Alcohol, drugs, caffeine, tobacco, and environmental contaminant exposure: Reproductive health consequences and clinical implications

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Abstract
Reproductive function and fertility are thought to be compromised by behaviors such as cigarette smoking, substance abuse, and alcohol consumption; however, the strength of these associations are uncertain. Furthermore, the reproductive system is thought to be under attack from exposure to environmental contaminants, particularly those chemicals shown to affect endocrine homeostasis. The relationship between exposure to environmental contaminants and adverse effects on human reproductive health are frequently debated in the scientific literature and these controversies have spread into the lay press drawing increased public and regulatory attention. Therefore, the objective of the present review was to critically evaluate the literature concerning the relationship between lifestyle exposures and adverse effects on fertility as well as examining the evidence for a role of environmental contaminants in the purported decline of semen quality and the pathophysiology of subfertility, polycystic ovarian syndrome, and endometriosis. The authors conclude that whereas cigarette smoking is strongly associated with adverse reproductive outcomes, high-level exposures to other lifestyle factors are only weakly linked with negative fertility impacts. Finally, there is no compelling evidence that environmental contaminants, at concentrations representative of the levels measured in contemporary biomonitoring studies, have any effect, positive or negative, on reproductive health in the general population. Further research using prospective study designs with robust sample sizes are needed to evaluate testable hypotheses that address the relationship between exposure and adverse reproductive health effects.

Keywords: Alcohol; environmental contaminants; fertility; reproductive; tobacco; toxicity

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1. Introduction

Through daily interaction with our environment we are exposed to a wide variety of naturally occurring and man-made chemicals in our drinking water or food, the air, or, in some cases, the drugs we take. Although it has long been recognized that certain compounds are teratogenic, the effects of dietary compounds, some of which are hormonally active, on sexual maturation, fecundity (the potential to conceive), and fertility (the capacity to give birth) are frequently overlooked or not considered. It has only been in the last decade that reproductive toxicology has emerged as a distinct field of study apart from teratology. Through reproductive toxicology, the consequences of exposure to environmentally relevant levels of compounds on all aspects of reproductive physiology throughout the course of life are examined and risks characterized. Although considerable strides have been made in understanding the mechanisms of chemical action on reproductive physiology, function, health and development, many data gaps persist, making definitive conclusions about the health risk associated with exposure to many agents uncertain. The consequences of these exposures on reproduction are not well understood and the clinical relevance is often unknown. Therefore, the purpose of this paper is to systematically review the effects and risks to human reproduction of a selected number of compounds based on the strength of evidence for their adverse effects, the high frequency and extent of human exposure, and the consequences of those exposures to human reproductive health. The human health relevance of the data for men and women attempting to conceive will be highlighted in an effort to provide clinicians with helpful direction for their patients faced with these exposures.

2. Substance use and abuse

There is ample evidence that use of addictive drugs, whether legal (nicotine and ethanol) or illicit (opiates, cocaine, cannabinoids, and others), pose real risks to the developing fetus and neonate. Recognized effects include disorders such as the fetal alcohol syndrome, withdrawal syndromes, and growth retardation (Frank et al., 1990; Lumeng et al., 2007; Rivkin et al., 2008) in newborns. It is generally accepted that these developmental exposures in utero have known or suspected long-term adverse consequences for exposed children (Shankaran et al., 2007). Issues of teratology and developmental toxicology are extremely important matters of human health, but are tangential to the theme of this review, which is an appraisal of possible reproductive deficiencies due to adult exposure to these classes of agents.

2.1. Addictive drugs

An explicit determination of potential effects of adult exposures to addictive drugs on fertility is severely compromised by outright addiction to one or more of these agents. Not only is it extremely difficult to attribute a particular outcome to use of a specific agent, at some specific doses or some particular pattern of use, but the general consequences of addiction confound interpretation. From the perspective of the physiological impact on an individual, addiction to one or more of these agents includes an array of debilitating stressors such as inadequate nutrition (in terms of both macronutrients and micronutrients), exposure to infectious diseases, lack of shelter, inadequate health care, or poor decision making leading to late access of health care, physical abuse, multiple other psychosocial stressors, etc. Thus, it is biologically plausible that addicted individuals may be so metabolically compromised that essential steps in normal reproduction would be adversely affected. For the purposes of our review, we will not address the reproductive effects of addiction. Instead, we will consider the limited information about effects of these agents at lower levels of use.

Substance use is ubiquitous in society, with 60% to 80% of adults using alcohol, and approximately 10% of adults carry some form of substance use to the point of abuse or addiction. Herein we review the most common substances of abuse in developed countries, namely narcotics (opiates and cocaine), alcohol (ethanol), and marijuana (cannabinoids). Several other classes of agents such as amphetamines, chlor Diazepoxide, phencyclidine, and others may also be abused but are beyond the scope of the present review.

Because multidrug abusers predominate, it can be extremely difficult to isolate the effects of a single drug. The level of substance abuse has been suggested by a number of studies. For example, in the Midwest (Minneapolis–St Paul, MN), a study of over 1000 pregnant women (Yawn et al., 1994) found that 2.0% were cocaine positive, 2.6% tetrahydrocannabinol positive, and 1.2% opiates positive. Additionally, 22.6% reported that they smoked tobacco. In one study in Central California in the early 1990s (Gurnack and Paul, 1997), 11% of pregnant women tested positive for some type of substance. Of the substances found in the urine of those who tested positive, the most prevalent were barbiturates, marijuana, methamphetamine, and amphetamines. An epidemiological study of meconium for the metabolites of cocaine, morphine (opiates), and cannabinoid in over 3000 newborns found that 44% of them were positive for cocaine, morphine, or cannabinoid; 31% were positive for cocaine, 21% for morphine, and 12% for cannabinoid (Ostrea et al., 1992). Finally, in some studies there appears to be even higher rates of substance abuse, with analysis of meconium samples from newborns
in adult substance-abusing women are indicated by men-
sere developmental effects, reproductive disturbances
al nervous system (CNS) function. Aside from these
ences that can persist for months and may lead to
later increased risk of neurobehavioral effects and altered central nervous system (CNS) function. Aside from these
serious developmental effects, reproductive disturbances in adult substance-abusing women are indicated by menstrual abnormalities, especially amenorrhea in heroin users. Complications in individuals who abuse opiates include viral infection (human immunodeficiency virus [HIV], hepatitis), malnutrition, skin infections, and cardiac pathology (Oei and Van Thiel, 1977). Nevertheless, the fact that many infants are born of mothers who are regular abusers of these narcotics implies that the degree of reproductive compromise is not profound enough to obviate the frequent birth of exposed and affected infants. Such negative reasoning does not measure or even clearly imply the extent to which fertility in adult women or men may be diminished by use of narcotics, but certainly even abuse to the level of addiction does not reliably prevent pregnancies. However, the drug may tip the balance against those whose fertility was borderline before exposure, thereby pushing them "over the edge" into infertility. It is known that in adult mammals, opiates bind to endogenous opiate receptors in the hypothalamus, and this binding causes an inhibition in the secretion of luteinizing hormone (LH). Mild to modest gonadal suppression appears to result from this primary action in the CNS. In animal studies, adverse effects in males have been confirmed by observations of significant disturbance of spermatogenesis (Abel et al., 1989; George et al., 1996). Chronic cocaine exposure in female rhesus monkeys led to disruption of menstrual cycles (Mello et al., 1997). In females, the strongest evidences of adverse reproductive effects are obstetrical complications in human pregnancies such as pregnancy loss and placental abruption (Chasnoiff et al., 1985; Acker et al., 1983; Little et al., 1989).

In summary, the effect of narcotics on fecundity is unclear and not well studied. The frequency of narcotic use by individuals attempting to conceive is unknown and the consequences to their fertility remains to be determined. Furthermore, whereas the adverse effects of exposure to narcotics on the fetus are known, the specific future fertility effects of developmental exposure of such offspring is unknown.

