ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

REVIEW ARTICLE

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Keywords:
chronic exposures, diet, drug use, male infertility, pollution, spermatogenesis

Received: 6-May-2015
Revised: 7-Mar-2016
Accepted: 10-Mar-2016

doi: 10.1111/andr.12198

Chronic exposures and male fertility: the impacts of environment, diet, and drug use on spermatogenesis

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SUMMARY

Several recent studies have suggested that sperm concentrations and semen quality have been decreasing over the past several decades in many areas of the world. The etiology of these decreases is currently unknown. Acute events can have significant impacts on spermatogenesis and are often readily identified during the male fertility evaluation. The majority of male factor infertility, however, is idiopathic. Chronic, low-dose exposures to chemicals and nutrients are more difficult to identify, but are extremely prevalent. These exposures have been shown to have dramatic effects on both individual and community health and interest in the cumulative and synergistic impacts of such agents on spermatogenesis has been increasing. While our understanding of these potential hazards is evolving, it is clear that they may significantly influence male reproductive potential. This review explores the literature related to effects of chronic exposures from drug use, dietary intake, and the environment on spermatogenesis in humans and animals.

INTRODUCTION

Given the prevalence of male factor infertility, there has been increasing interest in the effects of diet, lifestyle, and environmental exposures on reproductive potential. Many retrospective studies suggest that there has been a decrease in semen quality over the past several decades in different countries throughout the world (Carlsen *et al.*, 1992; Auger *et al.*, 1995; Irvine *et al.*, 1996; Geoffroy-Siraudin *et al.*, 2012; Haimov-Kochman *et al.*, 2012). The etiology of this decrease is unclear. Public health research into the effects of chronic, low-dose exposures from sources such as dietary consumption, drug use, and the environment has surged as the cumulative effect of these exposures are being found to have dramatic impact on individual and community health. These accruing impacts can also impair spermatogenesis and male fertility. Indeed, male reproductive health may be a sensitive marker of pollution (Moline *et al.*, 2000) and environmental exposures (Nordkap *et al.*, 2012).

Spermatogenesis is a complex process, with many genes involved with and necessary for the production of spermatozoa. Additionally, spermatogenesis requires not only proper function of the testes but also intact hormonal stimulation from the hypothalamus and pituitary gland. Given the variables and intricacies of spermatozoa production, there are many opportunities and levels at which these chronic exposures may impact spermatogenesis.

While acute exposures of highly toxic substances can cause dramatic short- and long-term changes in semen parameters, these exposures are relatively rare and usually easily identified during the male fertility evaluation. The majority of male factor infertility is idiopathic, however, with no clear explanation for impaired spermatogenesis. Chronic, low-dose exposures may not have as profound effects as acute exposures. The sustained nature of such persistent exposures, however, could contribute to clinically significant impairments of spermatogenesis, reflected in alternations in semen parameters.

This review aims to provide a broad overview of the most prevalent and commonly studied environmental and lifestyle factors that impact spermatozoa production. Given the breadth of this topic, selected papers have been presented and pertinent reviews will be referenced for deeper discussion into specific factors. Those factors affecting spermatogenesis in humans are summarized in Table 1. The effects of prescription medication use will not be addressed [reviewed in (Samplaski & Nangia, 2015)]. The primary discussion will focus on alterations in sperm counts and changes in the reproductive hormones that drive spermatogenesis [i.e. testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH)].

Table 1 Summary of common chronic exposures associated with changes in spermatogenesis in humans

Alcohol, Tobacco, Diet, and Drugs	Ambient and Occupational Exposures
Alcohol	Water Pollution
Tobacco products	Disinfection byproducts (DBPs)
Methylxanthine derivatives	Persistent organochlorine pollutants (POPs)
Pentoxifylline[a]	Polychlorinated biphenyl (PCB)
Illicit drugs	CB-153[b]
Marijuana	*p,p'*-DDE[b]
Androgenic anabolic steroids	Pesticides
Narcotic opioids	1,2-dibromo-3-chloropropane (DBCP)
Cocaine	Paraquat
Dietary Intake, Micronutrients, and Supplements	Malathion
Obesity/Weight loss[a]	Ethylene dibromide
Micronutrients	Phthalates
Zinc deficiency	Monobutyl phthalate
Selenium deficiency	Dibutyl phthalate
Copper excess	Glycol ethers
Iron excess/deficiency	Heat
Manganese excess/deficiency	Radiation
Lead excess	Gamma radiation
Cadmium excess	Natural background radiation
Folate[a]	Electromagnetic field radiation
Coenzyme Q_{10}[a]	Hypoxia
Antioxidants[a]	Heavy exertion
Fish intake[b]/Omega-3 fatty acids	Psychological stress

While many of the exposures have been observed to have no effect on spermatogenesis in some studies, unmarked exposures have studies suggesting a negative effect, [a]a positive effect, and [b]an inconclusive (both positive and negative) effect on spermatogenesis.

INTERPRET WITH CARE

There are several significant obstacles to elucidating the role of chronic and sub-chronic exposures in human spermatogenesis. First, by nature many of these exposures are low dose and occur in combination with other exposures. In animal studies, the effects of specific exposures can be investigated prospectively and confounders can be well controlled. For ethical reasons, this is generally not possible in humans. As such, most studies are retrospective and attempts to control for confounders are limited. Additionally, in animal studies, the level of exposure is often significantly higher than those normally encountered by humans. Thus, extrapolation of animal data may be difficult and findings may be discordant between animal studies and clinically relevant impacts on human spermatogenesis.

Second, the choice of subjects and controls will have a significant impact on the results. For many reasons, the majority of studies in humans use men who present to infertility clinics for evaluation. Significant differences between this population and the general population have been noted (Lalos *et al.*, 2003; Muller *et al.*, 2004). To further complicate the matter, population-based studies have demonstrated regional and ethnic differences in semen analyses (Jørgensen *et al.*, 2002; Li *et al.*, 2009).

Finally, most studies use ejaculated semen samples to evaluate spermatogenesis in humans. This can be problematic for several reasons. As spermatogenesis takes 72–81 days in humans (Adler, 1996), the ejaculated sample will be more of an average of events that happened approximately 10–12 weeks earlier rather than an accurate reflection of what happened given an isolated exposure. The effects of finite exposures that may affect specific stages in spermatogenesis may become diluted over the course of spermatogenesis and ultimately have minimal impact on ejaculated semen parameters. Additionally, a variety of factors can impair delivery of spermatozoa to the ejaculate independently of spermatogenesis. While some factors are easily identified (e.g. vasectomy), incomplete collection and factors causing emission and/or ejaculatory impairment may not be easily identified, thus, resulting in artificially low semen parameters.

ALCOHOL, TOBACCO, AND DRUGS

Alcohol

Alcohol use is prevalent in society: 56% of adults in the United States reported alcohol use within the prior 30 days, with 25% of the population qualifying as binge drinkers, and 7% qualifying as heavy drinkers (>5 drinks per day during at least 5 days of the previous 30 days) (Substance Abuse and Mental Health Services Administration 2014). Alcohol has been shown to impact spermatogenesis on multiple levels. Alcohol suppresses the hypothalamic–pituitary–testis (HPT) axis in mice as well as humans and has direct toxic effects on Leydig and Sertoli cells (Emanuele & Emanuele, 1998), thus affecting spermatogenesis at both the level of the pituitary and the testes.

In postmortem studies of men 25–54 years of age who died suddenly, 17 of the 19 men who were found to have impaired spermatogenesis on testicular pathology were classified as moderate to heavy drinkers; the majority of those with moderately to severely impaired spermatogenesis consumed more than 32 drinks per week (Kuller *et al.*, 1978). Additionally, one-third of the men who were classified as daily drinkers were found to have at least moderately decreased spermatogenesis (Kuller *et al.*, 1978). In living subjects, chronic alcohol use was found to decrease sperm count with increasing alcohol intake in some (Villalta *et al.*, 1997; Jensen *et al.*, 2014), but not all studies (Li *et al.*, 2009). In mice, abstinence from alcohol restored alcohol-impaired spermatogenesis (Anderson *et al.*, 1985), and case reports in humans suggest alcohol-associated azoospermia may also be reversible (Sermondade *et al.*, 2010).

Tobacco

Tobacco use remains prevalent, particularly outside Western countries (Asma *et al.*, 2015). Approximately, 33% of U.S. adults reported using tobacco products in 2013 (Substance Abuse and Mental Health Services Administration 2014). Most studies have reported a negative association between smoking tobacco and spermatogenesis. Smoking has been associated with significant decreases in sperm counts in fertile men presenting for vasectomy (Pasqualotto *et al.*, 2006) and decreased sperm counts in healthy young men presenting for military physical evaluations (Richthoff *et al.*, 2008). A meta-analysis of more than 2500 men from five separate studies revealed a significant decrease in sperm concentrations of current smokers compared with those who had never smoked (Ramlau-Hansen *et al.*, 2007). Likewise, among 2100 men presenting for fertility evaluation, smoking was associated with significant decreases in sperm concentrations (Künzle *et al.*, 2003). On the other hand, a large, population-based cohort of healthy, Han Chinese men did not find a significant difference in sperm counts among those who smoked more than 10 cigarettes per day compared to those who smoked fewer (Li *et al.*, 2009). Of note, this study did not compare non-smokers vs. smokers, so one may speculate that smoking fewer than 10 cigarettes per day may have the same degree of impairment

as smoking more than 10 per day. Smokeless tobacco has also been associated with decreased sperm counts and concentrations in a dose-dependent fashion (Said *et al.*, 2005; Sunanda *et al.*, 2014).

There are several possible mechanisms by which smoking tobacco may lead to decreased spermatogenesis. More than 4700 different chemicals have been identified in tobacco smoke (Borgerding & Klus, 2005), several of which have known effects on spermatogenesis. Tobacco smoke may alter blood and seminal fluid heavy metal concentrations (see below under the subsection *Micronutrients* for further discussion). Lifetime smoking estimate was significantly and positively associated with seminal plasma lead levels (Benoff *et al.*, 2003), and smoking is currently the most common source of cadmium exposure in the general population (Jurasović *et al.*, 2004). Furthermore, tobacco smoke contains polycyclic aromatic hydrocarbons (PAH) and other chemicals known to cause mutagenesis, apoptosis, and cell death in rapidly dividing cells, leading to decreased rates of cell division and spermatogenesis (reviewed in Dai *et al.*, 2015). Finally, tobacco smoke may increase carbon monoxide levels and induce relative hypoxia within the testes (Koskinen *et al.*, 2000) (the impact of hypoxia on spermatogenesis will be discussed in further detail below under the subsection *Hypoxia*).

Methylxanthine derivatives

Caffeine, theobromine, theophylline, and pentoxifylline are derivatives of methylxanthine. While the majority of caffeine consumption comes from coffees, teas, and colas (Lim *et al.*, 2015), other sources include supplements, energy drinks, and chocolate. Theobromine is found in chocolate, tea leaves, and the cola nut. Theophylline and pentoxifylline are prescription medications used to treat chronic lung diseases and peripheral vascular disease, respectively, although pentoxifylline is also used in an 'off-label' fashion for a variety of other conditions.

