

## INTRODUCTION

In recent times male infertility and deteriorating semen quality has been an increasingly prevalent issue; researchers point towards changing environmental and lifestyle conditions as arguably the most significant cause of this phenomenon. Environmental and lifestyle exposure to a wide variety of factors may stress the male reproductive system throughout a man's lifespan, from gestation to advanced adult age (**Fig. 5.1**). Ultimately, male infertility may be the result of exposure to any combination of factors such as chemical toxins, smoking, alcohol, diet, exercise, obesity, different types of stress, and the increasing prevalence of cell phone and ionizing radiation.

While spermatogenesis is a function of only mature testes, the effects of maternal or paternal exposure to adverse environmental factors can be projected to the future offspring, resulting in poor semen quality in the male offspring years later. Parental exposure in conjunction with the exposure of the adult offspring can amplify the effects. While adverse environmental effects during adulthood are believed to be reversible, damage done before and up to puberty is considered by many to be irreversible.<sup>1,2</sup>

Despite the lack of conclusive studies tracking effects of the environment and lifestyle of an individual throughout life, there is reason enough to believe that the environment and lifestyle plays a significant role in the quality of male gamete production and thus male fertility as a whole. This argument is evidenced by the fact that over the last 50 years mean sperm counts in the general population have decreased by 50 percent while dramatic environmental and lifestyle changes have occurred during this same period. The expansion of the chemical industry in every facet of modern life in both developed and developing countries is one such major change.<sup>3-5</sup> While there may be arguments that state otherwise, the implications of these issues are significant and warrant increased awareness and the implementation of precautions that may help reverse diminishing male fertility.

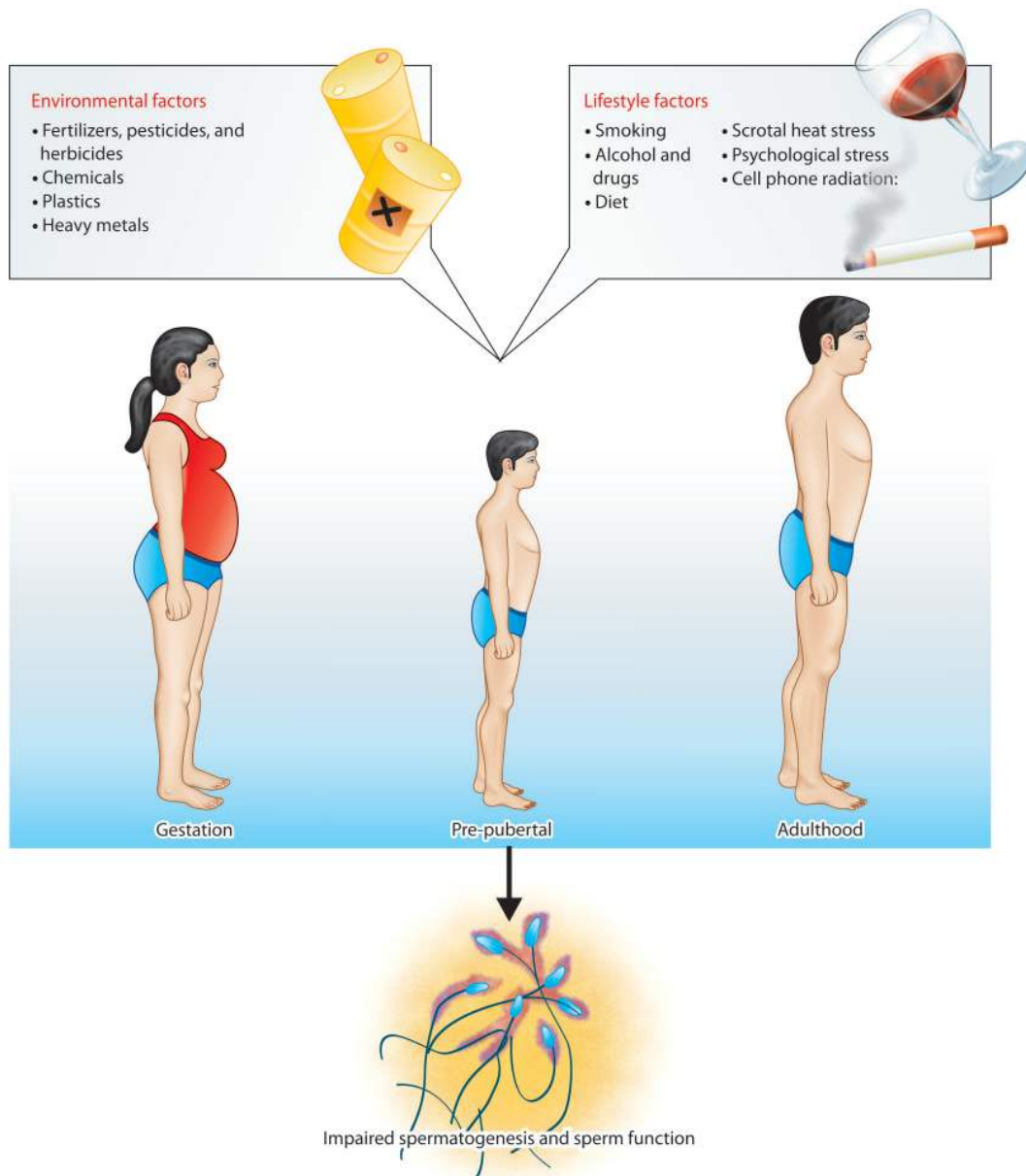
## MATERNAL AND INFANT EXPOSURE

Not only male infertility can be caused during adulthood, but also it can be a result of maternal and pre-pubertal toxic exposure (**Fig. 5.1**). Sperm production begins during puberty and