2.2. Ethanol

The feminization seen in alcoholic men seems to be related to liver dysfunction (Lloyd and Williams, 1948) and probably reflects reduced hepatic clearance of estrogens (Lester and Van Thiel, 1977). In men who consume lower amounts of ethanol, some effects on sex steroids can still occur. In men and male animals, chronic ethanol exposure has been associated with lower serum testosterone levels (Lester and Van Thiel, 1977; Van Thiel et al., 1975a; Gordon et al., 1978; Badr and Bartke, 1974; Mendelson et al., 1978b), increased clearance of testosterone from the plasma (Gordon et al., 1976; Rubin et al., 1976), increased levels of plasma sex hormone–binding globulin and higher prolactin (Van Thiel et al., 1975b), and increased estradiol levels (Lindholm et al., 1978). The reduction in plasma testosterone levels correlate with decreased responsiveness to human chorionic gonadotropin, suggesting that ethanol damages the Leydig cell compartment of the testis, as has been seen in ethanol-treated animals (Van Thiel et al., 1975a; Klassen and Persaud, 1978). LH levels are generally in the normal range in alcoholic men (Lester and Van Thiel, 1977; Gordon et al., 1976; Van Thiel et al., 1975a), but because both androgens and estrogens can contribute to feedback regulation of LH, the physiological meaning is not obvious. In a more recent study, significantly reduced plasma concentrations of testosterone, LH, and follicle-stimulating hormone (FSH) were reported in male alcohol abusers (Maneesh et al., 2006).

Alcohol can cause significant deterioration in sperm concentration, semen volume, and sperm motility (Kucheria et al., 1985; Brzek, 1987). Distinct morphological abnormalities have been shown in semen samples of men consuming excessive amounts of alcohol (Dixit et al., 1983; Wichman, 1992; Muthusami, 2005). There is disruption of spermatogenesis (Lester and Van Thiel, 1977; Anderson et al., 1978) following acute consumption of ethanol at 0.4 to 0.8 g/kg. Semen analysis showed increased abnormal morphologies (Doepfmer and Hinckers, 1965), whereas chronic exposure is associated with a reduction in seminiferous tubular diameter and injury to the germinal epithelium (Van Thiel et al., 1975a; Klassen and Persaud, 1978; Arlit and Wells, 1917). In mice (Anderson et al., 1978), known fertile males were fed a liquid ethanol diet (5% v/v) for 26 days, then returned to a normal diet for 2 days and mated with unexposed females. Compared to litters sired by control males (pair fed with an isocaloric sucrose diet), litters sired by ethanol-exposed males had reduced litter size and lower postnatal viability.

Adverse effects of chronic ethanol consumption on male fertility have been demonstrated in experimental animals. However, it appears that prepubertal animals are more susceptible than adults. In one study (Cicero et al., 1990), prepubescent (25 days of age) and fully mature adult male rats were maintained on an alcohol liquid diet or were pair-fed a control diet. Alcohol significantly affected many of the primary indices of puberty and sexual maturation in the younger animals. The normal pubertal increases in serum testosterone levels, the weights of the testes and secondary sex organs and β-endorphin levels in the hypothalamus were substantially reduced in alcohol-exposed animals compared with controls. In the fully mature animals, the effects of alcohol on reproductive endpoints were transitory and of considerably less magnitude. After a 2-week alcohol-free period, male rats exposed to alcohol during development were
bred with unexposed primiparous females. Although litter numbers sired by alcohol-exposed or control males were the same, litter sizes were significantly smaller in alcohol-derived offspring than in controls. In another study (Klassen and Persaud, 1976), where chronic alcoholism had been induced in male rats by an oral self-administration technique, reduced reproductive performance and decreased serum testosterone levels were observed in the exposed animals. Thus there is clear evidence of the reproductive toxic effects of chronic alcohol exposure; however, the consequences of moderate alcohol use on fertility remain unclear.

Substantial evidence has been gathered over the last half-century that reveals that excessive ethanol intake is detrimental to nongravid and gravid women and that lesser exposures adversely affect the human fetus. Alcohol remains the substance most frequently abused by pregnant women, and the fetal alcohol syndrome (FAS) has been well described. FAS is characterized by intrauterine growth retardation, facial abnormalities, congenital defects, musculoskeletal abnormalities, and dysfunction of the CNS (Spohr et al., 2007; Calhoun and Warren, 2007). It is generally accepted that an average daily maternal intake of 3 oz of pure alcohol is sufficient to significantly increase the incidence of FAS in the offspring, whereas consumption of less than 1 oz per day appears to have little or no associated increased risk of FAS. In terms of birth defects, intake of six drinks of ethanol per day leads to a 50% chance of birth defects, or approximately 10 times the normal incidence (Rementeria, 1977). Nevertheless, the fertility impact of ethanol use or abuse in nonalcoholic women is uncertain. The most recent and convincing observation is that in a group of 124 healthy volunteer women, the probability of conception was reduced by about 50% in cycles in which women had consumed one or more drinks of ethanol per week (Hakim et al., 1998).

Comparable effects have also been documented in female animals. In the rat and mouse, ethanol has been reported to inhibit ovulation (Van Thiel et al., 1978). Plasma estradiol and progesterone levels are suppressed (Blake, 1974). Rats maintained on a 5% ethanol liquid diet manifest decreased ovarian function, including cessation of estrous cycles and decreased ovarian and uterine weights (Bo et al., 1982). Another study assessed the effects of a similar diet in immature female rats (Krueger et al., 1982). Vaginal patency was significantly delayed in ethanol-treated rats compared to controls. The rats that received ethanol for 16 weeks exhibited more irregular estrous cycles (both less than 4 and greater than 6 days) than controls. After 16 weeks of treatment, the rats were mated, and ethanol was not given during pregnancy. The average number of pups per litter and body weight of the offspring was similar for all groups. These data show that although ethanol alters normal cyclic activity, it does not totally suppress ovarian function, since alcohol-treated rats were capable of mating and delivering viable offspring.

In a nonhuman primate study, female macaque monkeys self-administered high doses of alcohol (2.9 to 4.4 g per kilogram per day, which is approximately equivalent to an average person consuming 7 to 10 oz of alcohol a day) for 3 to 6½ months (Mello et al., 1983). Chronic alcohol intoxication led to amenorrhea, uterine atrophy, decreased ovarian weights, and suppression of LH levels. These reproductive system effects in female nonhuman primates seemed to mimic the findings in clinical studies of alcoholic women.

Although the primary site of action in chronic exposure studies may not be clear, acute effects of ethanol appear to be due to hypothalamic effects. In rats, infusion of alcohol (95% ethanol representing 36% of the total calories provided in the liquid diet) inhibited spontaneous LH release, but did not affect LH release in response to gonadotropin-releasing hormone (GnRH) (Van Thiel et al., 1978; Kieffer and Ketchel, 1970). This suggests that ethanol acts at the level of the CNS to inhibit LH secretion by the pituitary and thus ovulation. Rettori et al. (1987) fed female rats either an alcohol or an isocaloric control liquid diet regimen beginning on the first day of diestrus. Animals receiving the control diets showed uninterrupted estrous patterns, whereas those animals receiving the alcohol diet remained in diestrus. Additionally, the alcohol-treated animals showed an increase in hypothalamic luteinizing hormone–releasing hormone (LHRH) content, with a concomitant decrease in serum LH. No significant differences were detected in serum FSH levels or pituitary LHRH receptor content. We conclude that there is clear evidence of the reproductive and developmental toxicity of chronic alcohol exposure and although less compelling, the data suggest that even acute low-dose exposure should be avoided in women attempting to conceive.

2.3. Cannabis
The pathophysiological effects of marijuana smoke and its bioactive cannabinoids were first reported from in vitro and in vivo experimental studies (Nahas and Latour, 1992). In animals, marijuana or its major psychoactive constituent, Δ⁹-tetrahydrocannabinol (THC), produces symptoms of neurobehavioural toxicity, disrupts all phases of gonadal or reproductive function and is fetotoxic.

In an early descriptive report, Kolodny et al. (1974) observed that 6 out of 17 marijuana smokers had sperm counts below 30 million/ml. Chronic marijuana users have been reported to show abnormal sperm morphology, including reduced nuclear size, increased condensation of chromatin, disorganization of acrosomal structure, and absence of acrosomes (Issidoriedes, 1980). In two small trials based on men who were regular marijuana users (Hembree et al., 1976, 1980), a period of 3 to 4 weeks of marijuana abstinence followed by a period of 4 weeks of use produced a subsequent transient decrease in sperm numbers without obvious effects on motility or morphology.