In mouse studies, high-dose caffeine administration resulted in decreased testicular size but increased sperm concentrations (Anon 1997); however, the authors noted that the sperm concentrations of the control group were notably lower than those of subsequent experimental control groups in their laboratory. In rats, caffeine induced occasional degeneration of spermatogenic cells in the testes (Gans, 1984). High-dose theobromine, though, is associated with diffuse destruction of spermatogenic cells with many seminiferous tubules containing Sertoli cells only (Gans, 1984; Funabashi *et al.*, 2000).

Studies in humans have yielded less clear results in part because of the difficulty in quantifying caffeine and theobromine intake. In a large group of young Danish military recruits, overall caffeine consumption did not have a statistically significant effect on sperm counts (even >800 mg/day, equivalent to at least four cups of coffee daily); however, high intake of cola was associated with a significant decrease in sperm counts and other markers of spermatozoa quality (Jensen *et al.*, 2010). Given the relatively low caffeine content of cola compared to that of coffee, it is unclear how much caffeine alone contributes to this effect as opposed to theobromine or other chemicals found specifically in colas. Likewise, coffee consumption was not associated with decreased sperm concentrations in men presenting for vasectomy (Sobreiro *et al.*, 2005). Conversely, in a randomized controlled trial in men with idiopathic oligoasthenoteratospermia (OAT), pentoxifylline increased sperm concentrations 63% compared to placebo (Safarinejad, 2011b).

Illicit drugs

Illicit drug use is highest during puberty, a crucial time for testicular development, and the reproductive years (Substance Abuse and Mental Health Services Administration 2014). Thus, not only may these substances have significant, direct immediate impacts on spermatogenesis, there may also be long-term impairment from abnormal development of the testes. Drugs associated with male factor infertility include marijuana, androgenic anabolic steroids (AAS), opioid narcotics, cocaine, and methamphetamines.

Marijuana

Marijuana is the most frequently used illicit drug, with 12.5% of U.S. adults reporting use within the past year (Substance Abuse and Mental Health Services Administration 2014). Use, however, is even more prevalent among adolescents, with 23% of 9th to 12th graders reporting use within the previous month (Substance Abuse and Mental Health Services Administration 2015). Marijuana acutely decreases LH levels (Cone *et al.*, 1986), while chronic use is associated with decreased basal LH levels and decreased responsiveness to gonadotropin-releasing hormone (GnRH) stimulation (Vescovi *et al.*, 1992). Chronic, intensive marijuana usage has been associated with dramatically decreased serum testosterone levels in a dose-dependent manner and oligospermia was found in 35% of the men who provided semen samples (Kolodny *et al.*, 1974). Another study, however, did not confirm the testosterone-related findings and semen parameters were not assessed (Mendelson *et al.*, 1974). Human spermatozoa express the cannabinoid 1 receptor and in vitro studies exposing human spermatozoa to marijuana extracts have demonstrated decreased sperm motility, viability, and function (Schuel *et al.*, 2002; Rossato *et al.*, 2005; Whan *et al.*, 2006).

Androgenic, anabolic steroids

An estimated 3 million U.S. adults use AAS (Evans, 2004). They are frequently used among reproductive age males, particularly on the younger end of the spectrum, with 1.7% of adolescent males reporting use within the past year (van den Berg *et al.*, 2007). While AAS use is commonly attributed to professional athletes and body builders, two-thirds of men use AAS recreationally for cosmetic appearances and other non-competitive reasons (Evans, 2004). AAS are of particular concern because of their similarity to testosterone and the supraphysiologic levels at which they are used. They mimic testosterone resulting in hypogonadotrophic hypogonadism [reviewed in (de Souza & Hallak, 2011)]. In short, elevated serum androgens activate a negative feedback loop by which the pituitary gland decreases LH and FSH production. This, in turn, leads to decreased testicular production of testosterone and, consequently, decreased intratesticular testosterone levels. Decreased FSH and intratesticular testosterone levels result in impaired spermatogenesis. AAS use is associated with decreased sperm count, normal morphology, and normal motility, and in some circumstances, complete azoospermia (Schürmeyer *et al.*, 1984; Knuth *et al.*, 1989). In most cases, recovery of spermatozoa to the ejaculate is seen

within 4–12 months following cessation of AAS (Schürmeyer *et al.*, 1984; Knuth *et al.*, 1989); however, recovery may take 2 years or longer (Liu *et al.*, 2006).

Androgen-associated impairment of spermatogenesis is not limited to illicit use of AAS. Exogenous testosterone replacement therapy in a hypogonadal or healthy male can also lead to suppression of spermatogenesis through similar mechanisms (Roth *et al.*, 2013).

Narcotic opioids

Opiate addicted men were found to have significantly lower total sperm counts than matched controls (Safarinejad *et al.*, 2013), although this finding may be drug specific (Cicero *et al.*, 1975). Serum testosterone levels are significantly lower in opiate users (Cicero *et al.*, 1975; Safarinejad *et al.*, 2013). Additionally, functional δ-, κ-, and μ-opioid receptors have been demonstrated in human spermatozoa (Agirregoitia *et al.*, 2006), which may explain decreased motility seen in opiate users and variability in the other semen parameters reported in the literature (Cicero *et al.*, 1975; Safarinejad *et al.*, 2013).

Opiate addiction is treated with long-acting opiates, opiate antagonists, and buprenorphine. Methadone is the most commonly used long-acting opiate, and as with other opiates, is associated with decreased serum LH and testosterone levels and decreased sperm counts in humans (Cicero *et al.*, 1975; Hallinan *et al.*, 2009). Opiate antagonists (e.g. naloxone, naltrexone, and nalmefene) have been shown to increase LH pulsatility and serum testosterone levels in humans (Graves *et al.*, 1993), and naloxone has been shown to reverse the in vitro effects of opiates on sperm motility (Agirregoitia *et al.*, 2012). Buprenorphine is a mixed opioid agonist and antagonist and has been associated with hypogonadism, although not as frequently as methadone (Hallinan *et al.*, 2009). Effects of the opiate antagonists and buprenorphine on spermatogenesis have not been reported.

Cocaine

Inhaled crack cocaine decreases testicular volume, numbers of Sertoli cells, and impairs spermatid differentiation in mice (Pires *et al.*, 2012). Chronic cocaine administration decreases seminiferous tubule diameter and spermatid count in rats (George *et al.*, 1996), and has been shown to decrease cAMP responsive element modulator (CREM) expression, which is essential for spermatogenesis (Li *et al.*, 2003). In humans, cocaine use was found to be twice as common among oligospermic men (Bracken *et al.*, 1990). Although cocaine has been shown to bind human spermatozoa in vitro, this does not appear to affect motility or viability (Yazigi *et al.*, 1991).

Amphetamines

Methamphetamine decreases sperm counts in rats and induces apoptosis in cells involved in most stages of spermatogenesis (Nudmamud-Thanoi & Thanoi, 2011). A specific amphetamine, (±)-3,4-methylenedioxymethamphetamine (also known as MDMA or 'ecstasy'), impairs hypothalamic and gonadal function in rats by decreasing GnRH mRNA and serum testosterone levels during both acute and chronic administration of the drug (Dickerson *et al.*, 2008). The effects of amphetamines on sperm counts have not been reported in humans.

DIETARY INTAKE, MICRONUTRIENTS, AND SUPPLEMENTS

Caloric intake

In rodents, significant food restriction results in decreased serum testosterone and LH levels, decreased epididymal weights, and degeneration of spermatocytes (Rehm *et al.*, 2008). In young Rhesus Macaques, 30% caloric restriction was not associated with significant differences in semen parameters or mean testosterone levels (Sitzmann *et al.*, 2010), but the power to detect a difference may have been limited by sample size. In humans, similar levels of caloric restriction without malnutrition are associated with significantly decreased testosterone levels compared to controls on a Western diet (Cangemi *et al.*, 2010); however, no effect on spermatogenesis has been reported.

On the other hand, obesity in humans is also associated with decreased testosterone levels and sperm counts with significant improvement seen in both parameters with diet- and exercise-induced weight loss (Håkonsen *et al.*, 2011). Bariatric surgery in obese men increases total testosterone and FSH, but does not have a significant impact on semen parameters (Reis *et al.*, 2012). These data suggest that diet quality may have a more pronounced effect on spermatogenesis than absolute caloric intake.

Micronutrients

While the studies below investigate individual micronutrients, it is important to remember that intake of a particular micronutrient may affect intake and excretion of others. This is particularly true for divalent cations, which have been of interest given their role in oxidative stress and other human diseases. For example, high molybdenum levels are associated with decreased serum zinc and copper levels in humans (Meeker *et al.*, 2008). A zinc-deficient diet in rats also significantly decreases serum magnesium and selenium levels while increasing copper and cadmium levels (Omu *et al.*, 2015), and seminal zinc levels are highly correlated with seminal selenium levels in humans (Oldereid *et al.*, 1998; Camejo *et al.*, 2011). Aluminum chloride increases testicular aluminum and copper levels while decreasing zinc and iron levels in rats (Zhu *et al.*, 2014). Thus, whether the findings described reflect changes in the particular micronutrient itself or relative levels of micronutrients is unclear.

Zinc

Zinc is an important divalent metal in biologic processes, including DNA replication and free radical scavenging [reviewed in (Valko *et al.*, 2015)]. Zinc concentration is high in the reproductive tract, particularly in the prostate and testes (Bedwal & Bahuguna, 1994). Seminal plasma zinc concentrations do not vary before and after vasectomy and vasovasostomy, suggesting the prostate is the predominant source of zinc in the ejaculate (Parrish *et al.*, 1987).

Recent studies in rats demonstrate that zinc deficiency causes decreased testosterone, increased serum markers of oxidative stress, and increased apoptosis of spermatogonia, spermatocytes, and spermatids (Omu *et al.*, 2015). Increased apoptosis of non-spermatogonial cells in the testes has also been reported (Kumari *et al.*, 2011).

In humans, seminal zinc levels are higher in men with normospermia compared to men with asthenospermia, oligospermia, and teratospermia, and seminal zinc levels are highly correlated

with total sperm count (Chia *et al.*, 2000; Camejo *et al.*, 2011; Atig *et al.*, 2012). Zinc supplementation increases total sperm count and decreases anti-spermatozoa antibodies in men with asthenospermia (Omu *et al.*, 1998). Likewise, in a double-blind, placebo-controlled trial in men with OAT, combination folate and zinc supplementation was found to increase sperm concentration (Raigani *et al.*, 2014).

While zinc is associated with decreased oxidative stress and improved spermatogenesis, too much may be toxic. Intratesticular zinc injections have been investigated for use as a low-cost sterilization agent in animals as they have been shown to impair spermatogenesis in dogs (Oliveira *et al.*, 2007), bears (Brito *et al.*, 2011), and cats (Fagundes *et al.*, 2014).

Selenium

Selenium is a cofactor of glutathione peroxidase, and therefore, helps protect cells from oxidative stress. In the human male reproductive tract, selenium concentration is highest in the testes, followed by the seminal vesicles and then the prostate (Oldereid *et al.*, 1998). In the rat, testicular uptake of selenium increases significantly around the time of puberty (Behne *et al.*, 1986). Interestingly, with selenium deficiency testicular selenium levels are maintained at the expense of other tissues, emphasizing its importance to testicular function (Behne & Höfer-Bosse, 1984).