continues until death. Due to the complexity of germ cell development, proper sperm manufacturing relies on optimal conditions. During this critical period errors in spermatogenesis are more likely than usual when influenced by environmental factors. Spermatogenesis, only occurring in mature testis, can be disrupted either directly in germ cells throughout adulthood, or indirectly via environmental insults; indirect injuries include maternal exposure during pregnancy that affect events preceding gamete production in the offspring. Early damage may impair testicular development in the male fetus as well as during infancy and these impacts may manifest themselves in adulthood.

In all phases until puberty, any environment that affects Sertoli cell proliferation may lead to impaired spermatogenesis and a diminished final number of cells, thus ultimately impacting sperm counts. Disorders such as cryptorchidism, hypospadias, and testicular germ cell cancer have a fetal precursor triggered by testicular malformation, which is a result of insults during developmental stages.<sup>6</sup> While it is difficult to study the final outcomes of maternal exposure in humans because of the time span between the discovery of male infertility and maternal exposure, animal models can be helpful. Results from these animal models supported the theory that environmental injuries that occur during development can determine spermatogenesis and fertility in adulthood.<sup>1</sup> A study conducted by Mocarelli et al. concluded that toxin exposure until puberty affected semen quality while adult exposure revealed no effect, thus making early exposure an especially noteworthy issue.<sup>2</sup>

Since hormones regulate fetal development, outside influences on hormone regulation can have dramatic effects. Maternal lifestyle that involves exposure to environmental chemicals with endocrine-disrupting properties, especially anti-androgenic activity, can influence testicular development and spermatogenesis in the adult offspring. Maternal smoking and obesity are two more factors that reduce sperm counts in developing male offspring. Significant sperm count reductions were reported in male offspring whose mothers smoked substantially during pregnancy.<sup>7,8</sup> It is believed that polycyclic aromatic hydrocarbons (PAHs) and other components of cigarette smoke activate the aryl hydrocarbon receptor and antagonize the androgen receptor mediated action. Thus, smoking during pregnancy reduces Sertoli cell number.<sup>9,10</sup> Meanwhile, maternal obesity during pregnancy can theoretically encroach on testicular development via



**Figure 5.1:** Male infertility can be attributed to maternal insults or exposure as well as adult exposure

increased aromatization, thereby disrupting the testosterone/estrogen ratio of the developing fetus. Furthermore, harmful herbicides and pesticides are lipophilic and therefore can accumulate in the fat of obese expecting mothers. During pregnancy and lactation these accumulated compounds can be delivered to the fetus and neonate.<sup>1,11</sup> Maternal diet is yet another area that the environment influences and thus affects the developing fetus. Anabolic steroids can be present in meat consumed by expectant mothers and these steroids have been linked to the reduction of spermatogenesis in the mature testis of the future son.<sup>11</sup>

After gestation damage may still occur. Toxins may still be passed on to an infant via breastfeeding. Also during infancy,

certain types of diapers may cause testicular heat stress. It has been found that disposable plastic lined diapers cause higher scrotal temperatures than reusable cotton diapers. This heat stress may be partially responsible for declining sperm counts.<sup>12,13</sup>

## ADULT EXPOSURE

### Environmental Effects on Mature Men

The mature human male, unlike females, is capable of reproducing throughout life. In the average fertile male millions of spermatozoa are produced daily until death. Injuries incurred to

the reproductive system early on may manifest themselves in the semen produced later. Unfortunately, evaluation of the results of environmental insults on spermatogenesis in human sperm studies is hampered by inconsistencies in biological analytical methods, in controlling factors and study design. Because of the shorter developmental period as well as being less varied in their spermatogenesis profile than humans, animals allow researchers to more clearly observe how external factors influence the reproductive process. It would be exceedingly difficult to isolate one specific environmental factor in the life of an individual who is constantly exposed to a mixture of chemicals and factors, therefore animal testing allows for controlled experiments.

Western lifestyle and worldwide environmental conditions have dramatically changed especially with respect to diet and exercise; dramatic change suggests that these factors may be involved in the etiology of declining male fertility and the impairment of sperm production. It is possible that such change caused this decline in fertility due to its rapid and widespread nature. Several of the following occupational and lifestyle factors to be discussed below are regarded as major areas of concern.

### Fertilizers, Pesticides, and Herbicides

With over six and a half billion people populating the world today, food production has been engineered to a large degree. While fertilizers and pesticides have revolutionized food production in recent times, both have also introduced new chemicals and possible toxins to millions. Chemical fertilizers such as nitrogen and ammonia are being extensively used in agriculture today. Nitric oxide has been found to reduce sperm motility, viability, and other semen parameters; it also has been found in some cases to impair the ability of spermatozoa to penetrate the oocyte.<sup>14</sup> Jurewicz suggested that there are consistent indications that pesticides like dichlorodiphenyltrichloroethane, better known as DDT, affect sperm counts in humans.<sup>15</sup> Also, herbicides such as lindane, methoxychlor, and dioxin-TCDD have all been linked with testicular oxidative stress and decreased sperm counts.<sup>16,17</sup> Food preservatives are yet another method for toxins to enter the bloodstream and cause fertility issues. Carbendazim is a systemic broad-spectrum fungicide commonly used on fruit and leather.<sup>18</sup> It has been found to have detrimental effects on male reproduction including decreased mean testicular weight and reduced seminiferous tubule diameters.<sup>19</sup> The vast prevalence of such pesticides, herbicides, and fertilizers utilized by the food industry today is a major fertility concern, one that will be difficult to overcome due to the necessity of large scale production.