Kolodny et al. (1974) reported decreased plasma testosterone levels in men who had been smoking marijuana for at least 6 months prior to testing. When marijuana users were given human chorionic gonadotropin (hCG), testosterone levels increased by more than 120%, indicating functional Leydig cell capacity. Following a 2-week period of marijuana abstinence, testosterone levels also increased. Other
contemporary reports (Hembree et al., 1976; Mendelson et al., 1978, 1974; Schaefer et al., 1975; Cushman, 1975) found no significant alterations in plasma testosterone levels in marijuana users, suggesting no impairment of Leydig cell function. In terms of the effects of THC on male reproductive hormone levels, studies in rats and rhesus monkeys have reported consistent cannabinoid-induced decreases in plasma testosterone levels (Collu et al., 1975; Thompson et al., 1973, 1974; Mazarinei et al., 1978; Symons et al., 1976) and LH (Collu et al., 1975; Symons et al., 1976; Marks, 1973). Demonstration of effects of cannabinoids on male reproductive tract tissues has been less consistent. Cannabinoid effects on testis weight are inconsistent, with effects ranging from increases (Rosenkrantz et al., 1974, 1975) to no changes, or decreases in weight (Thompson et al., 1973; Dixit and Lohiya, 1975; Cushman, 1975; Vyas and Singh, 1976). Changes in testis function had been observed following treatment with cannabinoids, even in the absence of weight changes. In rats, seminiferous tubule degeneration and degenerative changes in spermatocytes and spermatids were produced by exposure to marijuana smoke (Thompson et al., 1973; Rosenkrantz et al., 1975; Rosendrantz and Hayden, 1979). Mice treated with THC for 5 days had dose-related increases in abnormal sperm, including heads lacking hooks, banana-shaped heads, amorphous heads, and folded heads (Zimmerman et al., 1979). Dixit et al., (1974) observed Leydig cell regression in mice following chronic exposure to cannabis extract. Several cannabinoids did not affect rat or mouse Leydig cell basal testosterone production or hCG binding in vitro (Jakubovic et al., 1979; Kolodny et al., 1974).

In summary, studies on animals treated with cannabinoids are consistent with studies in humans, which have reported testicular toxicity following use of marijuana. The significance of these observations for human fertility is uncertain, since treatment of male mice with THC for 4 weeks prior to and during mating did not significantly affect fertility, number of implantations per female, number of corpora lutea, or pre-implantation losses or resorption rates (Legato et al., 1976). Absence of adverse effects in the animal studies must be viewed cautiously, since the high fecundity of mice compared to humans may underestimate potential effects in humans.

One early report (Kolodny et al., 1979) indicated that women who were marijuana users had shorter menstrual cycles (26.8 days) than those who were not (28.8 days). Marijuana users had more menstrual cycles that were either anovulatory or characterized by a shorter luteal phase (38.3% versus 12.5%, respectively). Hormone levels did not reveal any statistically significant differences in serum LH, follicle-stimulating hormone (FSH), estrogen, or progesterone levels between marijuana users and non-marijuana users despite decreased luteal phase progesterone levels. However, serum prolactin levels were significantly reduced and serum testosterone levels were significantly increased in the marijuana users. One identified confounding factor was that the consumption of ethanol was more than 2-fold greater in the marijuana users. Subsequently, short-term exposure trials showed that manifestation of acute hormonal effects of THC may depend upon the endocrinological status of the exposed woman. On one hand, LH levels determined before and after administration of marijuana and placebo cigarettes were not significantly different and were within the range of normal values for healthy menopausal women (Mendelson et al., 1985). On the other hand, smoking a single 1-g marijuana cigarette induced a 30% suppression of plasma LH levels in nonmenopausal women during the luteal phase of the menstrual cycle (Mendelson et al., 1986).

Most of the insight concerning the effects of cannabinoids on female reproductive function is derived from studies in animals. Not all the findings have been consistent. In female rats, low-dose chronic administration of THC was shown to cause a delay of the onset of puberty and to reduce the number of ova on the day of first estrus (Wenger et al., 1992). It has been shown that cannabinoid agonists interfere with preimplantation development of the mouse embryo in vitro (Paria et al., 1995) and that actions in the mouse embryo are mediated by one of the two known cannabinoid receptors, specifically the CB1-R (Yang et al., 1996; Paria et al., 1998), but not the CB2-R.

In small laboratory animals, cannabinoids have consistently been shown to inhibit ovulation. For example, Asch and coworkers (Asch et al., 1979a) reported a dose-dependent inhibition of ovulation by Δ9-tetrahydrocannabinol (THC) in female rabbits, and the blockade of ovulation could be overcome by administration of hCG. Administration of Δ1-tetrahydrocannabinol (Δ1-THC), the principal psychoactive ingredient of cannabis, to proestrus rats between 12.00 and 16.00 h suppressed the prostrous rise in the plasma levels of LH, FSH, and prolactin (Prl) and caused a 24-h delay in ovulation (Ayalon et al., 1977). Daily administration of cannabis extract for 30 days stopped ovulation in rats and mice (Fujimoto et al., 1979). In both ovariectomized (Marks, 1973; Smith et al., 1979; Besch et al., 1977; Tyrey, 1978) and intact (Chakravarty et al., 1975; Ayalon et al., 1977; Nir et al., 1973) animals, THC suppression of plasma LH levels appears to be the primary mechanism by which THC exposure inhibits ovulation. Because GnRH-induced LH secretion is unaffected by THC, the action of the drug appears to depend upon a hypothalamic rather than a hypophysial site of action. The antiovulatory effect of THC appears to result from an inhibition of LH secretion that does not involve the direct blockade of LHRR release (Murphy and Tyrey, 1986). Unlike women who smoke marijuana, plasma FSH levels are not reduced, suggesting that either dose or pattern of exposure may be important in mediating the adverse effects of THC compared to non-marijuana smokers (Kolodny et al., 1976). In comparison, THC does decrease plasma FSH in rats (Ayalon et al., 1977; Chakravarty et al., 1979) and monkeys (Smith et al., 1979).

There is evidence to indicate that THC can act directly on the ovary. THC treatment reduces ovarian responsivity to LH in experimental animals (Nir et al., 1973). THC-inhibited progesterone synthesis was demonstrated in rat luteal cell culture in vitro (Burstein et al., 1979). However, in vivo daily administration of THC had no effect on plasma progesterone
levels or luteal phase length in nonhuman primates (Asch et al., 1979b).

In a series of studies in nonhuman primates with doses of THC that plausibly relate to human levels of use, a number of key insights were revealed regarding the actions of THC in female reproductive physiology. In a first study, THC was administered to five regularly cycling rhesus monkeys from days 1 to 18 of the cycle (Asch et al., 1981). Animals treated with vehicle had normal cycle lengths (26, 26, 29, 30, and 34 days), but those treated with THC had abnormal lengths (145, 76, 22, 94, and 59 days). All vehicle-treated cycles were ovulatory, whereas four out of five THC cycles were anovulatory, and all five THC-treated animals were anovulatory in the posttreatment cycle. In a second study, administration of THC on day 20, 21, or 22 of the menstrual cycle caused a significant decrease in progesterone levels during the next 24-h period (Almirez et al., 1983). This decrease was reversed by the administration of human chorionic gonadotropin (hCG) at 6 h after THC administration. Additionally, neither marijuana extract nor THC had any effect on basal progesterone production by monkey luteal cells in vitro. These data indicated that the inhibitory effects of THC on luteal-phase progesterone levels were not mediated by a direct effect of the drug on ovarian steroid production. In a third and extremely important study, Smith et al. (1983) showed that despite chronic use of THC, reversible suppressive effects of the drug on the menstrual cycle can occur. Long-term exposure of sexually mature female rhesus monkeys (Macaca mulata) to thrice weekly injections of THC resulted in a disruption of menstrual cycles that lasted for several months. This period was marked by an absence of ovulation and decreased basal concentrations of gonadotropin and sex steroids in the plasma. After this period, and despite continued twice weekly dosing with THC, normal cycles and hormone concentrations were reestablished. These results help to explain the lack of cycle disruption in many women who are chronic users of cannabis.

3. Caffeine

Caffeine (1,3,7-trimethylxanthine) is naturally occurring in the leaves, seeds, or fruits of more than 63 plant species worldwide. The most commonly known sources of caffeine are coffee, tea leaves, soft drinks, and chocolate. Following consumption, it is readily absorbed and distributed throughout the body and has been detected in saliva, breast milk, and embryos, and in the blood of neonates (Sieber and Fabro, 1971; Yesair et al., 1984). Caffeine produces a number of biologic effects in the human, including CNS stimulation, increase in heart rate, relaxation of smooth muscles, and increased secretion of catecholamines. Based on data from both animal and human studies associating caffeine intake with spontaneous abortion, intrauterine growth retardation, birth defects, and possibly other fetotoxic effects, the impact of caffeine on other reproductive processes, including fertility itself, has been a focus of concern. Biologic plausibility for this is provided by information suggesting that through alterations in the reproductive hormone profile, caffeine may hinder ovulation and that caffeine intake is positively correlated with sex hormone–binding globulin concentrations (Petridou et al., 1992; London et al., 1991; Kotsopoulos et al., 2009). Some of these concerns have recently been revisited in a study using telephone interviews (Fenster et al., 1999) and have not been corroborated. To date, there have been a number of prospective studies and retrospective analyses addressing the issue of caffeine consumption on fertility rates. However, randomized clinical studies evaluating this question have not yet been carried out.