Selenium deficiency is associated with decreased basal and GnRH- and LH-stimulated testosterone levels in rats (Behne *et al.*, 1996). Prior to starting a selenium-deficient diet, the first generation of these rats were selenium sufficient and differences in testicular size and spermatogenesis were not observed even after selenium-deficiency was induced. With the second generation of rats continued on the same selenium-deficient diet, however, smaller testicular volumes and impaired spermatogenesis were noted (Behne *et al.*, 1996).

In humans, selenium concentrations in the seminal fluid are positively correlated with sperm concentrations in some (Oldereid *et al.*, 1998; Camejo *et al.*, 2011; Eroglu *et al.*, 2014), but not all (Atig *et al.*, 2012; Li *et al.*, 2012) studies. Oral administration of selenium (100–300 mcg/day) was shown to increase serum and seminal selenium levels, but had no effect on sperm concentrations or other semen parameters in normospermic (Hawkes & Turek, 2001; Hawkes *et al.*, 2009) or oligospermic (Scott & Yates, 1998) males.

Copper

Copper is an essential trace element and widely distributed in the human body. It is a critical component of multiple enzymes, particularly those involved in oxidative stress detoxification, including catalase and the copper/zinc superoxide dismutase (Cu-, Zn-SOD) (Uauy *et al.*, 1998). Copper can be absorbed from the gastrointestinal tract, lungs, and skin; however, diet is the most common source under normal conditions (Agency for Toxic Substances and Disease Registry, ATSDR 2004).

Molybdenum-induced copper deficiency has been reported to decrease sperm concentration in rams (Van Niekerk & Van Niekerk, 1989). Copper deficiency is relatively rare in humans and is mainly limited to infants (Uauy *et al.*, 1998). Thus, the effects of copper deficiency on spermatogenesis have not been reported in humans.

High-dose copper gavage decreased sperm concentrations in rats and was associated with decreased germ cells and collapse of the seminiferous tubules (Sakhaee *et al.*, 2012). In humans, seminal copper levels negatively correlate with sperm concentrations (Li *et al.*, 2012). Seminal copper and iron levels are higher in subfertile than in fertile males and are associated with increased oxidative stress (Aydemir *et al.*, 2006). On the other hand, a population-based study of 1179 Chinese men exploring the relationship between serum copper levels and sperm concentrations did not identify a significant association (Yuyan *et al.*, 2008). The discordant findings between these groups, however, may be because of differential regulation of serum copper and seminal copper concentrations. The effects of genetic causes of severe copper overload (e.g. Wilson's disease) on spermatogenesis have not been reported.

Iron

While iron is a required cofactor for many metalloenzymes, including those involved with spermatogenesis, it also induces oxidative stress by catalyzing the production of reactive oxygen species (Tvrda *et al.*, 2015). As the body has no regulated mechanism of eliminating excess iron, iron homeostasis must be maintained through tight control of dietary uptake and storage which is mediated by the iron export protein, ferroportin (Hentze *et al.*, 2010). Ferroportin expression is largely restricted to the cells involved in regulating body and serum iron levels. Interestingly, the Sertoli cell has been reported to express ferroportin (Leichtmann-Bardoogo *et al.*, 2012) and iron is exquisitely regulated in the testes [reviewed in (Griffin *et al.*, 2005)] suggesting a critical need for maintaining iron homeostasis for proper testicular function.

Iron overload negatively impacts spermatogenesis. Acute iron overload in mice is associated with degeneration of the seminiferous tubules (Lourdes de Pereira & Garcia e Costa, 2003). In humans, seminal plasma iron levels are higher in infertile compared to fertile men (Aydemir *et al.*, 2006). Severe iron overload, as seen in hereditary hemochromatosis and beta thalassemia, is associated with profound hypogonadotrophic hypogonadism and testicular atrophy (Mula-Abed *et al.*, 2008; Crownover & Covey, 2013; Kim *et al.*, 2013b). Total sperm counts are dramatically lower in men with homozygous beta thalassemia compared to controls (Safarinejad, 2008).

Conversely, iron deficiency is also associated with impaired spermatogenesis. For example, in men with iron deficiency anemia, intravenous iron supplementation resulted in a doubling of sperm count and improvement in all semen parameters (Soliman *et al.*, 2014). Whether this reflects a direct effect of iron or improvement in oxygenation because of resolution of the anemia remains unclear. Nonetheless, balance between too much and too little iron appears to be important for spermatogenesis.

Manganese

Manganese is ubiquitous in the environment and can be absorbed through dietary intake and via air and dust exposure (Aschner & Aschner, 2005). Manganese plays a role in mitigating oxidative stress through its incorporation into manganese superoxide dismutase (Mn-SOD), but excessive manganese intake can increase oxidative stress (Bonke *et al.*, 2015). Oral manganese was found to decrease sperm concentrations in mice in a dose-dependent manner; however, no discrete changes were seen on histologic examination of the testes (Ponnapakkam *et al.*, 2003). In humans, men with the lowest and highest serum manganese

concentrations were found to have decreased sperm concentrations (Wirth *et al.*, 2007), suggesting that an optimal manganese level is also necessary for spermatogenesis.

Lead

Environmental exposure to lead can come from many sources including old house paint, leaching from brass water fixtures, ceramic coatings, or smoking. In mice, lead in the drinking water decreased sperm concentrations by decreasing layers of germ cells and disrupting germ cell alignment (Wang *et al.*, 2013). In humans, lead workers have significantly lower total sperm counts without significant changes in serum LH, FSH, or testosterone levels (Alexander *et al.*, 1996; Telisman *et al.*, 2000). In the general population, lead levels in seminal plasma have been associated with decreased sperm concentrations in some (Benoff *et al.*, 2003; Pant *et al.*, 2003, 2014a; Li *et al.*, 2012), but not all (Xu *et al.*, 2003; Telisman *et al.*, 2007) studies. Interestingly, low-level lead exposure has been associated with higher testosterone levels (Telisman *et al.*, 2007).

Cadmium

Exposure to cadmium in the general population derives predominantly from smoking (Jurasović *et al.*, 2004). Cadmium acutely decreases sperm counts in rats in a dose-dependent manner (Laskey *et al.*, 1984). Increasing urinary cadmium levels are associated with increased LH and testosterone in occupationally exposed workers (Zeng *et al.*, 2002); however, semen parameters in this population have not been published. Low-dose cadmium exposure, however, is also associated with decreased total sperm counts in the general population in some (Pant *et al.*, 2003, 2014a; Xu *et al.*, 2003), but not all (Hovatta *et al.*, 1998; Jurasović *et al.*, 2004; Benoff *et al.*, 2009) studies.

Other metals

Elevated aluminum (Zhu *et al.*, 2014), molybdenum (Meeker *et al.*, 2008), nickel (Danadevi *et al.*, 2003), arsenic (Li *et al.*, 2012), and chromium (Danadevi *et al.*, 2003) levels have been associated with decreased sperm counts. Nickel deficiency has also been associated with decreased epididymal sperm counts in rats (Yokoi *et al.*, 2003).

Non-metal micronutrients

Boron

Boron is obtained through contact with soil, dietary intake, and inhalation. Sub-chronic and chronic exposures to boron are toxic to the testes and impair spermatogenesis in animals [reviewed in (Scialli *et al.*, 2010)]. Boron was found to accumulate in semen in humans, but exposure has not been associated with impaired spermatogenesis, even in industrial boron workers (Robbins *et al.*, 2010; Scialli *et al.*, 2010).

Folate

Folate is critical for DNA and protein synthesis, and therefore may play a role in spermatogenesis. Folate supplementation alone and in combination with zinc trended toward increased sperm concentrations in a small double-blind, placebo-controlled trial enrolling men with OAT (Raigani *et al.*, 2014).

Coenzyme Q_{10}

Coenzyme Q_{10} supplementation modestly increased sperm count and motility in men with idiopathic OAT (Safarinejad, 2009). In a small study of azoospermic men with maturation arrest on testicular biopsy, nine of 24 men developed spermatozoa in their ejaculate with the combined administration of multivitamins, micronutrients, and coenzyme Q_{10} (Singh *et al.*, 2010). Another study, however, found no correlation between seminal coenzyme Q_{10} levels and sperm concentrations (Eroglu *et al.*, 2014).

Antioxidants

Although reactive oxygen species (ROS) have been shown to negatively impact spermatogenesis, most studies involving oral intake of antioxidants, however, have been disappointing. Nonetheless, linear regression of combined fertile and infertile men demonstrated a positive correlation between serum antioxidant status with sperm concentration, motility, and normal morphology (Benedetti *et al.*, 2012; Eroglu *et al.*, 2014). In a randomized, controlled trial in men with idiopathic OAT, selenium, and/or N-acetyl cysteine supplementation was associated with transient increases in sperm counts (Safarinejad & Safarinejad, 2009). No significant difference was seen in sperm concentrations in a study investigating combined selenium and vitamin E administration (Keskes-Ammar *et al.*, 2003).

Other supplements and specific dietary factors

Total fish intake and dietary omega-3 fatty acid supplementation are associated with higher sperm counts in humans (Safarinejad, 2011a; Afeiche *et al.*, 2014); however, a negative association has been reported in several studies, attributed to build up of persistent organochlorine pollutants (POPs) especially in fatty fish (see *Persistent organochlorine pollutants* section below). Erythrosine (FD&C Red No 3), used to add color to foods and cosmetics, decreased epididymal sperm counts by approximately 50% in mice (Abdel Aziz *et al.*, 1997).

AMBIENT AND OTHER OCCUPATIONAL EXPOSURES

Air pollution

Prenatal and post-natal exposures to ambient air pollution decrease testicular size and impair some stages of spermatogenesis in mice (Pires *et al.*, 2011). In humans, while abnormalities in sperm motility, morphology, and DNA abnormalities have been reported, air pollution has not been associated with changes in sperm counts in most studies (Selevan *et al.*, 2000; Rubes *et al.*, 2005; Hammoud *et al.*, 2010; Hansen *et al.*, 2010). In some specific populations (e.g. those with increased exposure to vehicle exhaust fumes), however, air pollution has been negatively associated with sperm counts (Guven *et al.*, 2008).

Water pollution

Contaminants contributing to water pollution not only include factors entering the public water supply from wastewater, but the purification process itself has been reported to have negative effects on public health. Absorption can come not only from ingestion but also from hand washing, bathing, and boiling water. Disinfection byproducts (DBPs) are produced when the disinfecting agent (e.g. chlorine and ozone) interact with naturally occurring substances in the water (e.g. organic matter,

bromides, iodides). More than 600 types of DBPs have been identified in chlorinated drinking water, the most common being trihalomethanes and haloacetic acids [reviewed in (Richardson *et al.*, 2007)]. Given the number of chemicals involved, it is not surprising that there are conflicting data in the literature with regard to spermatogenesis. In rats, daily exposure to dichloroacetic acid is associated with testicular damage and decreased sperm counts (Linder *et al.*, 1997) and dibromoacetic acid administration is associated with delayed pubertal development and atrophy of the seminiferous tubules (Klinefelter *et al.*, 2004). In humans, the data are less clear. Using urinary trichloroacetic acid as a marker of exposure, increased exposure was associated with lower sperm concentrations in Chinese men (Zeng *et al.*, 2014). A separate study in China found a trend toward lower sperm concentrations with higher serum trihalomethane levels (Zeng *et al.*, 2013). Three studies, however, found no significant effect of trihalomethane exposure on sperm counts (Fenster *et al.*, 2003; Luben *et al.*, 2007; Iszatt *et al.*, 2013).