### Chemicals, Toxins, and Endocrine Active Compounds

Environmental chemicals and toxins have the potential to negatively affect fertility. Some of these chemicals have estrogenic properties and thus are considered toxic because they affect the normal functioning state of the endocrine system. Such compounds can affect LH stimulated Leydig cells which influence androgen secretion and thus interfere with the proper

endocrine regulation of spermatogenesis. The ideal ratio of testosterone and estrogen can be shifted as a result of such endocrine disrupters; this can lead to errors in feedback and regulation of the hypothalamus-pituitary-gonadal axis. In addition the pro-oxidant and anti-oxidant system of cells can also be thrown out of harmony. Such a disturbance could lead to the generation of free radicals and Reactive Oxygen Species (ROS). These free radicals could destabilize the electrolytic balance within cells. Spermatozoa are especially susceptible to ROS and lipid peroxidation due to the large amount of polyunsaturated fatty acids found in their membranes. Therefore, chemical toxins that generate ROS in spermatozoa are quite significant.

### Chemicals in Plastics

*Plastic:* The material of modern times. The increasing amount of plastic in contemporary products is a concern because of the toxicity of the chemicals infused to give the products certain desirable qualities. Plasticizers are polyphenolic chemical additives used to enhance the flexibility and toughness of plastic and are found in all clear, heat-resistant and unbreakable plastics. These compounds have been reported to be toxic to the male reproductive system. Another similarly common chemical is Bisphenol A (BPA); it is used to improve polycarbonate plastics and is found in disposable plastic ware, especially in the lids of food containers. BPA from such containers can migrate into food and become circulated in the body.<sup>20,21</sup> It has been estimated that approximately 90 percent of Americans have BPA present in their blood. Since this is such a prevalent chemical that is known to reduce sperm count, motility, and viability, it is a significant environmental threat to male fertility. Chitra reported that BPA generates ROS in various rat tissues including the reproductive organs.<sup>22</sup> BPA was shown to increase hydrogen peroxide levels in testicular tissue. This subsequently leads to the depletion of the antioxidant defense system. Kabuto found that BPA caused an overproduction of hydrogen peroxide in the kidneys, liver, and testes of rats.<sup>23</sup>

Some common plastic products including plastic bags, inflatable recreational toys, blood storage bags, plastic clothing, soaps, and shampoos have phthalate esters in them to improve the flexibility of the plastic. Animal studies concluded that a prevalent phthalate ester commonly used named Di (2-ethylhexyl) phthalate caused testicular atrophy in animals, but the effects on humans is still in question.<sup>24-26</sup>

Yet another commonly found chemical that has been under scrutiny is Nonylphenol. Nonylphenol is a synthetic plastic additive that has estrogenic properties and can accumulate in tissues due to its lipophilic nature. It is often found in detergents, paints, personal care products, food processing, and the packaging industry. Adult exposure to this chemical may reduce sperm counts.

The vast prevalence of such chemicals in numerous facets of daily life is a key concern. Further studies are required to definitively determine the effects of such chemicals, but currently it is believed that these common chemicals and others are harmful to the male reproductive system.

### Heavy Metal Toxicity

Several studies reported that heavy metal toxicity in men impaired spermatogenesis and decreased sperm counts.<sup>27-30</sup> Metals such as lead, cadmium, and mercury are three metals of concern while the effects of aluminum and vanadium are being investigated for possible adverse effects on male fertility. Inorganic lead can disturb the pro-oxidant and anti-oxidant balance and cause oxidative stress.<sup>28</sup> Before being banned, lead was found in a variety of common products, the most well known being lead paint; mercury was also found to accumulate in fish, which provides means of over exposure. Like lead, cadmium has been strongly linked to infertility; much higher cadmium levels were found in both the seminal plasma and the blood of infertile men when compared to those of fertile men. A strong negative correlation exists between cadmium and sperm concentrations due to its antisteroidogenic effects that lower testosterone production.<sup>31</sup>

Metal workers and other men who are exposed to such metals through their occupation may be rendered less fertile due to the toxic effects of these metals.

### LIFESTYLE EXPOSURE

Exposure to certain lifestyle and occupational factors can influence the adult testis directly and lead to impaired spermatogenesis. A few of the most common issues will be subsequently discussed; often these issues can be avoided by implementing the right precautions.