The influence of caffeine on human reproduction was initially reported in a prospective investigation (Wilcox et al., 1988) that revealed that daily consumption of the typical amount of caffeine found in a cup of coffee was associated with a 50% decrease in per cycle conception rates. Women consuming greater quantities of caffeine had consistently lower pregnancy rates, thus demonstrating a dose-related effect. Caffeine consumption was calculated based on the assumption that 1 cup of coffee, 2 cups of tea, and 2.5 glasses of soda each contain 100 mg caffeine. This small study evaluated 104 women who had not conceived during the first 3 months of trying to conceive. The major limitation of this study is that it was not primarily designed to evaluate the effect of caffeine on fertility. As a result, the measures for caffeine intake were relatively crude and attention to potential confounding variables was cursory. Another problem with the study is that the authors only evaluated individuals who had failed to conceive for 3 months. Enrollment of couples who have stopped contraception, together with assessment of their caffeine intake from this time on, would have allowed for determination of any caffeine effect during the first 3 months of trying to conceive. This would be a period when pregnancy rates are highest and perhaps when any impact of caffeine may be the most significant.

A second prospective study evaluating 259 women not working in medical hospitals was published in 1994 (Florack et al., 1994). Comprehensive data in this Dutch study was collected from the study subjects and their spouses from the onset of trying to conceive. As with the previous study, the primary objective was not to study the impact of caffeine consumption on fertility. The primary aim was to evaluate the relationship between occupational factors and early pregnancy failure. The same assumptions regarding caffeine content in drinks was used as in a previous study (Wilcox et al., 1988). Fecundity was not found to be affected by moderate caffeine intake in either partner or even by heavy intake by the female. Only when the male partner consumed more than 7 cups of coffee per day was an adverse influence on fertility detected. The significance of this observation is questionable, since it is likely that in most populations few individuals routinely drink more than 7 cups of coffee per day. The finding that caffeine could influence male fertility corroborated a prior study that suggested the negative impact of caffeine, combined with smoking, on sperm mobility and viability (Marshburn et al., 1989).
The other two prospective studies, which were designed to specifically evaluate the relationship between caffeine intake and fertility, were published in 1998. The first evaluated 187 women who had been trying to conceive for 3 months or less (Caan et al., 1998) and apart from an increase in fertility among tea drinkers with the consumption of more than one half of a cup per day, the result did not detect any significant effect of caffeine on fertility. This is contrary to the majority of other published studies and probably a reflection of other lifestyle characteristics of this cohort, rather than of the tea itself. For example, they were found to smoke less, eat less fat, and exercise more than coffee drinkers. Smoking, alcohol intake, and body weight were controlled for, but other factors such as stress were not well evaluated.

The largest prospective study to evaluate the effect of caffeine on fertility was published in 1998 by a Danish group of investigators (Jensen et al., 1998a). The detailed assessment of caffeine intake was notable in that it included an assessment of not only beverage, but also chocolate bar intake. The impact of smoking on fertility was also addressed. A total of 1596 cycles from 423 couples trying to achieve their first pregnancy was evaluated. The study population included metalworkers, office workers, nurses, and daycare workers aged 20 to 35 years. Forty-seven cycles in which couples reported no intercourse from days 11 to 20 were excluded. It is interesting that the authors chose this period to represent when conception is likely. Exclusion of couples without intercourse on days 9 to 16 may have been more appropriate for 28-day cycles (Barrett and Marshall, 1969; Royston, 1982; Wilcox et al., 1995). No adverse effect of caffeine on fecundability among female or male smokers was found. In nonsmoker males and females, a statistically significant progressive decrease in fecundability was detected with increasing caffeine consumption. The fecundability was also significantly decreased (by 44%) in men consuming more than 699 mg/day caffeine. The authors postulate that the lack of effect of caffeine on fertility in smokers is due to more rapid metabolism of caffeine in these individuals by the hepatic enzyme induction, which is absent in nonsmokers, suggesting some plausible biological interactions between caffeine and smoking (Yesair et al., 1984; Brown et al., 1988; Dlugosz and Bracken, 1992). A number of retrospective studies have been published on this topic. In general, they are prone to recall biases and other methodological problems. For example, some studies are based on caffeine intake during early pregnancy as a model for measuring caffeine intake while trying to conceive. Because many women reduce consumption of caffeine during pregnancy, this may be an inaccurate assessment of pre-pregnancy consumption (Hatch and Bracken, 1993; Christianson et al., 1989; Williams et al., 1990). In addition, the reduction in caffeine consumption with pregnancy has been shown to be greater in women who conceive within the first 2 months of trying compared to those who took longer (Caan and Coates, 1994). Other studies failed to account for caffeine intake from soft drinks (Olsen, 1991) or from any source other than coffee (Christianson et al., 1989).

More recently the association between ovulatory disorder infertility and consumption of caffeinated beverages was examined (Chavarro et al., 2009). The study involved 18,555 married women without a history of infertility who were followed for 8 years as they attempted to become (or became) pregnant. Diet was assessed twice during this period and prospectively related to the incidence of ovulatory disorder infertility. The results failed to demonstrate any relationship between caffeine consumption, impaired ovulation, and decreased fertility.

Studies on the association between caffeine and miscarriages showed that an increase in daily caffeine intake may be associated with an increased risk of recurrent pregnancy loss. Sata et al. (2005) showed that increase in daily caffeine intake of 100 to 300 mg or more (which is equal to around 1 to 3 cups, respectively), may be associated with a significantly increased risk of recurrent pregnancy loss among women who have homozygous CYP1A21F alleles. One study suggests that an increased dose of daily caffeine intake of less than 200 mg or more during pregnancy increased the risk of miscarriage in the general population independent of pregnancy-related symptoms (Weng et al., 2008). However, there are conflicting findings. In an epidemiological study, women were recruited before or early in pregnancy and interviewed regarding sources of caffeine, including assessment of changes over the perinatal period. The authors analyzed 2407 clinically recognized pregnancies resulting in 258 pregnancy losses and found that there is little indication of possible harmful effects of caffeine on miscarriage risk (Savitz et al., 2008).

A review of more than 20 human epidemiological studies by Leviton (1988) on the relationship between caffeine consumption by pregnant women and risk of miscarriage, low birth weight, preterm delivery, and congenital malformations found no evidence that caffeine consumption at moderate levels has any discernible adverse effect on pregnancy outcome. It is considered that the previous warning on caffeine consumption and the risk of reproductive hazards was based on findings that gavage feeding of large doses of caffeine to rats resulted in a particularly high incidence of facial cleft palate. However, the latest review on the effects of restricted caffeine intake by mother on fetal, neonatal, and pregnancy outcome in Cochrane Database System Review found insufficient evidence to confirm or refute the effectiveness of caffeine avoidance on birth weight or other pregnancy outcomes (Jahanfar and Sharifah, 2009). Lack of scientific evidence showing any association between caffeine consumption and adverse effect on pregnancy outcome led the US Food and Drug Administration (FDA) agency to conclude that caffeine, as currently used in foods, does not carry a health risk. However, the agency continues to recommend that pregnant women consume caffeine in moderation.

In summary, the data regarding the effect of caffeine on reproduction in humans are conflicting. Although it is not possible to give a clear recommendation for couples trying to conceive, it would be prudent to suggest those consuming the equivalent of more than 3 cups of coffee per day to reduce their consumption. The American College of Obstetricians
and Gynecologists recommends that pregnant women limit consumption to the caffeine equivalent of 1 to 2 cups of coffee. Given the high prevalence of caffeine intake by women of childbearing age, it is clear that further research is required.

4. Tobacco

By smoking tobacco, individuals expose themselves (primary smokers) and those around them (passive smokers, second hand smoke exposure) to a large number of toxicants and carcinogens. Cigarette smoke contains over 4000 compounds, including dozens of carcinogens or poisons and over 300 polycyclic aromatic hydrocarbons. Among other chemicals, smoke contains cadmium, arsenic, butane, ammonia, lead, acetone, carbon monoxide, pesticide residues such as DDT, polycyclic aromatic hydrocarbons (PAHs), and formaldehyde.