Another rising concern with water pollution is the increasing presence of detectable levels of pharmaceuticals, pharmaceutical byproducts, pesticides, and other manmade chemicals in the water supply [reviewed in (Richardson, 2007)]. Because of their unique chemical structures, many require specialized tests for identification so the extent of contamination is not well understood, and many are not removed by standard wastewater treatment (Stackelberg *et al.*, 2004). While most are present only in trace amounts thought not to pose significant reproductive or other health risks, there is an increasing frequency of reports of endocrine-disrupting chemicals and xenoestrogens impacting aquatic life downstream of wastewater treatment plants [e.g., see (Jobling *et al.*, 2006)]. The extent to which this may affect spermatogenesis is unknown. Although the effects of individual compounds in drinking water may be small or non-detectable, the cumulative or synergistic effects of thousands of chemicals may play an increasingly important role as these concentrations continue to increase.

Persistent organochlorine pollutants

Persistent organocholorine pollutants (POPs) are synthetic chemicals that are resistant to degradation, and include pesticides (see also *Pesticides* below), industrial chemicals, and solvents (e.g. polychlorinated biphenyl (PCB), dioxins, and dibenzofurans) and their metabolites. These accumulate as one ascends the food chain with animal sources, particularly fish, being major sources of exposure in humans (Liem *et al.*, 2000). While many studies have reported a negative impact on sperm motility, few studies have reported significant findings between POPs and sperm counts in the general population. In a subgroup of men with normal semen parameters, elevated serum polychlorinated biphenyl (PCB) metabolite levels were associated with decreased sperm concentrations (Dallinga *et al.*, 2002). Remarkably, a detailed analysis of POPs in serum samples found a *positive* association between several POPs and sperm counts (Mumford *et al.*, 2014). A weak positive correlation between sperm concentrations and CB-153 and *p,p'*-DDE levels was reported in a geographic subgroup of men in northern Norway (Haugen *et al.*, 2011), but no significant effect was seen in a U.S. cohort (Hauser *et al.*, 2003).

Pesticides

For the majority of people, pesticides are taken in via dietary consumption; however, pesticides can also be absorbed through the skin and by inhalation, particularly in occupationally exposed men. Pesticides have been shown to have a wide range of effects on male fertility and are potent endocrine disruptors [reviewed in (Bretveld *et al.*, 2007)].

Occupational exposures to pesticides have demonstrated dramatic effects on spermatogenesis because of the high levels and long durations of exposure. 1,2-dibromo-3-chloropropane (DBCP) is the most well-known pesticide associated with impaired fertility. The initial report that identified this link demonstrated that 14 of 25 workers in a DBCP factory had azoospermia or oligospermia with elevated FSH and LH levels and normal testosterone levels (Whorton *et al.*, 1977). Impressively, the men with severe oligospermia or azoospermia had all worked at the factory at least three years, whereas none of those with sperm counts >40 million/mL had worked there longer than 3 months (Whorton *et al.*, 1977). These findings were confirmed by a large scale, worldwide study of banana and pineapple plantation workers that found up to 90% of men were azoospermic or oligospermic after 3 years of exposure (Slutsky *et al.*, 1999).

Dramatic decreases in sperm counts have also been reported for occupational exposures to other pesticides including paraquat and malathion (Hossain *et al.*, 2010), and ethylene dibromide (Ratcliffe *et al.*, 1987). Additionally, Danish greenhouse workers with high pesticide exposure were found to have a 60% decrease in total sperm count compared to those with low exposure (Abell *et al.*, 2000).

There is also evidence to suggest that pesticides may impair spermatogenesis in those who do not have occupational-based exposures. For example, urinary concentrations of three organophosphate metabolites were found to be negatively associated with total sperm counts (Melgarejo *et al.*, 2015). Additionally, high pesticide residue fruit and vegetable intake was associated with lower sperm counts in men presenting to a fertility clinic, while total fruit and vegetable intake was not associated with changes in semen parameters (Chiu *et al.*, 2015).

Phthalates

Phthalates are used as plasticizers in a number of consumer and personal care products, and millions of tons are produced annually. They are commonly used in food and water containers, and as such, the majority of human exposure comes from dietary contamination. Exposure is nearly ubiquitous, as reflected by a study of 634 German men and women where 99% were found to have detectable levels of phthalates in their urine (Wittassek *et al.*, 2007). Some phthalates and phthalate metabolites have demonstrated anti-androgenic properties in both animals and humans. Administration of the most commonly used plasticizer, dibutyl phthalate (DBP) to rats decreased serum FSH and testosterone levels, testicular weights, and sperm counts in a dose-dependent manner (Aly *et al.*, 2015). Furthermore, these rats were found to exhibit increased oxidative stress and decreased antioxidant capacity within the testes along with associated testicular atrophy (Aly *et al.*, 2015). In humans, urinary DBP is positively associated with serum estradiol levels and estradiol: testosterone ratio (Fong *et al.*, 2015) and negatively associated with serum testosterone levels and sperm counts in most (Hauser *et al.*, 2006; Pant *et al.*, 2014b; Specht *et al.*, 2014), but not all

(Huang *et al.*, 2011) studies. Urinary monobutyl phthalate (MBP) concentrations are associated with decreased sperm counts (Hauser *et al.*, 2006) while DBP concentrations were not (Huang *et al.*, 2011). At least, part of the study outcome differences may be because of the mechanism by which phthalates were measured (estimated exposure vs. direct assessment of blood or urine concentrations) and the specific phthalate/metabolite investigated.

Glycol ethers

Glycol ethers are found in industrial solvents, thinners, deicers, and other products and have been associated with reproductive toxicity in male animals since the 1930s [reviewed in (Hardin, 1983)]. Exposures through all routes studied, including inhalational, transcutaneous, and dietary, appear to have potentially adverse effects (Hardin, 1983). In particular, occupational exposure to 2-ethoxyethanol and 2-methoxyethanol (commonly found in solvents) has been associated with increased risk of oligospermia and azoospermia in men (Welch *et al.*, 1988; Ratcliffe *et al.*, 1989; Veulemans *et al.*, 1993).

Heat

Spermatogenesis requires temperatures 2–4 °C below core body temperature (Ivell, 2007). Heat is well-known to affect all domains of spermatogenesis in both the acute and chronic setting through increased apoptosis of germ cells and increased DNA damage [reviewed in (Durairajanayagam *et al.*, 2015) and (Kim *et al.*, 2013a)]. Endogenous sources of elevated testicular temperatures include obesity, varicocele, fever, and cryptorchidism. The extent to which heat independently impairs spermatogenesis remains unknown, as other factors have also been shown to impair spermatogenesis in these conditions. Nonetheless, obese men and men with varicocele have increased scrotal temperatures and decreased total sperm counts with increased FSH levels compared to controls (Garolla *et al.*, 2015). External sources, such as sauna or hot tub usage, have also been associated with impaired spermatogenesis (Sheynkin *et al.*, 2005; Garolla *et al.*, 2013). For example, transient scrotal hyperthermia (scrotal warming for 30 min daily in a 43 °C water bath) induced a dramatic decline in total sperm counts and progressive motility without altering serum reproductive hormone levels (Rao *et al.*, 2015). Some exposures appear to have transient effects on spermatogenesis, while chronic exposures (e.g. varicocele, cryptorchidism) may be associated with a permanent decline in sperm production.

Radiation

Radiation is a well-known cause of impaired spermatogenesis. Clifton, *et al.*, demonstrated that gamma irradiation to the testes of healthy men suppressed sperm production and depleted Type A spermatogonia in a dose-dependent fashion. Recovery of sperm production also varied in a dose-dependent manner; however, all recovered sperm production if adequate, follow-up data were available (Clifton & Bremner, 1983). In a small study, 50% of men exposed to radiation either during or immediately following the Chernobyl nuclear power plant disaster were azoospermic or oligospermic (Birioukov *et al.*, 1993). Even natural background levels of radiation may impair spermatogenesis as elevated levels of radiation are associated with increased frequency of random mutations in the azoospermia factor a, b, and c regions on the Y chromosome in men; however, the significance of this finding is unknown as semen parameters were not reported (Premi *et al.*, 2009).

Low-intensity electromagnetic field (EMF) radiation exposure from cell phones has been controversially associated with impaired spermatogenesis. EMF radiation has been associated with ultrastructural changes in rat testes (Çelik *et al.*, 2012). Four recent meta-analyses of cell phone usage and semen quality have been conducted with varying results: two found significant decreases in sperm concentrations in humans (La Vignera *et al.*, 2012; Dama & Bhat, 2013), one was equivocal (Adams *et al.*, 2014), and one found significant decreases in sperm counts in rats but not in humans (Liu *et al.*, 2014). Increased exposure to EMFs (all sources, not just mobile phones) was also associated with decreased sperm concentrations in a population-based, case–control study (Li *et al.*, 2010).

Hypoxia

Spermatocytes are particularly sensitive to hypoxia, as even short-term (1 h) experimental torsion and reperfusion in rats specifically causes apoptosis of germ cells and not Sertoli and Leydig cells (Turner *et al.*, 1997). In humans, unilateral torsion is also associated with significant, long-term impairment of sperm counts despite surgical correction [reviewed in (Visser & Heyns, 2003)].

Impaired spermatogenesis is also noted in less severe degrees of hypoxia, such as altitude-associated hypobaric hypoxia. Hypobaric hypoxia induces sloughing of seminiferous tubules and increases spermatogonial apoptosis in rats (Liao *et al.*, 2010). Interestingly, chronic hypobaric hypoxia in rats increased intratesticular temperature by approximately 1.5 °C (Farías *et al.*, 2005), suggesting that hypoxia may also secondarily impair spermatogenesis by increasing intratesticular temperatures. In humans, moving from low altitude to high altitude is associated with decreased testosterone levels and significantly decreased sperm concentrations (Donayre *et al.*, 1968; Okumura *et al.*, 2003; Verratti *et al.*, 2008). Fertility is not impaired among individuals native to high altitudes (Gonzales, 2007), though, suggesting that the human body is able to compensate with time. Hypoxia may also be partly responsible for impaired spermatogenesis associated with cigarette smoking, iron deficiency, and varicocele (Collin *et al.*, 1995; Koskinen *et al.*, 2000; Reyes & Farias, 2012).

Other exposures

In a prospective study of men planning to attempt pregnancy, occupational heavy exertion was associated with decreased sperm counts with a near doubling of the rate of oligospermia (Eisenberg *et al.*, 2015). Noise, vibration, prolonged sitting, extreme heat, night work, and rotating shifts were not associated with decreased sperm counts in this study.

Impairment of spermatogenesis is not just limited to physical exposures. Occupation-related burnout, tension, listlessness, and cognitive weariness were all significantly higher in men with male factor infertility compared to controls, suggesting work-related psychological stress can contribute to infertility as well (Sheiner *et al.*, 2002).