### SMOKING

It is well established that smoking has detrimental effects on spermatogenesis as it has been correlated with significantly lower sperm counts, decreased motility, and impaired morphology.<sup>32-34</sup> Smoking not only interferes with oxygen supply, but also exposes smokers to thousands of potentially harmful substances such as alkaloids, nitrosamines, nicotine, and hydroxycotinine to name a few. These substances can lead to the formation of ROS and reactive nitrogen species, which leads to oxidative stress and ultimately infertility.<sup>33-35</sup> Saleh et al. demonstrated that cigarette smoking causes an increase in ROS levels and a decrease in ROS-TAC scores in semen. A 100-fold increase in oxidative stress was observed in the semen of smokers. Cadmium levels were also five times the normal level.<sup>33,35</sup> Furthermore, smokers have decreased levels of seminal plasma antioxidants such as Vitamin C and Vitamin E.<sup>36,37</sup> Besides the numerous other health issues caused by smoking, it has been clearly identified that smoking significantly reduces fertility in men due to the toxins in cigarettes.

### ALCOHOL AND DRUGS

There is a growing body of evidence suggesting that alcohol is a lifestyle factor that impacts spermatogenesis. Moderate alcohol consumption has not shown any significant impact on sperm count, however, chronic alcohol consumption appears to

harm spermatogenesis and male fertility. Impotence, testicular atrophy, and loss of sexual interest are associated with alcoholism, and reduced FSH, LH, and testosterone levels have been found as a result of excessive drinking.<sup>38</sup> It was also reported that decreased numbers of morphologically normal sperm as well as semen volume were present in alcoholics as apposed to individuals who drank moderately.<sup>39</sup> Alcohol was found to induce oxidative stress; ROS molecules are generated in response to the metabolism of ethanol by the microsomal ethanol-oxidizing system (MEOS).<sup>40,41</sup> Alcohol metabolism results in NADH formation, which enhances activity in the respiratory chain including heightened oxygen use and ROS formation.<sup>42</sup> Tissues are also at increased risk of damage due to the fact that alcohol induces hypoxia.<sup>43</sup>

Like alcohol, certain drugs whether therapeutic, recreational, or performance enhancing can have adverse effects on spermatogenesis. Several prescription drugs used for therapeutic purposes, especially when used chronically, can impact the development of sperm. Antibiotics and chemotherapy can damage germinal epithelium.<sup>44</sup> Many antibacterial drugs (e.g. tetracycline derivatives, sulfa drugs) impair spermatogenesis and chronic use can lead to infertility.<sup>45,46</sup> One especially interesting study showed that men who switched or stopped treatment of the most common medications (allergy relief, antiepileptic, antibiotics) had a 93 percent improvement in semen quality.<sup>46</sup> The class of therapeutic agent used, as well as the dose and duration of the therapy were obviously pertinent factors, but all concluded that these common drugs were contributing in some fashion to infertility whether it was short term reversible infertility or longer lasting.

Concrete evidence for the effect and mechanism of recreational drug abuse such as marijuana and cocaine on sperm production has yet to be found, however, some believe there are links to such drugs and infertility; some studies show endocrine disruption from excessive recreational drug use.<sup>48</sup>

The use of anabolic steroids, predominantly used to enhance body image or improve performance, is on the increase.<sup>47</sup> Steroids often lead to oligozoospermia because they suppress LH secretion and consequently suppress intratesticular testosterone levels. Hypogonadotropic hypogonadism is therefore the most common cause for impairment of sperm production in this group. This damage can be reversed in mature men after a few months of discontinued use.<sup>49</sup> Once again the widespread use of alcohol and such drugs prompt the need for awareness.

### DIET AND OBESITY

Diet and obesity are two important lifestyle factors that can influence spermatogenesis. Accompanying modern Westernized lifestyles are changes in diets and eating habits that are a result of a fast paced lifestyle. People are eating more highly refined carbohydrate rich foods and simultaneously consuming less fresh fruits and vegetables. The importance of fresh vegetables in a well balanced diet was noted in a study that decreased subjects' intake of certain nutritional substances, like fruits and vegetables; a correlation between this lack of nutrients and sub fertility



was found.<sup>50-53</sup> Besides containing antioxidants and essential nutrients, vitamins and folate are found in fruit and vegetables; these substances are involved in DNA and RNA synthesis and thus play an important role in spermatogenesis by protecting the sperm's DNA from free radical damage.<sup>52</sup>

Nutritionally deficient diets, lacking antioxidant vitamins and synergistic minerals do not enable the quenching of reactive oxygen molecules. For example, vitamin C and vitamin E are essential antioxidants that protect the body's cells from damage due to oxidative stress and free radicals. Vitamin C is the most abundant antioxidant in the semen of fertile men and contributes to the maintenance of healthy sperm by protecting the sperm's DNA from free radical damage.<sup>50-53</sup> Vitamin E is a fat-soluble vitamin that helps protect the sperm's cell membrane from damage. Studies show that vitamin E improves sperm motility and morphology while vitamin C regenerates vitamin E; thereby these vitamins work together to improve sperm function.<sup>50-53</sup> Selenium is a mineral that also functions as an antioxidant; Selenium supplements have also been shown to increase motility. Combinations of these three nutrients have been shown to improve sperm parameters in infertile men.<sup>54</sup>

Obese and overweight individuals with high body mass index (BMI) are at risk of infertility.<sup>55-57</sup> Men with a BMI higher than 25 are considered three times more at risk of infertility due to the reduction in sperm count and increase of DNA fragmentation.

