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REVIEW ARTICLE

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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)].

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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

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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

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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

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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

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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,

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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

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(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, de-icers, 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

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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.

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