CONCLUSIONS AND FUTURE DIRECTIONS

Interest continues to grow to better understand the effects of chronic and sub-chronic exposures on spermatogenesis. Many

lifestyle and dietary choices, and environmental and occupational exposures have been associated with changes in spermatogenesis. While animal models have been used to delineate the role of specific exposures, extrapolation of these exposures to humans remains a challenge and considerable conflicting data have been published.

Although human data are often limited to retrospective studies and subject to many known and unknown confounders, they have yielded valuable information to this point. There is an abundance of data available that will continue to generate hypotheses and help guide public health investigations.

Large prospective, randomized controlled trials would be ideal to determine the effects of individual chronic exposures; however, these studies may be difficult to conduct because of the ethical and financial challenges. To help decrease bias and confounders, population-based studies may be helpful. Another, less utilized avenue for investigating these effects would be identification of populations that have high levels of isolated exposures, whether ambient, genetic, or occupational. In these populations, higher doses and more chronic exposures may also help to reduce the impact of confounders. Improving study quality by recruiting from the general population, appropriately controlling for confounding factors, and attempting to identify those populations with unique exposure profiles will help to increase our understanding of the factors that impact spermatogenesis and thus, will allow us to focus on optimization of male reproductive potential in the future.

REFERENCES

Abdel Aziz AH, Shouman SA, Attia AS & Saad SF. (1997) A study on the reproductive toxicity of erythrosine in male mice. *Pharmacol Res* 35, 457–462.

Abell A, Ernst E & Bonde JP. (2000) Semen quality and sexual hormones in greenhouse workers. *Scand J Work Environ Health* 26, 492–500.

Adams JA, Galloway TS, Mondal D, Esteves SC & Mathews F. (2014) Effect of mobile telephones on sperm quality: a systematic review and meta-analysis. *Environ Int* 70, 106–112.

Adler ID. (1996) Comparison of the duration of spermatogenesis between male rodents and humans. *Mutat Res* 352, 169–172.

Afeiche MC, Gaskins AJ, Williams PL, Toth TL, Wright DL, Tanrikut C, Hauser R & Chavarro JE. (2014) Processed meat intake is unfavorably and fish intake favorably associated with semen quality indicators among men attending a fertility clinic. *J Nutr* 144, 1091–1098.

Agency for Toxic Substances and Disease Registry (ATSDR) (2004) *Toxicological Profile for Copper*. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Agirregoitia E, Valdivia A, Carracedo A, Casis L, Gil J, Subiran N, Ochoa C & Irazusta J. (2006) Expression and localization of delta-, kappa-, and mu-opioid receptors in human spermatozoa and implications for sperm motility. *J Clin Endocrinol Metab* 91, 4969–4975.

Agirregoitia E, Subiran N, Valdivia A, Gil J, Zubero J & Irazusta J. (2012) Regulation of human sperm motility by opioid receptors. *Andrologia* 44(Suppl 1), 578–585.

Alexander BH, Checkoway H, van Netten C, Muller CH, Ewers TG, Kaufman JD, Mueller BA, Vaughan TL & Faustman EM. (1996) Semen quality of men employed at a lead smelter. *Occup Environ Med* 53, 411–416.

Aly HA, Hassan MH, El-Beshbishy HA, Alahdal AM & Osman A-MM. (2015) Dibutyl phthalate induces oxidative stress and impairs spermatogenesis in adult rat. *Toxicol Ind Health*, 0748233714566877.

Anderson RA, Willis BR & Oswald C. (1985) Spontaneous recovery from ethanol-induced male infertility. *Alcohol* 2, 479–484.

Anon (1997) Reproductive toxicology. Caffeine. *Environ Health Perspect* 105(Suppl 1), 281–282.

Aschner JL & Aschner M. (2005) Nutritional aspects of manganese homeostasis. *Mol Aspects Med* 26, 353–362.

Asma S, Mackay J, Song SY, Zhao L, Morton J, Palipudi KM, Bettcher D, *et al.* (2015) *The GATS Atlas*. CDC Foundation, Atlanta, GA.

Atig F, Raffa M, Habib B-A, Kerkeni A, Saad A & Ajina M. (2012) Impact of seminal trace element and glutathione levels on semen quality of Tunisian infertile men. *BMC Urol* 12, 6.

Auger J, Kunstmann JM, Czyglik F & Jouannet P. (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 332, 281–285.

Aydemir B, Kiziler AR, Onaran I, Alici B, Ozkara H & Akyolcu MC. (2006) Impact of Cu and Fe concentrations on oxidative damage in male infertility. *Biol Trace Elem Res* 112, 193–203.

Bedwal RS & Bahuguna A. (1994) Zinc, copper and selenium in reproduction. *Experientia* 50, 626–640.

Behne D & Höfer-Bosse T. (1984) Effects of a low selenium status on the distribution and retention of selenium in the rat. *J Nutr* 114, 1289–1296.

Behne D, Duk M & Elger W. (1986) Selenium content and glutathione peroxidase activity in the testis of the maturing rat. *J Nutr* 116, 1442–1447.

Behne D, Weiler H & Kyriakopoulos A. (1996) Effects of selenium deficiency on testicular morphology and function in rats. *J Reprod Fertil* 106, 291–297.

Benedetti S, Tagliamonte MC, Catalani S, Primiterra M, Canestrari F, De Stefani S, Palini S & Bulletti C. (2012) Differences in blood and semen oxidative status in fertile and infertile men, and their relationship with sperm quality. *Reprod Biomed Online* 25, 300–306.

Benoff S, Centola GM, Millan C, Napolitano B, Marmar JL & Hurley IR. (2003) Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. *Hum Reprod* 18, 374–383.

Benoff S, Hauser R, Marmar JL, Hurley IR, Napolitano B & Centola GM. (2009) Cadmium concentrations in blood and seminal plasma: correlations with sperm number and motility in three male populations (infertility patients, artificial insemination donors, and unselected volunteers). *Mol Med* 15, 248–262.

van den Berg P, Neumark-Sztainer D, Cafri G & Wall M. (2007) Steroid use among adolescents: longitudinal findings from Project EAT. *Pediatrics* 119, 476–486.

Birioukov A, Meurer M, Peter RU, Braun-Falco O & Plewig G. (1993) Male reproductive system in patients exposed to ionizing irradiation in the Chernobyl accident. *Arch Androl* 30, 99–104.

Bonke E, Zwicker K & Dröse S. (2015) Manganese ions induce H2O2 generation at the ubiquinone binding site of mitochondrial complex II. *Arch Biochem Biophys* 580, 75–83.

Borgerding M & Klus H. (2005) Analysis of complex mixtures–cigarette smoke. *Exp Toxicol Pathol* 57(Suppl 1), 43–73.

Bracken MB, Eskenazi B, Sachse K, McSharry JE, Hellenbrand K & Leo-Summers L. (1990) Association of cocaine use with sperm concentration, motility, and morphology. *Fertil Steril* 53, 315–322.

Bretveld R, Brouwers M, Ebisch I & Roeleveld N. (2007) Influence of pesticides on male fertility. *Scand J Work Environ Health* 33, 13–28.

Brito LFC, Sertich PL, Rives W, Knobbe M, Del Piero F & Stull GB. (2011) Effects of intratesticular zinc gluconate treatment on testicular dimensions, echodensity, histology, sperm production, and testosterone secretion in American black bears (*Ursus americanus*). *Theriogenology* 75, 1444–1452.

Camejo MI, Abdala L, Vivas-Acevedo G, Lozano-Hernández R, Angeli-Greaves M & Greaves ED. (2011) Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters. *Biol Trace Elem Res* 143, 1247–1254.

Cangemi R, Friedmann AJ, Holloszy JO & Fontana L. (2010) Long-term effects of calorie restriction on serum sex-hormone concentrations in men. *Aging Cell* 9, 236–242.

Carlsen E, Giwercman A, Keiding N & Skakkebaek NE. (1992) Evidence for decreasing quality of semen during past 50 years. *BMJ* 305, 609–613.

Çelik S, Aridogan IA, Izol V, Erdoğan S, Polat S & Doran S. (2012) An evaluation of the effects of long-term cell phone use on the testes via light and electron microscope analysis. *Urology* 79, 346–350.

Chia SE, Ong CN, Chua LH, Ho LM & Tay SK. (2000) Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. *J Androl* 21, 53–57.

Chiu YH, Afeiche MC, Gaskins AJ, Williams PL, Petrozza JC, Tanrikut C, Hauser R & Chavarro JE. (2015) Fruit and vegetable intake and their pesticide residues in relation to semen quality among men from a fertility clinic. *Hum Reprod* 30, 1342–1351.

Cicero TJ, Bell RD, Wiest WG, Allison JH, Polakoski K & Robins E. (1975) Function of the male sex organs in heroin and methadone users. *N Engl J Med* 292, 882–887.

Clifton DK & Bremner WJ. (1983) The effect of testicular x-irradiation on spermatogenesis in man. A comparison with the mouse. *J Androl* 4, 387–392.

Collin O, Kilter S & Bergh A. (1995) Tobacco smoke disrupts testicular microcirculation in the rat. *Int J Androl* 18, 141–145.

Cone EJ, Johnson RE, Moore JD & Roache JD. (1986) Acute effects of smoking marijuana on hormones, subjective effects and performance in male human subjects. *Pharmacol Biochem Behav* 24, 1749–1754.

Crownover BK & Covey CJ. (2013) Hereditary hemochromatosis. *Am Fam Physician* 87, 183–190.

Dai JB, Wang ZX & Qiao ZD. (2015) The hazardous effects of tobacco smoking in male fertility. *Asian J Androl* 17, 954–960.

Dallinga JW, Moonen EJC, Dumoulin JCM, Evers JLH, Geraedts JPM & Kleinjans JCS. (2002) Decreased human semen quality and organochlorine compounds in blood. *Hum Reprod* 17, 1973–1979.

Dama MS & Bhat MN (2013) Mobile phones affect multiple sperm quality traits: a meta-analysis. *F1000Res* 2, 40.

Danadevi K, Rozati R, Reddy PP & Grover P. (2003) Semen quality of Indian welders occupationally exposed to nickel and chromium. *Reprod Toxicol* 17, 451–456.

Dickerson SM, Walker DM, Reveron ME, Duvauchelle CL & Gore AC. (2008) The recreational drug ecstasy disrupts the hypothalamic-pituitary-gonadal reproductive axis in adult male rats. *Neuroendocrinology* 88, 95–102.

Donayre J, Guerra-García R, Moncloa F & Sobrevilla LA. (1968) Endocrine studies at high altitude. IV. Changes in the semen of men. *J Reprod Fertil* 16, 55–58.

Durairajanayagam D, Agarwal A & Ong C. (2015) Causes, effects and molecular mechanisms of testicular heat stress. *Reprod Biomed Online* 30, 14–27.

Eisenberg ML, Chen Z, Ye A & Buck Louis GM. (2015) Relationship between physical occupational exposures and health on semen quality: data from the Longitudinal Investigation of Fertility and the Environment (LIFE) study. *Fertil Steril* 103, 1271–1277.

Emanuele MA & Emanuele NV. (1998) Alcohol's effects on male reproduction. *Alcohol Health Res World* 22, 195–201.