There are many links between obesity and infertility: firstly, excess adipose tissue leads to the conversion of more testosterone to estrogen. This subsequently results in the development of secondary hypogonadism through hypothalamic-pituitary-gonadal axis inhibition, thereby decreasing the levels of circulating testosterone and increasing the levels of estradiol.<sup>58</sup> This decrease in testosterone is most likely responsible for impaired spermatogenesis. Secondly, accumulation of suprapubic and inner thigh fat in severely obese men can lead to infertility due to the insulating effects of fat deposits near the scrotum, which causes testicular heat stress. Fat deposits around scrotal blood vessels can impair blood cooling and elevate temperatures. Obese men also tend to be more sedentary which would exacerbate any temperature increases. Finally, obesity and several of its accompanying complications, namely insulin resistance and dyslipidemia, are associated with systemic proinflammatory states and increased oxidative stress.<sup>59,60</sup> Oxidative stress causes sperm membrane lipid peroxidation, which results in the impairment of sperm motility, DNA damage, and impaired sperm-oocyte interaction.<sup>61,62</sup> Conversely, adipose tissue releases pro-inflammatory adipokines that increase leukocyte production of ROS, which negatively impacts sperm function.<sup>63</sup>

Poor endocrine and exocrine functions of the testis are believed to be directly proportional to increased BMI and obesity in men around the world. Lowering BMI in obese men can be a solution to some infertility issues.

## SCROTAL HEAT STRESS

The exteriorization of the male gonads in the scrotum is a uniquely mammalian feature. The most plausible evolutionary

explanation is that optimal spermatogenesis requires a temperature approximately 2°C cooler than core body temperature (37°C). It is widely accepted that increased scrotal temperatures impair spermatogenesis. In rats, testicular temperatures elevated via exposure to warm bath water showed deterioration in spermatogenesis.<sup>64</sup>

During puberty the testes descend into the very bottom of the scrotum—as far as possible from the higher temperature of the body's core. Also, counter-current blood exchange evolved to reduce the temperature of blood coming towards the testes and heating the cooled blood returning to the core of the body. Finally, the corrugated scrotal surface is a third mechanism through which heat is dissipated to cool the testes.

The lower temperature leads to reduced rates of oxidative DNA damage and consequently fewer mutations in resulting sperm cells.<sup>65</sup> Sperm are stored in the epididymis for many days or even weeks. Storage occurs specifically in the cauda epididymis, which by no coincidence is the coolest area of the scrotum, thereby reducing metabolic rates and oxidative damage of these spermatozoa.<sup>66</sup>

Scrotal pathologies such as varicocele and cryptorchidism can increase testicular temperature excessively, however, lifestyle and occupation can also lead to chronically elevated scrotal temperatures that can contribute to the global trend in declining male reproductive parameters.<sup>67</sup>

Occupational exposure in certain professions, for example bakers, welders, furnace operators, and professional drivers, has been shown to directly relate to levels of infertility because of the increase in scrotal temperatures, often for extended periods of time. Such workers have been found to have poor semen quality compared to men with similar lifestyles, but who are not exposed to such temperatures during work.<sup>68</sup> Some studies show short lasting infertility or no infertility caused by this type of heat stress, but if there is the possibility it is worth implementing precautions such as cooling breaks for these workers.

Another area of study is the boxers versus briefs dilemma. The tightness of underpants has been determined to cause scrotal heating. Constantly wearing tighter underpants that leads to elevated scrotal temperatures in conjunction with the effects of obesity, sedentary lifestyle, and certain occupations mentioned above compound and exacerbate potential heat stress and infertility. Loose fitting underpants and clothing have been found to keep scrotal temperatures at an optimal compared to the elevating effects of restrictive clothing.<sup>69</sup>

Other lifestyle factors like hot baths, sauna use, and excessive exercise can cause testicular heat stress especially in combination with previously mentioned conditions. Moderate exercise has been found to allow air circulation and thus cooling of the scrotum, but on the other hand extreme exercise may raise temperatures, for example, moderate biking and walking was found to be beneficial, whereas very competitive levels of biking were found to generate heat stress.<sup>70</sup>

Testicular heat stress can be an easily avoidable phenomenon with the implementation of a few simple lifestyle changes. Daily modifications may reduce previous heat stress and allow the return of any lost fertility.

## PSYCHOLOGICAL STRESS

One issue that is unfortunately all too prevalent in societies across the globe is mental stress. Not only can it reduce your quality of life, but also impair the quality of your semen. Mental stress is associated with lower levels of antioxidants such as glutathione (GSH) and SOD, as well as higher levels of pro-oxidants, which can create oxidative stress.<sup>71</sup> Various studies have shown correlations between poor semen quality and stress: one study showed that students have lower sperm counts and quality during highly stressful periods of exams.<sup>72</sup> Eskiocak was able to link intervals of psychological stress with a reduction in sperm quality mediated by an increase in seminal plasma ROS generated and a reduction in antioxidant protection. It has also been said that stress can lead to increased levels of glucocorticoids and decreased levels of testosterone.<sup>73</sup>

Numerous individuals struggle with psychological stress each day as a result of work, home life, and a variety of issues. This psychological phenomenon can be an acute stress on reproductive functions and has adverse affects on general health.

## CELL PHONE RADIATION

Another health concern is the use of cell phones and the effects of ionizing radiation on male fertility. Since cell phones are constantly being used across the globe and are often placed in the pockets of pants, mere centimeters from the testes, these phones are a very noteworthy topic.

