; Stillman, 1982). More than 30 different drugs can have an effect on the developing fetus if taken during pregnancy (Schardein, 1993).

Is there cause for concern?

Whereas this review has concentrated on the adverse reproductive effects of occupational and environmental contaminants, the opposite view can be proposed. The ubiquitous environmental contaminant with known toxicity, TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), was released into the atmosphere in Seveso, Italy in 1976, resulting in the highest known TCDD exposure in a human residential population (Eskenazi *et al.*, 2003). No change in fertility was observed, neither was the rate of spontaneous abortions increased (OR 0.8, CI 0.6–1.2). In India, Africa and parts of Mexico where DDT (Attaran and Maharaj, 2000; Ayotte *et al.*, 2001) is still being applied to control mosquitoes, there has not been a report of a decline in fertility. Similarly, no significant decline in fertility was observed in those countries where the DDE levels in tree bark, an index of exposure, were elevated (Cocco, 2002; Garcia, 2003). The argument that developed countries with heavy industries produce more pollutants which are associated with decline in fertility is tempered by two studies showing that human fertility is not declining in Sweden (Akre *et al.*, 1999) nor in Great Britain, where it is increasing (Joffe, 2000) despite evidence of declining sperm counts (Lunenfeld and Van Steirteghem, 2004).

Although the weight of evidence to date suggests that DDE may have an inhibitory effect on physiological processes, the highly controversial position could be taken that DDE is beneficial based on the following evidence. In a follow-up study, Hauser *et al.* (2003) were not able to confirm their earlier preliminary results (Hauser *et al.*, 2002) showing an association between DDE serum levels and abnormal sperm parameters. In the study by Cohn *et al.* (2003), 16% of the women studied showed a beneficial effect of DDE, i.e. TTP was reduced in women with elevated serum levels of DDE. DDE was shown to stimulate the aromatase enzyme system of human granulosa cells and to have a synergistic effect with FSH (Younglai *et al.*, 2004a). This could be interpreted as having a beneficial effect or as having an adverse effect through premature production of estradiol which is implicated in oocyte maturation. However, we have recently found that DDE stimulates calcium uptake in human granulosa cells (Younglai *et al.*, 2004b), again pointing to a putative beneficial effect since calcium is necessary for many physiological processes such as sperm capacitation and the acrosome reaction. Further evidence is seen from the studies by Tielemans *et al.* (2000) who reported that paternal pesticide exposure was significantly associated with increased implantation rates in IVF and Hjollund *et al.* (2004b) who found an average of 20.7% spontaneous abortions in Danish IVF pregnancies where 192 of the male partners were exposed to herbicides, fungicides, pesticides and growth retardants. This was in contrast to the reference group of 2925 pregnancies where the spontaneous abortion rate was 28.4%.

In another IVF study (Jarrell *et al.*, 1993b) there was a strong positive relationship of embryo cleavage with HCB levels ($r = 7.10$), suggesting that HCB may actually stimulate cleavage rates. This is reminiscent of a study in which heavy female smoking was associated with more previous pregnancies and higher fertilization rate in IVF when compared with non-smokers (Hughes *et al.*, 1992, 1994). Similarly, Zenzes and Reed (1996), in a study of 147 couples and 1094 embryos, found that smokers produced higher rates of best quality embryos and that there was a positive correlation between high cotinine levels and good embryo quality. More mature oocytes were found in younger female smokers compared to non-smokers (Zenzes *et al.*, 1997). The data from our laboratory (Younglai *et al.*, 2002) revealed two unusual and unexpected positive correlations: (i) between follicular fluid PCB 49 and pregnancy; and (ii) between seminal plasma mirex and follicular fluid cadmium in the fluids of the couples who became pregnant but were not detectable in the group where no fertilizations occurred. These positive correlations of environmental contaminants with favourable outcomes point to the difficulties associated with interpreting the significance of conflicting findings and multiple potential interpretations.

Summary and conclusions

This review highlights the paucity of studies on the exposure of couples to environmental insults including environmental contaminants and the association with IVF success, the problems associated with the interpretation of such data sets and the need for further well-designed studies. Across each domain examined in this review there is little consistency among study findings. Moreover, the paucity of literature makes it difficult to draw any firm conclusions other than to suggest that, despite growing

concern, the evidence linking environmental factors and impaired human fertility is weak. In order to advance this field of study and establish a link between environmental contaminant exposure and effects on fertility several steps must be accomplished. These include but are not limited to: (i) definitive exposure data preceding adverse effects followed by confirmation in animal studies; (ii) evidence that levels present in the environment as mixtures could have effects; (iii) geographic, gender and ethnic differences in response to exposures. Such studies could provide data to conclude that occupational exposure to environmental hazards pose a real risk for adverse IVF outcomes. Even with increasing information and better-designed studies to assess exposure, the rapidly advancing technological field and production of new pharmaceuticals will present more challenges.

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