Eroglu M, Sahin S, Durukan B, Ozakpinar OB, Erdinc N, Turkgeldi L, Sofuoglu K & Karateke A. (2014) Blood serum and seminal plasma selenium, total antioxidant capacity and coenzyme q10 levels in relation to semen parameters in men with idiopathic infertility. *Biol Trace Elem Res* 159, 46–51.

Evans NA. (2004) Current concepts in anabolic-androgenic steroids. *Am J Sports Med* 32, 534–542.

Fagundes AKF, Oliveira ECS, Tenorio BM, Melo CCS, Nery LTB, Santos FAB, Alves LC, Douglas RH & Silva VA. (2014) Injection of a chemical castration agent, zinc gluconate, into the testes of cats results in the impairment of spermatogenesis: a potentially irreversible contraceptive approach for this species? *Theriogenology* 81, 230–236.

Farías JG, Bustos-Obregón E & Reyes JG. (2005) Increase in testicular temperature and vascularization induced by hypobaric hypoxia in rats. *J Androl* 26, 693–697.

Fenster L, Waller K, Windham G, Henneman T, Anderson M, Mendola P, Overstreet JW & Swan SH. (2003) Trihalomethane levels in home tap water and semen quality. *Epidemiology* 14, 650–658.

Fong J-P, Lee F-J, Lu I-S, Uang S-N & Lee C-C. (2015) Relationship between urinary concentrations of di(2-ethylhexyl) phthalate (DEHP) metabolites and reproductive hormones in polyvinyl chloride production workers. *Occup Environ Med* 72, 346–353.

Funabashi H, Fujioka M, Kohchi M, Tateishi Y & Matsuoka N. (2000) Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats 22). Effects of 2- and 4-week administration of theobromine on the testis. *J Toxicol Sci* 25 Spec No, 211–221.

Gans JH. (1984) Comparative toxicities of dietary caffeine and theobromine in the rat. *Food Chem Toxicol* 22, 365–369.

Garolla A, Torino M, Sartini B, Cosci I, Patassini C, Carraro U & Foresta C. (2013) Seminal and molecular evidence that sauna exposure affects human spermatogenesis. *Hum Reprod* 28, 877–885.

Garolla A, Torino M, Miola P, Caretta N, Pizzol D, Menegazzo M, Bertoldo A & Foresta C. (2015) Twenty-four-hour monitoring of scrotal temperature in obese men and men with a varicocele as a mirror of spermatogenic function. *Hum Reprod* 30, 1006–1013.

Geoffroy-Siraudin C, Loundou AD, Romain F, Achard V, Courbière B, Perrard M-H, Durand P & Guichaoua M-R. (2012) Decline of semen quality among 10 932 males consulting for couple infertility over a 20-year period in Marseille, France. *Asian J Androl* 14, 584–590.

George VK, Li H, Teloken C, Grignon DJ, Lawrence WD & Dhabuwala CB. (1996) Effects of long-term cocaine exposure on spermatogenesis and fertility in peripubertal male rats. *J Urol* 155, 327–331.

Gonzales GF. (2007) Peruvian contributions to the study on human reproduction at high altitude: from the chronicles of the Spanish conquest to the present. *Respir Physiol Neurobiol* 158, 172–179.

Graves GR, Kennedy TG, Weick RF & Casper RF. (1993) The effect of nalmefene on pulsatile secretion of luteinizing hormone and prolactin in men. *Hum Reprod* 8, 1598–1603.

Griffin KP, Ward DT, Liu W, Stewart G, Morris ID & Smith CP. (2005) Differential expression of divalent metal transporter DMT1 (Slc11a2) in the spermatogenic epithelium of the developing and adult rat testis. *Am J Physiol Cell Physiol* 288, C176–C184.

Guven A, Kayikci A, Cam K, Arbak P, Balbay O & Cam M. (2008) Alterations in semen parameters of toll collectors working at motorways: does diesel exposure induce detrimental effects on semen? *Andrologia* 40, 346–351.

Haimov-Kochman R, Har-Nir R, Ein-Mor E, Ben-Shoshan V, Greenfield C, Eldar I, Bdolah Y & Hurwitz A. (2012) Is the quality of donated semen deteriorating? Findings from a 15 year longitudinal analysis of weekly sperm samples. *Isr Med Assoc J* 14, 372–377.

Håkonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, Bungum M, Ernst EH, Hansen ML, Ernst EH & Ramlau-Hansen CH. (2011) Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health* 8, 24.

Hallinan R, Byrne A, Agho K, McMahon CG, Tynan P & Attia J. (2009) Hypogonadism in men receiving methadone and buprenorphine maintenance treatment. *Int J Androl* 32, 131–139.

Hammoud A, Carrell DT, Gibson M, Sanderson M, Parker-Jones K & Peterson CM. (2010) Decreased sperm motility is associated with air pollution in Salt Lake City. *Fertil Steril* 93, 1875–1879.

Hansen C, Luben TJ, Sacks JD, Olshan A, Jeffay S, Strader L & Perreault SD. (2010) The effect of ambient air pollution on sperm quality. *Environ Health Perspect* 118, 203–209.

Hardin BD. (1983) Reproductive toxicity of the glycol ethers. *Toxicology* 27, 91–102.
Haugen TB, Tefre T, Malm G, Jönsson BAG, Rylander L, Hagmar L, Bjørsvik C, Henrichsen T, Sæther T, Figenschau Y & Giwercman A. (2011) Differences in serum levels of CB-153 and p, p'-DDE, and reproductive parameters between men living south and north in Norway. *Reprod Toxicol* 32, 261–267.
Hauser R, Chen Z, Pothier L, Ryan L & Altshul L. (2003) The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p, p'-DDE. *Environ Health Perspect* 111, 1505–1511.
Hauser R, Meeker JD, Duty S, Silva MJ & Calafat AM. (2006) Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. *Epidemiology* 17, 682–691.
Hawkes WC & Turek PJ. (2001) Effects of dietary selenium on sperm motility in healthy men. *J Androl* 22, 764–772.
Hawkes WC, Alkan Z & Wong K. (2009) Selenium supplementation does not affect testicular selenium status or semen quality in North American men. *J Androl* 30, 525–533.
Hentze MW, Muckenthaler MU, Galy B & Camaschella C. (2010) Two to tango: regulation of Mammalian iron metabolism. *Cell* 142, 24–38.
Hossain F, Ali O, D'Souza UJA & Naing DKS. (2010) Effects of pesticide use on semen quality among farmers in rural areas of Sabah, Malaysia. *J Occup Health* 52, 353–360.
Hovatta O, Venäläinen ER, Kuusimäki L, Heikkilä J, Hirvi T & Reima I. (1998) Aluminium, lead and cadmium concentrations in seminal plasma and spermatozoa, and semen quality in Finnish men. *Hum Reprod* 13, 115–119.
Huang L-P, Lee C-C, Hsu P-C & Shih T-S. (2011) The association between semen quality in workers and the concentration of di(2-ethylhexyl) phthalate in polyvinyl chloride pellet plant air. *Fertil Steril* 96, 90–94.
Irvine S, Cawood E, Richardson D, MacDonald E & Aitken J. (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 312, 467–471.
Iszatt N, Nieuwenhuijsen MJ, Bennett J, Best N, Povey AC, Pacey AA, Moore H, Cherry N & Toledano MB. (2013) Chlorination by-products in tap water and semen quality in England and Wales. *Occup Environ Med* 70, 754–760.
Ivell R. (2007) Lifestyle impact and the biology of the human scrotum. *Reprod Biol Endocrinol* 5, 15.
Jensen TK, Swan SH, Skakkebaek NE, Rasmussen S & Jørgensen N. (2010) Caffeine intake and semen quality in a population of 2,554 young Danish men. *Am J Epidemiol* 171, 883–891.
Jensen TK, Gottschau M, Madsen JOB, Andersson A-M, Lassen TH, Skakkebaek NE, Swan SH, Priskorn L, Juul A & Jørgensen N. (2014) Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *BMJ Open* 4, e005462.
Jobling S, Williams R, Johnson A, Taylor A, Gross-Sorokin M, Nolan M, Tyler CR, van Aerle R, Santos E & Brighty G. (2006) Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. *Environ Health Perspect* 114 (Suppl 1), 32–39.
Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen A-G, Andersson A-M, Haugen TB, Horte A, Jensen TK, Magnus Ø, Petersen JH, Vierula M, Toppari J & Skakkebaek NE. (2002) East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Hum Reprod* 17, 2199–2208.
Jurasović J, Cvitković P, Pizent A, Colak B & Telisman S. (2004) Semen quality and reproductive endocrine function with regard to blood cadmium in Croatian male subjects. *Biometals* 17, 735–743.
Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, Zghal K, Fki H, Damak J & Bahloul A. (2003) Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 49, 83–94.
Kim B, Park K & Rhee K. (2013a) Heat stress response of male germ cells. *Cell Mol Life Sci* 70, 2623–2636.
Kim MK, Lee JW, Baek KH, Song KH, Kwon HS, Oh KW, Jang EH, Kang MI & Lee KW. (2013b) Endocrinopathies in transfusion-associated iron overload. *Clin Endocrinol (Oxf)* 78, 271–277.
Klinefelter GR, Strader LF, Suarez JD, Roberts NL, Goldman JM & Murr AS. (2004) Continuous exposure to dibromoacetic acid delays pubertal development and compromises sperm quality in the rat. *Toxicol Sci* 81, 419–429.
Knuth UA, Maniera H & Nieschlag E. (1989) Anabolic steroids and semen parameters in bodybuilders. *Fertil Steril* 52, 1041–1047.
Kolodny RC, Masters WH, Kolodner RM & Toro G. (1974) Depression of plasma testosterone levels after chronic intensive marihuana use. *N Engl J Med* 290, 872–874.
Koskinen LO, Collin O & Bergh A. (2000) Cigarette smoke and hypoxia induce acute changes in the testicular and cerebral microcirculation. *Ups J Med Sci* 105, 215–226.
Kuller LH, May SJ & Perper JA. (1978) The relationship between alcohol, liver disease, and testicular pathology. *Am J Epidemiol* 108, 192–199.
Kumari D, Nair N & Bedwal RS. (2011) Testicular apoptosis after dietary zinc deficiency: ultrastructural and TUNEL studies. *Syst Biol Reprod Med* 57, 233–243.
Künzle R, Mueller MD, Hänggi W, Birkhäuser MH, Drescher H & Bersinger NA. (2003) Semen quality of male smokers and nonsmokers in infertile couples. *Fertil Steril* 79, 287–291.
La Vignera S, Condorelli RA, Vicari E, D'Agata R & Calogero AE. (2012) Effects of the exposure to mobile phones on male reproduction: a review of the literature. *J Androl* 33, 350–356.
Lalos A, Daniels K, Gottlieb C & Lalos O. (2003) Recruitment and motivation of semen providers in Sweden. *Hum Reprod* 18, 212–216.
Laskey JW, Rehnberg GL, Laws SC & Hein JF. (1984) Reproductive effects of low acute doses of cadmium chloride in adult male rats. *Toxicol Appl Pharmacol* 73, 250–255.
Leichtmann-Bardoogo Y, Cohen LA, Weiss A, Marohn B, Schubert S, Meinhardt A & Meyron-Holtz EG. (2012) Compartmentalization and regulation of iron metabolism proteins protect male germ cells from iron overload. *Am J Physiol Endocrinol Metab* 302, E1519–E1530.
Li H, Dunbar JC & Dhabuwala CB. (2003) Expression of cAMP-responsive element modulator (CREM) in rat testes following chronic cocaine administration. *J Environ Pathol Toxicol Oncol* 22, 111–116.
Li Y, Lin H, Ma M, Li L, Cai M, Zhou N, Han X, Bao H, Huang L, Zhu C, Li C, Yang H, Rao Z, Xiang Y, Cui Z, Ao L, Zhou Z, Xiong H & Cao J. (2009) Semen quality of 1346 healthy men, results from the Chongqing area of southwest China. *Hum Reprod* 24, 459–469.
Li D-K, Yan B, Li Z, Gao E, Miao M, Gong D, Weng X, Ferber JR & Yuan W. (2010) Exposure to magnetic fields and the risk of poor sperm quality. *Reprod Toxicol* 29, 86–92.
Li P, Zhong Y, Jiang X, Wang C, Zuo Z & Sha A. (2012) Seminal plasma metals concentration with respect to semen quality. *Biol Trace Elem Res* 148, 1–6.
Liao W, Cai M, Chen J, Huang J, Liu F, Jiang C & Gao Y. (2010) Hypobaric hypoxia causes deleterious effects on spermatogenesis in rats. *Reproduction* 139, 1031–1038.
Liem AK, Fürst P & Rappe C. (2000) Exposure of populations to dioxins and related compounds. *Food Addit Contam* 17, 241–259.
Lim HS, Hwang JY, Choi JC & Kim M. (2015) Assessment of caffeine intake in the Korean population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 32, 1786–1798.
Linder RE, Klinefelter GR, Strader LF, Suarez JD & Roberts NL. (1997) Spermatotoxicity of dichloroacetic acid. *Reprod Toxicol* 11, 681–688.
Liu PY, Swerdloff RS, Christenson PD, Handelsman DJ & Wang CHormonal Male Contraception Summit Group. (2006) Rate, extent, and modifiers of spermatogenic recovery after hormonal