The addictive chemical nicotine and its metabolites can cause vasoconstriction, reduce tissue oxygenation, and collect in blood, urine, saliva, follicular fluid, and other reproductive tissues (Jensen et al., 1991; Wall et al., 1988). Measurements in the serum and follicular fluid of cigarette smoke constituents and their metabolites such as benzo[a]pyrene (B[a]P), but not other polycyclic aromatic hydrocarbons, correlate with subjective measures of pregnancy rates in women exposed to mainstream and second hand smoke compared to nonsmokers (Neal et al., 2008). These chemicals have been detected in both primary and passive smokers. Cadmium has also been detected in human ovaries and follicular fluid (Zenzes et al., 1995; Younglai et al., 2002). These studies demonstrate that a number of organs intimately involved in reproduction are exposed to the products of tobacco smoke in smokers and those around them.

Biologic plausibility for cigarette smoke to interfere with reproduction comes from a number of in vivo and in vitro animal and human studies evaluating the impact of cigarette smoke exposure and its constituents or metabolites on functions known to be important in reproduction. Animal studies have shown cadmium to interfere with a number of cellular processes, including chromosomal anomalies in gametes and embryos and a decrease in the number of oocytes reaching metaphase II that have important implications for oocyte quality, embryo development, and fertility (Watanabe et al., 1979; Zenzes, 2000; Thompson and Bannigan, 2008). Additional animal studies have shown cigarette smoke to increase the rate of follicular destruction and therefore, not surprisingly, accelerate the cessation of reproductive functioning (Mattison et al., 1989; Jurisicova et al., 2007; Tuttle et al., 2009). Human studies have confirmed that women who smoke have an accelerated loss of ovarian follicles (Sharara et al., 1994; Cooper et al., 1995) and decreased ovarian reserve with an earlier menopause (Sharara et al., 1994; Adeno and Gallagher, 1982; Cooper et al., 1999). These studies have relied upon the demonstration of either higher basal or stimulated FSH levels in primary smokers compared to passive smokers who, in turn, have higher levels than age-matched controls. Because cigarette smoke has also been shown to decrease human granulosa cell aromatase production (Barbieri et al., 1986), it is possible that the elevated FSH levels in smokers are a direct result of decreased ovarian estradiol production. It is likely that a substance other than nicotine or cotinine is responsible for the effect of cigarette smoke on aromatase activity (Weiss and Eckert, 1989). Exposure of rat preantral follicles to different concentrations of B[a]P in vitro showed an inhibition of follicle growth and a dose-dependent decrease in the level of estradiol production (Neal et al., 2007). Other human studies have demonstrated that smoking may affect the meiotic maturation of oocytes (Gruber et al., 2008), affect fetal health (Leonardi-Bee et al., 2008), and increase zygote triploidy (Zenzes et al., 1995b), and there is a likelihood of spontaneous abortion (Windham et al., 1999; George et al., 2006).

Several epidemiologic studies have evaluated the impact of smoking on fertility in women. Most of these studies have been retrospective surveys or designed case-control studies. Obvious problems with such designs include recall bias and the particularly difficult assessment of confounders. Although some studies have found conflicting results, in general, the majority of studies support the conclusion that there is an increased prevalence of infertility or subfertility in smokers compared to nonsmokers. Specifically, it takes smokers a longer time to conceive than nonsmokers. The first and largest prospective cohort study was conducted by the Oxford Family Planning Association (Howe et al., 1985). A total of 4104 women who stopped contraception for the purpose of achieving pregnancy were studied. The participants were interviewed at enrollment, where the magnitude of their smoking behavior was assessed, and subsequently the time taken from stopping contraception to delivery of a child was recorded. A consistent and highly significant trend of decreasing fertility with increasing numbers of cigarettes smoked per day was detected. The authors estimated that at 5 years after stopping contraception, 10.7% of smokers of more than 20 cigarettes per day, and only 5.4% of nonsmokers, had not delivered a child. Because of the study design, it is not possible to determine whether the lower fertility rate in smokers represents a delay in conception or is due to an increase in spontaneous abortions.

The second prospective study (de Mouzon et al., 1988) of 1887 French couples failed to detect an effect of smoking on fertility (odds ratio [OR] 0.86, 95% confidence interval [CI] 0.63–1.19) when the confounding variables were considered. A total of 4200 couples volunteered for the study, but only 2362 returned the questionnaires and a surprising further 475 couples were excluded because of uninterpretable Basal Body Temperature charts. Loss of such a large number of study participants is unfortunate, since documentation of regular menstrual cycles may have been sufficient evidence of ovulation.

The most recent prospective study (Jensen et al., 1998b) evaluated 430 nulliparous Danish couples including the impact of caffeine on fertility (Jensen et al., 1998a). The couples were followed for a maximum of 6 months. The data collected included information on whether female participants had been exposed to cigarette smoke when in utero, through
their mother’s smoking. The most significant reduction in fecundability was seen in smokers who also had in utero exposure to cigarette smoke (OR 0.53, 95% CI 0.31–0.91) compared to nonsmokers who were not exposed in utero. The results for nonsmoking women exposed in utero (OR 0.70, 95% CI 0.48–1.03) were similar to those for smokers without a history of in utero exposure (OR 0.67, 95% CI 0.42–1.06). Unlike current smoking, in utero exposure to cigarette smoke also resulted in a decreased fecundability in the males (OR 0.68, 95% CI 0.48–0.97). The finding that in utero exposure to cigarette smoke does have an effect on potential fertility corroborates an earlier report that women whose mothers smoked while being pregnant with them may be substantially (50%) less fecund than women whose mothers did not smoke during pregnancy (Weinberg et al., 1989). Specific developmental defects in the offspring resulting in their decreased fertility with in utero exposure to smoking have yet to be determined. Ovaries of offspring born to mice exposed to PAHs were shown to contain only a third of the ovarian follicle pool compared with offspring of unexposed female mice (Jurisicova et al., 2007). Although the use of smoking cessation products during pregnancy is advocated for women unable to stop smoking, emerging evidence in the animal literature suggests that developmental exposure to nicotine at concentrations equivalent to those in women using nicotine containing patches increase the risk of low birth weight, over-weight offspring, insulin resistance, and hypertension (Gao et al., 2005; Bruin et al., 2007; Holloway et al., 2007). Moreover, fertility in female rats exposed to nicotine in utero and during lactation had impaired ovarian function and reduced fertility in later life (Holloway et al., 2006).

Reports for women that smoke while undergoing assisted reproduction indicate that they have decreased gonadotropin-stimulated estradiol production, a 50% decrease in implantation and ongoing pregnancy rate, fewer numbers of retrieved oocytes and numbers of embryos (Van Voorhis et al., 1996; Neal et al., 2005), more canceled cycles, undergo more cycles with failed fertilization than nonsmokers, and lower implantation rates (Cooper and Moley, 2008). Spontaneous abortion rates are also increased (Maximovich and Beyler, 1995). The data suggest that the effects may be transient, since past smokers have pregnancy rates similar to nonsmokers (Van Voorhis et al., 1996). A more recent meta-analysis provides compelling evidence for a significant negative effect of cigarette smoking upon clinical outcomes of assisted reproductive technologies (ARTs). Although a systematic literature review revealed that fertilization rates were not significantly different between smoking and nonsmoking groups in most studies, smoking patients demonstrated significantly lower odds of clinical pregnancy and live birth per cycle, and significantly higher odds of spontaneous miscarriage, and ectopic pregnancy (Waylen et al., 2009).

With regards to the impact of smoking on male fertility, numerous studies have evaluated the effects of cigarette smoking on crude parameters of semen quality. Decreased volume, sperm density, total count, normal forms, and motility have been reported (Vine et al., 1996; Saaranen et al., 1989; Rubes et al., 1998). It is doubtful whether the magnitude of this effect is sufficient to impact male fertility (de Mouzon et al., 1996; Hughes and Brennan, 1996; Bolumar et al., 1996; Jensen et al., 1998b). Regardless of the uncertainty, smoking in men should be considered as an infertility risk factor in the light of the impacts of cigarette smoke on conventional parameters of semen analysis.

Clearly, the potential for cigarette smoking to interfere with human reproductive processes is immense and affects all aspects of reproduction (Soares, 2009). Despite years of study, important clinically relevant questions remain to be addressed. Specifically, it is unclear if there is either a critical window or stage of development, threshold amount or duration of cigarette smoking that is crucial to human reproductive health. Moreover, guidance for clinicians is lacking concerning whether the adverse effects associated with cigarette smoking are reversible and if so, how long following smoking cessation before improvement in reproductive parameters can be expected. Responses to these questions could have important implications for physicians in assisted reproduction clinics and patients seeking fertility care hoping to maximize success of fertility treatments while minimizing costs to patients.