Stopczyk demonstrated that radiofrequency electromagnetic waves (RF-EMW) produced by cell phones significantly deplete SOD-1 activity, thereby increasing the concentration of malonyldialdehyde (MDA) after 1, 5, and 7 minutes of exposure in a suspension of human blood platelets.<sup>74</sup> This team concluded that oxidative stress after exposure to microwaves may be the reason for many adverse changes in cells and could cause a number of systemic disturbances in the human body.

Various epidemiological studies proposed that cell phone usage might cause decreases in sperm count and other sperm parameters.<sup>75,76</sup> A study by Friedman et al. revealed that cell phone radiation could lead to generation of ROS.<sup>77</sup> Results showed a significant increase in ROS production and a decrease in sperm motility, viability, and ROS-TAC score in exposed semen samples. A possible explanation for the production of ROS is the stimulation of the plasma membrane redox system of spermatozoa due to this radiation. Furthermore, the electromagnetic wave-dependent decrease in melatonin can predispose sperm to oxidative stress, which as mentioned results in poor semen quality.<sup>78</sup>

Considerable research is still required to conclude the exact effects of cell phones on male fertility. The close proximity of cell phones to the testes in men is a factor that may increase the risks of radiation. Since cell phones are constantly sending and receiving data regardless of whether they are actually being operated at the time by the owner, men may be constantly exposed to potentially harmful waves that could have negative effects on their reproductive success.

## CONCLUSION

The increase in defective spermatogenesis, testicular cancer, cryptorchidism, and numerous other male fertility issues over the course of the past few decades is a great cause of concern and has prompted the investigation of environmental and lifestyle factors that may be responsible. As societies increasingly introduce new chemical and potentially toxic substances into daily life, adverse effects may be amplified from one generation to the next. This is an issue that both developed and developing countries face.

These environmental factors can disrupt endocrine functions eventually leading to fertility problems. Exposure to certain toxins can lead to DNA damage, oxidative stress, and a host of other issues. Whether it occurred during gestation, the pre-pubertal age, or during adulthood, such exposure can affect fertility. The effects of exposure during each period are not fully understood, but information from animal models reveals that exposure itself and exposure at certain times is a topic worth investigating.

While assisted reproductive techniques have advanced in recent years and allows couples to bypass semen quality issues by directly injecting sperm into an egg, this only treats the symptom and not the issue itself. By eliminating or reducing certain environmental or lifestyle factors male fertility as a whole may increase.

## REFERENCES

1. Sharpe RM. Environmental/lifestyle effects on spermatogenesis. *Philosophical transactions of the Royal Society of London*. May 27;365(1546):1697-712.
2. Mocarelli P, Gerthoux PM, Patterson DG, Jr, Milani S, Limonta G, Bertona M, et al. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. *Environmental Health Perspectives* 2008;116(1):70-7.
3. Irvine DS. Declining sperm quality: a review of facts and hypotheses. *Bailliere's Clinical Obstetrics and Gynecology* 1997;11(4):655-71.
4. Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jorgensen E, Kjaerstad MB, et al. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. *Environmental Health Perspectives* 2008;116(4):566-72.
5. Swan SH, Liu F, Overstreet JW, Brazil C, Skakkebaek NE. Semen quality of fertile US males in relation to their mothers' beef consumption during pregnancy. *Human Reproduction (Oxford, England)* 2007;22(6):1497-502.
6. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction (Oxford, England)* 2001;16(5):972-8.
7. Storgaard L, Bonde JP, Ernst E, Spano M, Andersen CY, Frydenberg M, et al. Does smoking during pregnancy affect sons' sperm counts? *Epidemiology (Cambridge, Mass)* 2003;14(3):278-86.
8. Jensen MS, Mabeck LM, Toft G, Thulstrup AM, Bonde JP. Lower sperm counts following prenatal tobacco exposure. *Human Reproduction (Oxford, England)* 2005;20(9):2559-66.
9. Kizu R, Okamura K, Toriba A, Kakishima H, Mizokami A, Burnstein KL, et al. A role of aryl hydrocarbon receptor in the