male contraception: an integrated analysis. *Lancet* 367, 1412–1420.

Liu K, Li Y, Zhang G, Liu J, Cao J, Ao L & Zhang S. (2014) Association between mobile phone use and semen quality: a systemic review and meta-analysis. *Andrology* 2, 491–501.

Lourdes de Pereira M & Garcia e Costa F. (2003) Spermatogenesis recovery in the mouse after iron injury. *Hum Exp Toxicol* 22, 275–279.

Luben TJ, Olshan AF, Herring AH, Jeffay S, Strader L, Buus RM, Chan RL, Savitz DA, Singer PC, Weinberg HS & Perreault SD. (2007) The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. *Environ Health Perspect* 115, 1169–1176.

Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Paneth N & Wirth JJ. (2008) Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. *Environ Health Perspect* 116, 1473–1479.

Melgarejo M, Mendiola J, Koch HM, Moñino-García M, Noguera-Velasco JA & Torres-Cantero AM. (2015) Associations between urinary organophosphate pesticide metabolite levels and reproductive parameters in men from an infertility clinic. *Environ Res* 137, 292–298.

Mendelson JH, Kuehnle J, Ellingboe J & Babor TF. (1974) Plasma testosterone levels before, during and after chronic marihuana smoking. *N Engl J Med* 291, 1051–1055.

Moline JM, Golden AL, Bar-Chama N, Smith E, Rauch ME, Chapin RE, Perreault SD, Schrader SM, Suk WA & Landrigan PJ. (2000) Exposure to hazardous substances and male reproductive health: a research framework. *Environ Health Perspect* 108, 803–813.

Mula-Abed W-A, Hashmi Al H, Muslahi Al M, Muslahi Al H & Lamki Al M. (2008) Prevalence of endocrinopathies in patients with Beta-thalassaemia major – a cross-sectional study in Oman. *Oman Med J* 23, 257–262.

Muller A, La Rochebrochard De E, Labbé-Declèves C, Jouannet P, Bujan L, Mieusset R, Le Lannou D, Guerin JF, Benchaib M, Slama R & Spira A. (2004) Selection bias in semen studies due to self-selection of volunteers. *Hum Reprod* 19, 2838–2844.

Mumford SL, Kim S, Chen Z, Gore-Langton RE, Boyd Barr D & Buck Louis GM. (2014) Persistent organic pollutants and semen quality: the LIFE study. *Chemosphere* 135, 427–435.

Nordkap L, Joensen UN, Blomberg Jensen M & Jørgensen N. (2012) Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. *Mol Cell Endocrinol* 355, 221–230.

Nudmamud-Thanoi S & Thanoi S. (2011) Methamphetamine induces abnormal sperm morphology, low sperm concentration and apoptosis in the testis of male rats. *Andrologia* 43, 278–282.

Okumura A, Fuse H, Kawauchi Y, Mizuno I & Akashi T. (2003) Changes in male reproductive function after high altitude mountaineering. *High Alt Med Biol* 4, 349–353.

Oldereid NB, Thomassen Y & Purvis K. (1998) Selenium in human male reproductive organs. *Hum Reprod* 13, 2172–2176.

Oliveira ECS, Moura MR, Silva VA, Peixoto CA, Saraiva KLA, de Sá MJC, Douglas RH & de Pinho Marques A. (2007) Intratesticular injection of a zinc-based solution as a contraceptive for dogs. *Theriogenology* 68, 137–145.

Omu AE, Al-Azemi MK, Al-Maghrebi M, Mathew CT, Omu FE, Kehinde EO, Anim JT, Oriowo MA & Memon A. (2015) Molecular basis for the effects of zinc deficiency on spermatogenesis: an experimental study in the Sprague-dawley rat model. *Indian J Urol* 31, 57–64.

Omu AE, Dashti H & Al-Othman S. (1998) Treatment of asthenozoospermia with zinc sulphate: andrological, immunological and obstetric outcome. *Eur J Obstet Gynecol Reprod Biol* 79, 179–184.

Pant N, Upadhyay G, Pandey S, Mathur N, Saxena DK & Srivastava SP. (2003) Lead and cadmium concentration in the seminal plasma of men in the general population: correlation with sperm quality. *Reprod Toxicol* 17, 447–450.

Pant N, Kumar G, Upadhyay AD, Gupta YK & Chaturvedi PK. (2014a) Correlation between lead and cadmium concentration and semen quality. *Andrologia* 44, 370–375.

Pant N, Kumar G, Upadhyay AD, Patel DK, Gupta YK & Chaturvedi PK. (2014b) Reproductive toxicity of lead, cadmium, and phthalate exposure in men. *Environ Sci Pollut Res Int* 21, 11066–11074.

Parrish RF, Kessler R, Shapiro CE & Fair WR. (1987) Vasectomy and vasovasostomy have no effect on seminal plasma zinc concentrations. *J Urol* 137, 228–229.

Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB & Lucon AM. (2006) Cigarette smoking is related to a decrease in semen volume in a population of fertile men. *BJU Int* 97, 324–326.

Pires A, de Melo EN, Mauad T, Nascimento Saldiva PH & de Siqueira Bueno HM. (2011) Pre- and postnatal exposure to ambient levels of urban particulate matter (PM(2.5)) affects mice spermatogenesis. *Inhal Toxicol* 23, 237–245.

Pires A, Pieri P, Hage M, Santos ABG, Medeiros MCR, Garcia RCT, Yonamine M, Hallak J, Saldiva PHN, Zorzetto JC & Bueno HMS. (2012) Repeated inhalation of crack-cocaine affects spermatogenesis in young and adult mice. *Inhal Toxicol* 24, 439–446.

Ponnapakkam TP, Bailey KS, Graves KA & Iszard MB. (2003) Assessment of male reproductive system in the CD-1 mice following oral manganese exposure. *Reprod Toxicol* 17, 547–551.

Premi S, Srivastava J, Chandy SP & Ali S. (2009) Unique signatures of natural background radiation on human Y chromosomes from Kerala, India. *PLoS One* 4, e4541.

Raigani M, Yaghmaei B, Amirjannti N, Lakpour N, Akhondi MM, Zeraati H, Hajihosseinal M & Sadeghi MR. (2014) The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. *Andrologia* 46, 956–962.

Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G & Bonde JP. (2007) Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Hum Reprod* 22, 188–196.

Rao M, Zhao X-L, Yang J, Hu S-F, Lei H, Xia W & Zhu C-H. (2015) Effect of transient scrotal hyperthermia on sperm parameters, seminal plasma biochemical markers, and oxidative stress in men. *Asian J Androl* 17, 668–675.

Ratcliffe JM, Schrader SM, Steenland K, Clapp DE, Turner T & Hornung RW. (1987) Semen quality in papaya workers with long term exposure to ethylene dibromide. *Br J Ind Med* 44, 317–326.

Ratcliffe JM, Schrader SM, Clapp DE, Halperin WE, Turner TW & Hornung RW. (1989) Semen quality in workers exposed to 2-ethoxyethanol. *Br J Ind Med* 46, 399–406.

Rehm S, White TE, Zahalka EA, Stanislaus DJ, Boyce RW & Wier PJ. (2008) Effects of food restriction on testis and accessory sex glands in maturing rats. *Toxicol Pathol* 36, 687–694.

Reis LO, Zani EL, Saad RD, Chaim EA, de Oliveira LC & Fregonesi A. (2012) Bariatric surgery does not interfere with sperm quality–a preliminary long-term study. *Reprod Sci* 19, 1057–1062.

Reyes JG, Farias JG, Henríquez-Olavarrieta S, Madrid E, Parraga M, Zepeda AB & Moreno RD. (2012) The hypoxic testicle: physiology and pathophysiology. *Oxid Med Cell Longev*. doi:10.1155/2012/929285. Epub 2012 Sep 27.

Richardson SD. (2007) Water analysis: emerging contaminants and current issues. *Anal Chem* 79, 4295–4323.

Richardson SD, Plewa MJ, Wagner ED, Schoeny R & Demarini DM. (2007) Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res* 636, 178–242.

Richthoff J, Elzanaty S, Rylander L, Hagmar L & Giwercman A. (2008) Association between tobacco exposure and reproductive parameters in adolescent males. *Int J Androl* 31, 31–39.

Robbins WA, Xun L, Jia J, Kennedy N, Elashoff DA & Ping L. (2010) Chronic boron exposure and human semen parameters. *Reprod Toxicol* 29, 184–190.

Rossato M, Ion Popa F, Ferigo M, Clari G & Foresta C. (2005) Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. *J Clin Endocrinol Metab* 90, 984–991.

Roth MY, Ilani N, Wang C, Page ST, Bremner WJ, Swerdloff RS, Dart C, Sitruk-Ware R, Kumar N, Blithe D & Amory JK. (2013) Characteristics associated with suppression of spermatogenesis in a male hormonal contraceptive trial using testosterone and Nestorone(®) gels. *Andrology* 1, 899–905.

Rubes J, Selevan SG, Evenson DP, Zudova D, Vozdova M, Zudova Z, Robbins WA & Perreault SD. (2005) Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. *Hum Reprod* 20, 2776–2783.