In summary, a number of animal and human studies indicate that cigarette smoking strongly contribute to a decrease in natural fertility, and success rate of clinical outcomes of ART, which is thought to be the result of ovarian toxicity and decreased implantation rates. Because of the impact of cigarette exposure on fertility, the toxic effects of cigarette smoke during pregnancy, and the impact of smoking on health in general, women desiring conception should be advised to avoid exposure to both primary and passive smoking. Furthermore, given that complete cessation of smoking is difficult for most smokers, and that the negative effects of smoking on fertility are also dose related (Howe et al., 1985), any possible reduction in exposure to cigarette smoke should be encouraged. Advice on this topic should be a routine component of preconception counseling. Furthermore, emerging evidence from animal studies suggests that nicotine replacement therapy during pregnancy may pose important adverse health effects in offspring that may include impaired fertility in adulthood.

5. Environmental contaminants

Environmental contaminants are postulated to play a role in adverse reproductive outcomes such as spontaneous abortion, premature ovarian failure, infertility, decreased semen quality, polycystic ovarian syndrome (PCOS), endometriosis, altered birth sex ratio, cancers in steroid sensitive target tissues (breast, ovary, prostate, and testis), and developmental abnormalities of the male reproductive tract (reviewed in Sharara et al., 1994; Foster et al., 2008). Although the evidence of adverse reproductive outcomes resulting from accidental and occupational exposures has clearly been established, the evidence linking exposure to environmental contaminants at the concentrations found in contemporary studies is
equivocal. Although it is acknowledged that few studies have been specifically designed to explore the role, if any, of man-made chemicals on reproduction in the general population, those that have suffer from subject selection bias, failure to control for confounding variables, inadequate sample size, and absence of true control populations. Moreover, unlike studies designed to examine the relationship between adverse reproductive health outcome and exposure to drugs and lifestyle factors such as caffeine and tobacco where exposure timing such as age at exposure, duration, and concentration can be quantified, this is most often not the case with exposure to environmental contaminants where exposures are frequently subtle and most often unknown. Furthermore, exposure to environmental chemicals has not been measured directly in many studies, but estimated from diet histories, occupational questionnaires, self-reports of exposure, or residence in a contaminated region and thus the potential for error and misclassification is high. Indeed, poor or absent exposure data is commonly the weakest link in efforts to accurately assess the role of man-made chemicals in adverse reproductive outcomes. Regardless, results of biomonitoring studies reveal widespread exposure to persistent organic pollutants as well as endocrine active chemicals such as bisphenol A (an estrogenic chemical used as a plasticizer in the manufacture of polycarbonate plastics), phthalates (antiandrogenic chemicals used in plastics to make them pliable), polybrominated diphenyl ethers (chemical flame retardants), and perfluorinated compounds (stain guards for fabrics). However, it must be noted that exposure does not equate evidence of an adverse health effect. Indeed, concentration of contaminants in human tissues and fluids are remarkably low and their potency relative to endogenous hormones such as estradiol is also low. Despite these considerations, concern continues to grow regarding common every day exposure to man-made chemicals and their effects on reduced fecundity and the purported global decline in semen quality as well as their role in the pathophysiology of reproductive disorders such as PCOS and endometriosis.

5.1. Time-to-pregnancy

Few studies have addressed the issue of man-made chemical-induced decreased fecundity, and hence the impact of man-made chemicals on human infertility rates in the general population is essentially unknown. Although compelling evidence of increased infertility rates for the population as a whole is lacking, it is possible that the prevalence of reduced fecundity and infertility may be increasing in subpopulations. Women with a diagnosis of infertility (n = 281) were compared with 216 postpartum women for risk of infertility due to chemical exposures. After controlling for age at first pregnancy, cigarette use, and employment history, women with a history of working in the agricultural industry had an elevated risk of infertility, with an adjusted OR = 11.3, CI 2.6–48.8. The relationship held for women who resided on a farm and who were not employed directly in the agriculture industry (adjusted OR = 1.8, CI 1.2–2.7). We therefore postulated that if man-made chemicals adversely affect fecundity, then the time-to-pregnancy (TTP) should also be prolonged in couples with exposure to chemical contaminants. In the New York Anglers study (Buck et al., 1999), TTP and the impact of paternal sport caught fish consumption was assessed in 2445 female participants. No relationship between paternal fish consumption and longer TTP was found. In contrast, a survey of 4931 licensed anglers from Michigan counties bordering on the Great Lakes yielded a modest association between TTP and paternal fish consumption only, whereas maternal sport caught fish consumption was not associated with delayed conception (Courval et al., 1999). An adjusted odds ratio of 2.8 with a 95% confidence interval of 1.0–8.0 was obtained for men who consumed large numbers of fish meals (271 to 1127 meals). In contrast, Buck and colleagues (2000) suggest that maternal but not paternal consumption of contaminated fish may reduce fecundability among couples attempting pregnancy. Among the 1234 (50%) women who became pregnant, 895 (73%) had a known TTP. They found that maternal consumption of fish for 3 to 6 years was associated with reduced fecundability (fecundability ratio = 0.75; 95% CI = 0.59–0.91), as was more than a monthly fish meal (fecundability ratio = 0.73; 95% CI = 0.54–0.98). However, these studies assume that all participants consumed the same kind of sport-caught fish, with equal contamination, and that frequency of consumption and meal size was equivalent amongst the fish consumers. Method of preparation of the fish meal was also not considered in these studies. Other confounding factors that could contribute to longer TTP such as alcohol consumption, tobacco use, and consumption of caffeine beverages were not adequately controlled for. Thus we conclude that taken together these studies suggest that in anglers TTP may be delayed; however, the causative factors remain to be determined.

Exposure to heat through occupations such as professional drivers and bakers has also been explored with only a modest positive association uncovered (Thonneau et al., 1997). No relationship between TTP or infertility has been found for other occupations such as greenhouse workers, cosmetologists, welders, or employees with exposure to crude oil (Bull et al., 1999; Lauria et al., 2006; Peretz et al., 2009; Hjollund et al., 1998). Although no association was found between paternal welding and TTP, cigarette smoking and a history of welding was associated with decreased fecundity, thus illustrating the potential for interaction between exposures. Taken together we conclude that sport fish consumers and individuals with multiple chemical exposures should be regarded as potentially being at risk for delayed conception.

5.2. Semen quality

Intense interest and significant controversy surrounded the report that there was approximately a 50% reduction in semen quality worldwide over the last 50 years (Carlsen et al., 1992). A critical review of Carlsen’s work (Olsen et al., 1995) cast doubt on this interpretation. The most serious criticism of Carlsen’s data was the low number of studies published prior to 1970 that were included in the analyses and the large number of studies with inadequate sample size to
provide an accurate assessment of the true semen quality in a population. Many reports have subsequently been published (Table 1) with divergent findings, some of which show a small increase in semen quality whereas others report a decrease. These studies are important and informative because they highlight geographic differences in semen quality and suggest regions that may be at increased risk for subfertility.

Several factors should be considered in multicentered analyses of semen quality data. Among these are the extreme difficulty in obtaining samples from normal men, interlaboratory differences, small sample sizes, and regional differences. Interlaboratory variation has been demonstrated very convincingly by Matson (1995), who showed that among 20 laboratories, the variation in sperm densities was as much as 560-fold. Such a difference among reported mean concentrations in earlier studies incorporated in the Carlsen and colleagues (Carlsen et al., 1992) analysis contributed to the overall lack of confidence in the observed trends and conclusions reported in that study. Furthermore, all of these studies are retrospective and involve a select group of men from the population and thus are not reflective of the general population overall. In spite of this, reports frequently appear in the scientific literature as well as in the lay press claiming that such papers lend support to the theory that environmental pollutants are the most likely cause for the reported global decline in semen quality. Prospective studies involving men from the general population, with appropriate controls for potential confounding variables including but not limited to cigarette use, alcohol consumption, and health factors, and attention to interlaboratory variation as well as intrasubject variability, must be undertaken in order to resolve this issue.