- antiandrogenic effects of polycyclic aromatic hydrocarbons in LNCaP human prostate carcinoma cells. *Archives of Toxicology* 2003;77(6):335-43.
10. Barnes-Ellerbe S, Knudsen KE, Puga A. 2,3,7,8-Tetrachlorodibenzo-p-dioxin blocks androgen-dependent cell proliferation of LNCaP cells through modulation of pRB phosphorylation. *Molecular Pharmacology* 2004;66(3):502-11.
  11. Ramlau-Hansen CH, Nohr EA, Thulstrup AM, Bonde JP, Storgaard L, Olsen J. Is maternal obesity related to semen quality in the male offspring? A pilot study. *Human Reproduction (Oxford, England)* 2007;22(10):2758-62.
  12. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, Jr., et al. Male reproductive health and environmental xenoestrogens. *Environmental Health Perspectives* 1996;104(Suppl)4:741-803.
  13. Hughes PI. How vulnerable is the developing testis to the external environment? *Archives of Disease in Childhood* 2000;83(4):281-2.
  14. Wu TP, Huang BM, Tsai HC, Lui MC, Liu MY. Effects of nitric oxide on human spermatozoa activity, fertilization and mouse embryonic development. *Archives of Andrology* 2004;50(3):173-9.
  15. Jurewicz J, Hanke W, Radwan M, Bonde JP. Environmental factors and semen quality. *International Journal of Occupational Medicine and Environmental Health* 2009;22(4):305-29.
  16. Chitra KC, Sujatha R, Latchoumycandane C, Mathur PP. Effect of lindane on antioxidant enzymes in epididymis and epididymal sperm of adult rats. *Asian Journal of Andrology* 2001;3(3):205-8.
  17. Latchoumycandane C, Mathur PP. Induction of oxidative stress in the rat testis after short-term exposure to the organochlorine pesticide methoxychlor. *Archives of Toxicology* 2002;76(12):692-8.
  18. Selmanoglu G, Barlas N, Songur S, Kockaya EA. Carbendazim-induced haematological, biochemical and histopathological changes to the liver and kidney of male rats. *Human and Experimental Toxicology* 2001;20(12):625-30.
  19. Carter SD, Hess RA, Laskey JW. The fungicide methyl 2-benzimidazole carbamate causes infertility in male Sprague-Dawley rats. *Biology of Reproduction* 1987;37(3):709-17.
  20. Korasli D, Ziraman F, Ozyurt P, Cehreli SB. Microleakage of self-etch primer/adhesives in endodontically treated teeth. *Journal of the American Dental Association (1939)*. 2007;138(5):634-40.
  21. Le HH, Carlson EM, Chua JP, Belcher SM. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicology Letters* 2008;176(2):149-56.
  22. Chitra KC, Latchoumycandane C, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology* 2003;185(1-2):119-27.
  23. Kabuto H, Amakawa M, Shishibori T. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. *Life Sciences* 2004;74(24):2931-40.
  24. Gesler RM. Toxicology of di-2-ethylhexyl phthalate and other phthalic acid ester plasticizers. *Environmental Health Perspectives* 1973;3:73-9.
  25. Ishihara M, Itoh M, Miyamoto K, Suna S, Takeuchi Y, Takenaka I, et al. Spermato-genic disturbance induced by di-(2-ethylhexyl) phthalate is significantly prevented by treatment with antioxidant vitamins in the rat. *International Journal of Andrology* 2000;23(2):85-94.
  26. Peakall DB. Phthalate esters: Occurrence and biological effects. *Residue Reviews* 1975;54:1-41.
  27. Acharya UR, Acharya S, Mishra M. Lead acetate induced cytotoxicity in male germinal cells of Swiss mice. *Industrial Health* 2003;41(3):291-4.
  28. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. *Toxicology* 2002;180(1):33-44.
  29. Naha N, Chowdhury AR. Inorganic lead exposure in battery and paint factory: effect on human sperm structure and functional activity. *Journal of UOEH* 2006;28(2):157-71.
  30. Xu DX, Shen HM, Zhu QX, Chua L, Wang QN, Chia SE, et al. The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. *Mutation Research* 2003;534(1-2):155-63.
  31. Benoff S, Auburn K, Marmar JL, Hurley IR. Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. *Fertility and Sterility* 2008;89(2 Suppl):e73-9.
  32. Kunzle R, Mueller MD, Hanggi W, Birkhauser MH, Drescher H, Bersinger NA. Semen quality of male smokers and nonsmokers in infertile couples. *Fertility and Sterility* 2003;79(2):287-91.
  33. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ Jr. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertility and Sterility* 2002;78(3):491-9.
  34. Vine MF, Tse CK, Hu P, Truong KY. Cigarette smoking and semen quality. *Fertility and Sterility* 1996;65(4):835-42.
  35. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. *Journal of Andrology* 2002;23(6):737-52.
  36. Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA. Varicocele reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *International Journal of Andrology* 2001;24(5):261-5.
  37. Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. *Mutation Research* 1996;351(2):199-203.
  38. Boyden TW, Pamentor RW. Effects of ethanol on the male hypothalamic-pituitary-gonadal axis. *Endocrine Reviews* 1983;4(4):389-95.
  39. Goverde HJ, Dekker HS, Janssen HJ, Bastiaans BA, Rolland R, Zielhuis GA. Semen quality and frequency of smoking and alcohol consumption—an explorative study. *International Journal of Fertility and Menopausal Studies* 1995;40(3):135-8.
  40. Dahchour A, Lallemand F, Ward RJ, De Witte P. Production of reactive oxygen species following acute ethanol or acetaldehyde and its reduction by acamprosate in chronically alcoholized rats. *European Journal of Pharmacology* 2005;520(1-3):51-8.
  41. Lieber CS. The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. *Drug Metabolism Reviews* 2004;36(3-4):511-29.
  42. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology* 2005;43(11):963-74.

43. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *The Biochemical Journal* 1996;313 (Pt 1):17-29.
44. Shalet SM. Effects of cancer chemotherapy on gonadal function of patients. *Cancer Treatment Reviews* 1980;7(3):141-52.
45. Schlegel PN, Chang TS, Marshall FF. Antibiotics: potential hazards to male fertility. *Fertility and Sterility* 1991;55(2):235-42.
46. O'Morain C, Smethurst P, Dore CJ, Levi AJ. Reversible male infertility due to sulphasalazine: studies in man and rat. *Gut* 1984;25(10):1078-84.
47. Sikka SC, Wang R. Endocrine disruptors and estrogenic effects on male reproductive axis. *Asian Journal of Andrology* 2008;10(1):134-45.
48. Fronczak CM, Kim ED, Barqawi AB. The Insults of Recreational Drug Abuse on Male Fertility. *Journal of Andrology*, 2011.
49. Knuth UA, Maniera H, Nieschlag E. Anabolic steroids and semen parameters in bodybuilders. *Fertility and Sterility* 1989;52(6):1041-7.
50. Eskenazi B, Kidd SA, Marks AR, Slotter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. *Human reproduction (Oxford, England)* 2005;20(4):1006-12.
51. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proceedings of the National Academy of Sciences of the United States of America* 1991;88(24):11003-6.
52. Song GJ, Norkus EP, Lewis V. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. *International Journal of Andrology* 2006;29(6):569-75.
53. Therond P, Auger J, Legrand A, Jouannet P. alpha-Tocopherol in human spermatozoa and seminal plasma: relationships with motility, antioxidant enzymes and leukocytes. *Molecular Human Reproduction* 1996;2(10):739-44.
54. Hawkes WC, Turek PJ. Effects of dietary selenium on sperm motility in healthy men. *Journal of Andrology* 2001;22(5):764-72.
55. Koloszar S, Fejes I, Zavaczki Z, Daru J, Szollosi J, Pal A. Effect of body weight on sperm concentration in normozoospermic males. *Archives of Andrology* 2005;51(4):299-304.
56. Kort HI, Massey JB, Elsner CW, Mitchell-Leef D, Shapiro DB, Witt MA, et al. Impact of body mass index values on sperm quantity and quality. *Journal of Andrology* 2006;27(3):450-2.
57. Nguyen RH, Wilcox AJ, Skjaerven R, Baird DD. Men's body mass index and infertility. *Human Reproduction (Oxford, England)* 2007;22(9):2488-93.
58. Fejes I, Koloszar S, Zavaczki Z, Daru J, Szollosi J, Pal A. Effect of body weight on testosterone/estradiol ratio in oligozoospermic patients. *Archives of Andrology* 2006;52(2):97-102.
59. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation* 2005;111(11):1448-54.
60. Davi G, Falco A. Oxidant stress, inflammation and atherogenesis. *Lupus* 2005;14(9):760-4.
61. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertility and Sterility* 1997;68(3):519-24.
62. Twigg J, Fulton N, Gomez E, Irvine DS, Aitken RJ. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. *Human reproduction (Oxford, England)* 1998;13(6):1429-36.
63. Singer G, Granger DN. Inflammatory responses underlying the microvascular dysfunction associated with obesity and insulin resistance. *Microcirculation* 2007;14(4-5):375-87.
64. Jung A, Schuppe HC. Influence of genital heat stress on semen quality in humans. *Andrologia* 2007;39(6):203-15.
65. Werdelin L, Nilsson A. The evolution of the scrotum and testicular descent in mammals: a phylogenetic view. *Journal of Theoretical Biology* 1999;196(1):61-72.
66. Bedford JM. Anatomical evidence for the epididymis as the prime mover in the evolution of the scrotum. *The American Journal of Anatomy* 1978;152(4):483-507.
67. Ivell R. Lifestyle impact and the biology of the human scrotum. *Reprod Biol Endocrinol* 2007;5:15.
68. Thonneau P, Bujan L, Multigner L, Mieusset R. Occupational heat exposure and male fertility: a review. *Human Reproduction (Oxford, England)* 1998;13(8):2122-5.
69. Jung A, Leonhardt F, Schill WB, Schuppe HC. Influence of the type of undertrousers and physical activity on scrotal temperature. *Human Reproduction (Oxford, England)* 2005;20(4):1022-7.
70. Jung A, Schuppe HC. Influence of genital heat stress on semen quality in humans. *Andrologia* 2007;39(6):203-15.
71. Eskiocak S, Gozen AS, Kilic AS, Molla S. Association between mental stress and some antioxidant enzymes of seminal plasma. *The Indian Journal of Medical Research* 2005;122(6):491-6.
72. Eskiocak S, Gozen AS, Yapar SB, Tavas F, Kilic AS, Eskiocak M. Glutathione and free sulphhydryl content of seminal plasma in healthy medical students during and after exam stress. *Human Reproduction (Oxford, England)* 2005;20(9):2595-600.
73. Eskiocak S, Gozen AS, Taskiran A, Kilic AS, Eskiocak M, Gulen S. Effect of psychological stress on the L-arginine-nitric oxide pathway and semen quality. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica et al* 2006;39(5):581-8.
74. Stopczyk D, Gnitecki W, Buczynski A, Kowalski W, Buczynska M, Kroc A. Effect of electromagnetic field produced by mobile phones on the activity of superoxide dismutase (SOD-1)- in vitro researches. *Annales Academiae Medicae Stetinensis* 2005;51(Suppl)1:125-8.
75. Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, et al. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertility and Sterility*, 2008.
76. Deepinder F, Makker K, Agarwal A. Cell phones and male infertility: dissecting the relationship. *Reproductive Biomedicine Online* 2007;15(3):266-70.
77. Friedman J, Kraus S, Hauptman Y, Schiff Y, Seger R. Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. *The Biochemical Journal* 2007;405(3):559-68.
78. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Nocturnal excretion of a urinary melatonin metabolite among electric utility workers. *Scandinavian journal of work, environment and health* 1998;24(3):183-9.