Safarinejad MR. (2008) Evaluation of semen quality, endocrine profile and hypothalamus-pituitary-testis axis in male patients with homozygous beta-thalassemia major. *J Urol* 179, 2327–2332.

Safarinejad MR. (2009) Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. *J Urol* 182, 237–248.

Safarinejad MR. (2011a) Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. *Andrologia* 43, 38–47.

Safarinejad MR. (2011b) Effect of pentoxifylline on semen parameters, reproductive hormones, and seminal plasma antioxidant capacity in men with idiopathic infertility: a randomized double-blind placebo-controlled study. *Int Urol Nephrol* 43, 315–328.

Safarinejad MR & Safarinejad S. (2009) Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. *J Urol* 181, 741–751.

Safarinejad MR, Asgari SA, Farshi A, Ghaedi G, Kolahi AA, Iravani S & Khoshdel AR. (2013) The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity. *Reprod Toxicol* 36, 18–23.

Said TM, Ranga G & Agarwal A. (2005) Relationship between semen quality and tobacco chewing in men undergoing infertility evaluation. *Fertil Steril* 84, 649–653.

Sakhaee E, Emadi L, Abshenas J, Kheirandish R, Azari O & Amiri E. (2012) Evaluation of epididymal sperm quality following experimentally induced copper poisoning in male rats. *Andrologia* 44(Suppl 1), 110–116.

Samplaski MK & Nangia AK. (2015) Adverse effects of common medications on male fertility. *Nat Rev Urol* 12, 401–413.

Schuel H, Burkman LJ, Lippes J, Crickard K, Mahony MC, Giuffrida A, Picone RP & Makriyannis A. (2002) Evidence that anandamide-signaling regulates human sperm functions required for fertilization. *Mol Reprod Dev* 63, 376–387.

Schürmeyer T, Knuth UA, Belkien L & Nieschlag E. (1984) Reversible azoospermia induced by the anabolic steroid 19-nortestosterone. *Lancet* 1, 417–420.

Scialli AR, Bonde JP, Brüske-Hohlfeld I, Culver BD, Li Y & Sullivan FM. (2010) An overview of male reproductive studies of boron with an emphasis on studies of highly exposed Chinese workers. *Reprod Toxicol* 29, 10–24.

Scott M & Yates HD. (1998) The effect of oral selenium supplementation on human sperm motility. *BJU Int* 82, 76–80.

Selevan SG, Borkovec L, Slott VL, Zudová Z, Rubes J, Evenson DP & Perreault SD. (2000) Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. *Environ Health Perspect* 108, 887–894.

Sermondade N, Elloumi H, Berthaut I, Mathieu E, Delarouzière V, Ravel C & Mandelbaum J. (2010) Progressive alcohol-induced sperm alterations leading to spermatogenic arrest, which was reversed after alcohol withdrawal. *Reprod Biomed Online* 20, 324–327.

Sheiner EK, Sheiner E, Carel R, Potashnik G & Shoham-Vardi I. (2002) Potential association between male infertility and occupational psychological stress. *J Occup Environ Med* 44, 1093–1099.

Sheynkin Y, Jung M, Yoo P, Schulsinger D & Komaroff E. (2005) Increase in scrotal temperature in laptop computer users. *Hum Reprod* 20, 452–455.

Singh AK, Tiwari AK, Singh PB, Dwivedi US, Trivedi S, Singh SK, Agrawal NK & Deshpande SB. (2010) Multivitamin and micronutrient treatment improves semen parameters of azoospermic patients with maturation arrest. *Indian J Physiol Pharmacol* 54, 157–163.

Sitzmann BD, Leone EH, Mattison JA, Ingram DK, Roth GS, Urbanski HF, Zelinski MB & Ottinger MA. (2010) Effects of moderate calorie restriction on testosterone production and semen characteristics in young rhesus macaques (*Macaca mulatta*). *Biol Reprod* 83, 635–640.

Slutsky M, Levin JL & Levy BS. (1999) Azoospermia and oligospermia among a large cohort of DBCP applicators in 12 countries. *Int J Occup Environ Health* 5, 116–122.

Sobreiro BP, Lucon AM, Pasqualotto FF, Hallak J, Athayde KS & Arap S. (2005) Semen analysis in fertile patients undergoing vasectomy: reference values and variations according to age, length of sexual abstinence, seasonality, smoking habits and caffeine intake. *Sao Paulo Med J* 123, 161–166.

Soliman A, Yassin M & De Sanctis V. (2014) Intravenous iron replacement therapy in eugonadal males with iron-deficiency anemia: effects on pituitary gonadal axis and sperm parameters; A pilot study. *Indian J Endocrinol Metab* 18, 310–316.

de Souza GL & Hallak J. (2011) Anabolic steroids and male infertility: a comprehensive review. *BJU Int* 108, 1860–1865.

Specht IO, Toft G, Hougaard KS, Lindh CH, Lenters V, Jönsson BAG, Heederik D, Giwercman A & Bonde JPE. (2014) Associations between serum phthalates and biomarkers of reproductive function in 589 adult men. *Environ Int* 66, 146–156.

Stackelberg PE, Furlong ET, Meyer MT, Zaugg SD, Henderson AK & Reissman DB. (2004) Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Sci Total Environ* 329, 99–113.

Substance Abuse and Mental Health Services Administration (2014) *Results from the 2013 National Survey on Drug Use and Health: Mental Health Detailed Tables*. Substance Abuse and Mental Health Services Administration, Rockville, MD.

Substance Abuse and Mental Health Services Administration (2015) *Behavioral Health Barometer: United States, 2014*. Substance Abuse and Mental Health Services Administration, Rockville, MD.

Sunanda P, Panda B, Dash C, Ray PK, Padhy RN & Routray P. (2014) Prevalence of abnormal spermatozoa in tobacco chewing sub-fertile males. *J Hum Reprod Sci* 7, 136–142.

Telisman S, Cvitković P, Jurasović J, Pizent A, Gavella M & Rocić B. (2000) Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. *Environ Health Perspect* 108, 45–53.

Telisman S, Colak B, Pizent A, Jurasović J & Cvitković P. (2007) Reproductive toxicity of low-level lead exposure in men. *Environ Res* 105, 256–266.

Turner TT, Tung KS, Tomomasa H & Wilson LW. (1997) Acute testicular ischemia results in germ cell-specific apoptosis in the rat. *Biol Reprod* 57, 1267–1274.

Tvrda E, Peer R, Sikka SC & Agarwal A. (2015) Iron and copper in male reproduction: a double-edged sword. *J Assist Reprod Genet* 32, 3–16.

Uauy R, Olivares M & Gonzalez M. (1998) Essentiality of copper in humans. *Am J Clin Nutr* 67, 952S–959S.

Valko M, Jomova K, Rhodes CJ, Kuča K & Musílek K. (2015) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol* 90, 1–37.

Van Niekerk FE & Van Niekerk CH. (1989) The influence of experimentally induced copper deficiency on the fertility of rams. I. Semen parameters and peripheral plasma androgen concentration. *J S Afr Vet Assoc* 60, 28–31.

Verratti V, Berardinelli F, Di Giulio C, Bosco G, Cacchio M, Pellicciotta M, Nicolai M, Martinotti S & Tenaglia R. (2008) Evidence that chronic hypoxia causes reversible impairment on male fertility. *Asian J Androl* 10, 602–606.

Vescovi PP, Pedrazzoni M, Michelini M, Maninetti L, Bernardelli F & Passeri M. (1992) Chronic effects of marihuana smoking on luteinizing hormone, follicle-stimulating hormone and prolactin levels in human males. *Drug Alcohol Depend* 30, 59–63.

Veulemans H, Steeno O, Masschelein R & Groeseneken D. (1993) Exposure to ethylene glycol ethers and spermatogenic disorders in man: a case-control study. *Br J Ind Med* 50, 71–78.

Villalta J, Ballescà JL, Nicolás JM, Martínez de Osaba MJ, Antúnez E & Pimentel C. (1997) Testicular function in asymptomatic chronic alcoholics: relation to ethanol intake. *Alcohol Clin Exp Res* 21, 128–133.

Visser AJ & Heyns CF. (2003) Testicular function after torsion of the spermatic cord. *BJU Int* 92, 200–203.

Wang X, Wang M, Dong W, Li Y, Zheng X, Piao F & Li S. (2013) Subchronic exposure to lead acetate inhibits spermatogenesis and downregulates the expression of Ddx3y in testis of mice. *Reprod Toxicol* 42, 242–250.

Welch LS, Schrader SM, Turner TW & Cullen MR. (1988) Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. *Am J Ind Med* 14, 509–526.

Whan LB, West MCL, McClure N & Lewis SEM. (2006) Effects of delta-9-tetrahydrocannabinol, the primary psychoactive cannabinoid in marijuana, on human sperm function in vitro. *Fertil Steril* 85, 653–660.

Whorton D, Krauss RM, Marshall S & Milby TH. (1977) Infertility in male pesticide workers. *Lancet* 2, 1259–1261.

Wirth JJ, Rossano MG, Daly DC, Paneth N, Puscheck E, Potter RC & Diamond MP. (2007) Ambient manganese exposure is negatively associated with human sperm motility and concentration. *Epidemiology* 18, 270–273.

Wittassek M, Wiesmüller GA, Koch HM, Eckard R, Dobler L, Müller J, Angerer J & Schlüter C. (2007) Internal phthalate exposure over the last two decades–a retrospective human biomonitoring study. *Int J Hyg Environ Health* 210, 319–333.

Xu D-X, Shen H-M, Zhu Q-X, Chua L, Wang Q-N, Chia S-E & Ong C-N. (2003) The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. *Mutat Res* 534, 155–163.

Yazigi RA, Odem RR & Polakoski KL. (1991) Demonstration of specific binding of cocaine to human spermatozoa. *JAMA* 266, 1956–1959.

Yokoi K, Uthus EO & Nielsen FH. (2003) Nickel deficiency diminishes sperm quantity and movement in rats. *Biol Trace Elem Res* 93, 141–154.

Yuyan L, Junqing W, Wei Y, Weijin Z & Ersheng G. (2008) Are serum zinc and copper levels related to semen quality? *Fertil Steril* 89, 1008–1011.

Zeng X, Jin T, Zhou Y & Kong Q. (2002) Alterations of serum hormone levels in male workers occupationally exposed to Cadmium. *J Toxicol Environ Health A* 65, 513–521.

Zeng Q, Li M, Xie S-H, Gu L-J, Yue J, Cao W-C, Zheng D, Liu A-L, Li Y-F & Lu W-Q. (2013) Baseline blood trihalomethanes, semen parameters and serum total testosterone: a cross-sectional study in China. *Environ Int* 54, 134–140.

Zeng Q, Wang Y-X, Xie S-H, Xu L, Chen Y-Z, Li M, Yue J, Li Y-F, Liu A-L & Lu W-Q. (2014) Drinking-water disinfection by-products and semen quality: a cross-sectional study in China. *Environ Health Perspect* 122, 741–746.

Zhu YZ, Sun H, Fu Y, Wang J, Song M, Li M, Li YF & Miao LG. (2014) Effects of sub-chronic aluminum chloride on spermatogenesis and testicular enzymatic activity in male rats. *Life Sci* 102, 36–40.