There are a few important studies that merit discussion in relation to the potential for man-made chemicals to impact male fertility. Geographic differences have been detected in the USA (Fisch et al., 1996; Paulsen et al., 1996), Canada (YoungLai et al., 1998), the UK (Ginsburg et al., 1994), and France (Auger et al., 1995; Bujan et al., 1996). In the French study there was an urban-rural explanation for the difference. Furthermore, in the UK a difference between men residing within the Thames River Watershed area (TWA) versus those outside the TWA further underscore potential regional influences and point at possible man-made chemical influences (Ginsburg et al., 1994). In the Canadian study (YoungLai et al., 1998), a secular increase in semen quality was found in some centers and a decrease was found in others. It is also important to note that an increase in the semen quality was reported for Oslo (Bendvold et al., 1991) and Turku (Vierula et al., 1996), whereas a decrease was found for Stockholm (Bendvold et al., 1989) and Copenhagen (Bostofte et al., 1983), areas that one would not expect to differ markedly in environmental contaminant exposure. There is clearly a need to investigate the underlying cause for these divergent findings in different geographic settings. Despite these limitations, there are reports clearly demonstrating a positive association between exposure and decreased semen quality. A small American case-control study conducted among different study centers, using standardized methods and strict quality control, compared semen quality in fertile men in relation to geographical area and pesticide exposure. Despite the small sample size, the results suggested an association between current-use pesticides and reduced semen quality. Twenty-five fertile men (cases) from Columbia, Missouri, all showing low semen parameters (concentration, % normal morphology and % motile), were compared (controls) with an equal number of fertile men (from Columbia, Missouri), all showing semen parameters within normal limits. The urine samples provided at the time of semen collection were measured for metabolites of eight nonpersistent, current-use pesticides. Pesticide metabolite levels were found to be elevated in cases compared with controls for the herbicides alachlor and atrazine, and for the insecticide diazinon (2-isopropoxy-4-methyl-pyrimidinol) (p = .0007, .012, and .0004 for alachlor, atrazine, and diazinon, respectively). Considering that pesticides are commonly used in the Midwest, the study concluded that they might be the most important contributing factors to the reduced semen quality seen in those fertile men from mid-Missouri (Swan, 2006). However, the small sample size used in this study makes generalization of results to the population at large difficult.

In contrast to the preceding studies, which were either retrospective or suffered from poor exposure assessments, an effect of man-made chemicals was demonstrated in a study of infertile men in the general Israeli population of the mid-1980s (Pines et al., 1987). In this study a positive correlation was found for higher blood pesticide (p,p'-DDT
levels and lower sperm counts. In another study (Larsen et al., 1998), no difference in semen quality could be found in a comparison of Danish farmers who used pesticides versus organic farmers. Interestingly, an effect of season was the only positive finding in this study. Similarly, a seasonal variation in sperm concentration and total sperm count was found in a European study that looked at the regional differences in sperm quality of 1082 fertile men of four European cities, Copenhagen, Paris, Edinburgh, and Turku. Semen quality of a “standardized” man (30 years old, fertile, 96-h ejaculation abstinence) was estimated, and sperm concentrations \((\times 10^6/ml)\) for winter/summer were: Turku: 132/93; Edinburgh: 119/84; Paris: 103/73; and Copenhagen: 98/69. Although this finding might suggest that different environmental exposures or lifestyle in the four populations could result in the observed differences in semen quality, it remains to be investigated (Jorgensen et al., 2004). In a case-control study (Tielmans et al., 1999), the association between abnormal semen characteristics and occupational exposure to organic solvents, pesticides, and metals was evaluated in 899 male partners of couples consulting for infertility at two hospital clinics in The Netherlands. Occupational exposure was estimated by means of a job-specific questionnaire, job exposure matrix, and measurements of metals and solvent metabolites in the urine. An association between organic solvent exposure and impaired semen quality was found, with an odds ratio of 4.81 (1.32–17.54) for men in whom sperm concentration was \(<5 \times 10^6/ml\), <10% motile sperm, or <5% normal forms, and for azoospermic men 7.26 (1.41–37.54). The semen profile of the reference group was sperm concentration: 20 \(\times 10^6\) sperm/ml, motility: 50%, and normal morphology: 14%.

An important factor that has emerged recently and that requires consideration is age at the time of exposure. An inverse relationship between sperm concentration and year of birth has been found in a Danish study (Bonde et al., 1998), wherein the sperm count of 1196 men participating in 10 cross-sectional occupational studies in three regions of Denmark from 1986 through 1995 were studied. The median sperm count of the birth cohort 1937–1949 was 63 \(\times 10^6/ml\) compared to 52 \(\times 10^6/ml\) for the 1970–1995 birth cohort. These data are consistent with the hypothesis of a constant risk factor (such as exposure to chemical contaminant throughout pregnancy and covering relevant critical windows of development) acting in prenatal life. The data are also compatible with an age-related selection bias. Furthermore, in a recent study Mocarelli and colleagues (2008) demonstrated that the age at the time of exposure to dioxin contamination in the Seveso Italy chemical plant explosion have a negative impact on semen quality. Specifically, there was no effect of dioxin exposure in men who were adults at the time of exposure, whereas sperm counts were increased in men who were exposed in the peripubertal period. However, decreased sperm concentrations were observed in men who were between 1 and 9 years of age at the time of their exposure (mean ages at time of exposure = 6.2 years). Also noted in this study was a decrease in the number of motile sperm and the percentage of progressively motile sperm. These data suggest that there may be a critical window for contaminant exposure during which the developing reproductive tract is more sensitive. Finally, we must also appreciate that it is feasible for exposure to environmental toxicants to adversely affect semen quality in ways separate from simple sperm count. Of note, decreased semen quality has also been documented in young men from Teplice, a Czech Republic city where air quality is negatively impacted by the burning of coal for electricity and home heating, compared to Prachtice, a rural community with comparatively clean air (Selevan et al., 2000; Rubes et al., 2005). Although there was no evidence of any effect of exposure on sperm concentration, the percentage of motile sperm was decreased, the proportion of sperm with abnormal forms was increased, and sperm with abnormal chromatin structure was also increased in men from Teplice. Moreover, in another study conducted in Taiwan, sperm DNA damage was greater in coke oven workers compared to controls and correlated with PAH exposure (Hsu et al., 2006). In this study, ambient PAH exposure and urinary 1-hydroxypyrene, a marker of PAH exposure, were significantly higher in top-side coke oven workers compared to side oven workers. Moreover, the frequency of oligospermia (low sperm count; 18.8% versus 0%; \(p < .05\)) and morphologically abnormal sperm (32.3% versus 14.6%; \(p < .01\)) were greater in top-side coke oven workers compared to side oven workers. Although a relatively small study involving only 48 men (16 top-side oven and 32 side oven workers), the data suggest that occupational exposure to PAHs is a risk factor for sperm dysfunction. Occupational groups that are potentially at risk are men working as roofers, in the coking of coal, and paving crews.

In summary, the balance of the evidence suggests an absence of a decline in semen quality worldwide. The data do suggest, however, that there are regional influences that require further investigation in order to identify the causative factors. Genetic differences and lifestyle may play a significant role in the regional deviation in semen quality in addition to exposure to environmental contaminants. The available evidence also indicates that there are occupations such as farming and jobs involving solvent or PAH exposure that may place men at greater risk for lower sperm counts and sperm dysfunction. It will be important for future studies to include age at time of exposure as a potentially important variable. Finally, direct measures of exposure is of critical importance and should be integral to future studies.

5.3. Polycystic ovarian syndrome (PCOS)

Women with PCOS are characterized by the clinical features of hyperandrogenism, obesity, aganthosis nigrans, insulin resistance, and polycystic ovaries, some or all of which may be present in a single individual. Dysregulation of follicle growth and absence of a clear etiology has led to speculation that environmental contaminants may play a role in the pathogenesis of PCOS. Environmental contaminants have been quantified in the follicular fluid (Jarrell et al., 1993; YoungLai et al., 2002; Weiss et al., 2006) and bisphenol A
Endometriosis is an estrogen-dependent disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. It is a common gynecologic disorder and a major cause of infertility (Chedid et al., 1995) affecting approximately 14% of women of all reproductive ages (Vercellini et al., 1995). Coelomc metaplasia, proiferation of a progenitor stem cell, or retrograde menstruation of endometrial cells is thought to lead to the implantation and proliferation of ectopic endometrial cells. Although retrograde menstruation or bleeding into the peritoneal cavity during menstruation is widely accepted as a major contributing factor in the pathogenesis of this disease, it is a common phenomenon even in women without endometriosis (Halme et al., 1984). Hence, factors other than retrograde menstruation such as immune and endocrine (Sinaii et al., 2002) as well as genetic factors (human leukocyte antigen [HLA]-B7) are thought to contribute to the development and progression of endometriosis (Semino et al., 1995; Lee et al., 2005). Owing in part to the estrogenic dependence of endometriosis, the role of environmental contaminants in the pathobiology of this disease has received substantial attention from toxicologists.

Environmental contaminants have been implicated in the pathobiology of endometriosis through the results of several observational studies in women and animal studies (reviewed in Foster and Agarwal, 2002; Rier and Foster, 2002; Anger and Foster, 2008; Heilier et al., 2008). Over the last two decades numerous studies have sought to identify a relation between exposure to environmental toxicants and endometriosis. The resulting literature, however, is highly conflicted, with some case-control studies finding a positive association between exposure to polychlorinated biphenyls (Gerhard and Runnebaum, 1992; Louis et al., 2005; Propora et al., 2006; 2009), dioxins (Koninckx et al., 1994; Mayani et al., 1997; Heilier et al., 2004; 2005), and phthalates (Reddy et al., 2006), whereas others have been unable to demonstrate a significant relationship of increased risk (Boyd et al., 1995; Lebel et al., 1998; Pauwels et al., 2001; Eskenazi et al., 2002; Fierens et al., 2003; De Felip et al., 2004; Tsukino et al., 2005; Heilier et al., 2007; Hoffman et al., 2007; Niskar et al., 2009). Of note, although serum dioxin, antiestrogenic polychlorinated biphenyl (PCB), and 1,1 dichloro-2,2-bis(4-chlorophenyl)-ethene (p,p'-DDE) concentrations were greater in women with endometriosis compared to healthy controls, no increased risk for endometriosis could be demonstrated in the study population (Mayani et al., 1997; Louis et al., 2005; Propora et al., 2009, respectively). Indeed correction for confounding variables reduced 95% confidence intervals and bracketed 1.0 (Louis et al., 2005; Propora et al., 2009). Hence the role of environmental toxicants in the pathogenesis of endometriosis is unclear and comparing results across studies is complicated by differences in study design, methods of exposure assessment, analytical methods employed, and differences in definitions of controls (reviewed in Anger and Foster, 2008; Heilier et al., 2008). In addition, these studies are all relatively small, and thus may not have had the statistical power to detect differences if they were indeed present. It has been determined that a sample size of 286 subjects with endometriosis and 286 control subjects would be required to detect a 2-fold increase in the incidence of endometriosis assuming a 10% prevalence rate, significance level of .05, and power level of 90% (Mayani et al., 1997). Consequently, we suggest that the studies conducted to date are all under-powered and thus the association between exposure to environmental toxicants and endometriosis remains unsettled.

Apart from the above-mentioned limitations in study design, studies of endometriosis are complicated further by the delay between time of appearance of symptoms and diagnosis. There is often a substantial lag of approximately 10 years between the appearance of symptoms and diagnosis of endometriosis. This temporal disconnection between initiation of disease and diagnosis makes it difficult to identify factors important in the development of this enigmatic disease.
Moreover, although well recognized as an estrogen-dependent disease, endometriosis is far from a homogenous disorder but has many presentations, including white, red, gun powder burn implants, chocolate cysts, peritoneal endometriosis, deep infiltrating nodules, and ovarian endometriosis. Indeed, peritoneal endometriosis and deep infiltrating nodules are considered by some (Donnez et al., 1996) to be different diseases and thus may have different functional characteristics. The American Fertility Society (AFS) further stage the endometriosis on the basis of its severity using a 4-point scale. Rarely are the appearance and severity of disease considered in studies designed to test the relationship between exposure to environmental toxicants and endometriosis. Risk for endometriosis was increased in women with deep infiltrating nodules and peritoneal endometriosis with exposure to non-dioxin-like PCBs and dioxin-like compounds in serum (Heilier et al., 2004; 2005). However, Propora and colleagues (2009) found a positive relationship between serum concentration of \( p,p' \)-DDE and PCBs and all types of endometriosis, suggesting that these chemicals are potential risk factors for all types of endometriosis. It is troubling that there was no relationship between the concentrations of any chemical measured and severity of endometriosis (Propora et al., 2009). Similarly, in another study (Louis et al., 2005), AFS staging of endometriosis was recorded and although increased risk was detected for antiestrogenic PCB exposure in the highest tertile versus the lowest tertile (OR 3.77, 95% CI 1.12–12.68), there was no relationship with endometriosis severity. One would expect that with greater exposure to environmental toxicants that are thought to contribute to the pathophysiology of the disease, there would also be an increase in disease severity. Although increased risk remained elevated after adjusting for serum lipids and cigarette smoking, the 95% CI encompassed 1 (OR 3.0 and 95% CI 0.87–12.46). We therefore conclude that the weight-of-evidence at present suggests a weak association between exposure to environmental toxicants and endometriosis. Hence, the human data at present neither confirm nor refute the hypothesis that environmental contaminants play a role in the pathobiology of endometriosis.

Several animal models involving nonhuman primates, rats, and mice have been used to investigate the role of environmental contaminants in the development and progression of endometriosis and are reviewed in detail elsewhere (Rier and Foster, 2002; Foster and Agarwal, 2002; Anger and Foster, 2008). Although these models permit the prospective study of toxicant exposure on the development or progression of surgically induced endometriosis, which is impractical to do in epidemiological studies, the animal models have a number of important limitations. Specifically, the rodent models of endometriosis are of limited usefulness because these studies almost always involve surgical induction of endometriosis in either immune compromised mice or autotransplantation of uterine strips in rats in either intact or ovariecctomized. Translation of results from these studies to humans is complicated, since endometriosis is not established through this mechanism and the important contribution of the immune system is obviated in these models. Spontaneous development of endometriosis in rhesus monkeys (Rier et al., 1993) established a potential association between exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). The study has a number of strengths, including laparoscopic investigation of the pelvis by a gynecologist blinded to treatment groups, together with staging of the disease and subsequent residue analysis (Rier et al., 2001). There are, however, a number of important limitations, which include the small number of animals included in the study and the use of feral monkeys in this study whose exposure prior to inclusion in the study is unknown. It is also important to note that no relationship between TCDD exposure and endometriosis could be demonstrated in the follow-up study (Rier et al., 2001). However, 3,3’,4,4’ tetrachlorodibiphenyl (TCB), 3,3’,4,4’,5-pentachlorobiphenyl (PCB), and an increased total serum toxic equivalence quotient were associated with a higher prevalence of endometriosis. Moreover, the severity of endometriosis was associated with serum concentration of 3,3’,4,4’-TCB. We take these data to suggest that dioxin and dioxin-like PCBs may be important in the pathophysiology of endometriosis. In summary, the link between these exposures and endometriosis are best characterized as weak at this stage and additional studies are necessary to better define the role of dioxins in dysregulation of mechanisms thought to be central to the development and progression of endometriosis, such as increased aromatase expression and activity, dysregulation of apoptosis in the endometrium, angiogenesis, and immune dysfunction.

Finally, the evidence for a role of man-made chemicals in the pathophysiology of reproductive and developmental effects in the human population, at the residue levels commonly reported in contemporary studies, is conflicting and thus neither supports nor excludes this possibility. However, from the preceding sections, it is clear that there is sufficient evidence, both anecdotal and empirical, to advance testable hypotheses regarding the impact of man-made chemicals on human reproduction. The data are sufficient to suggest that certain occupational groups such as those involved in the agriculture and chemical industries are at greater risk. Patients are undoubtedly concerned about the potential impact of man-made chemicals on their reproductive health. In the absence of concrete evidence of an association between man-made chemicals and adverse effects in the general population, it may be wise to advise patients to avoid or reduce their potential exposure to man-made chemicals wherever possible through the use of protective equipment and attention to directions for product use. Replacement with substitute practices or less toxic chemicals is also recommended.

6. Summary and conclusions

Human reproduction depends upon a complex series of time-dependent endogenous events. These events must occur within specific compartments that are anatomically and physiologically competent to yield viable progeny that
are themselves reproductively competent. In spite of the fact that endogenous integration of these events is accomplished by precise signals such as reproductive hormones, exogenous physical and chemical agents do intersect these internal events. Because exposure of internal reproductive processes to some exogenous chemical agents has to some extent always occurred, the key considerations for assessing the impact of contemporary self-inflicted or environmental exposures lies in the dose and timing of exposures and the properties (modes of action) of the agent. On these bases, many reproductive aged humans are episodically exposed on extended intervals to relatively high doses of addicting or habituating drugs such as ethanol, opiates, cannabinoids, nicotine, and caffeine, whereas exposures to environmental chemicals are typically at low levels, but due to the biopersistence of many of the compounds concerned, the exposures are essentially continuous over prolonged periods. Although each of these groups of compounds have modes of action that imply that human reproduction could be affected, there is sufficient evidence to conclude that ethanol, opiates cannabinoids, or cigarette smoke can disrupt human reproduction. There is sufficient evidence to indicate that at the upper extreme of intake, caffeine may also have modest disruptive effects, whereas there is insufficient evidence to reach any conclusion about disruption of human reproduction by environmental chemicals at the levels to which the general population are exposed.

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