Lifestyle factors and oxidative stress in female infertility: is there an evidence base to support the linkage?


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At present, between 10 to 15% of couples are infertile, and half of all infertility cases are credited to a female factor. Determination of the source of the problem may hold the key to improving fertility for women. Emerging research demonstrates that reactive oxygen species and oxidative stress (OS) have strong connections with female reproductive function; increases in OS which is associated with certain lifestyle factors can negatively impact female fertility. Lifestyle factors including being obese or underweight, exercising, cigarette smoking, alcohol and caffeine consumption, drug use, psychological stress and environmental and occupational exposures can all have adverse effects on fertility due to their complex interactions and impact exerted via OS on female reproductive processes. Our review highlights these linkages to explain their impact on female fertility, as well as provide suggestions to reduce OS and improve reproductive potential in women.

KEYWORDS: antioxidants • assisted reproductive techniques • female reproduction • infertility • lifestyle factors • oxidative stress • reactive oxygen species.

The prevalence of lifestyle factors detrimental to fertility has increased significantly in the 21st century, particularly among women in the reproductive age. There has been a dramatic shift in the risk factors for infertility over the last few decades including delayed conception and advanced maternal age. These shifts have probably further brought to fore the impact of oxidative stress (OS) on female infertility by influencing the oxidative microenvironment within follicles with increasing age. Many lifestyle factors are reported to be associated with the generation of OS, which renders the physiological processes of female reproduction and embryo development vulnerable to damage. The present research aims to identify the complex relationship between lifestyles and OS-induced infertility, in order to provide recommendations for enhancement of natural and assisted fertility. OS may contribute to several disease states affecting female fertility, including endometriosis and polycystic ovary syndrome (PCOS). In addition, extremes of body weight, excessive physical activity, maternal smoking, alcohol and caffeine consumption, recreational drug abuse, psychological stress and environmental pollutants may negatively affect female fertility through OS mechanisms. The prevalence of some of those factors, such as smoking, have actually decreased while others, like obesity, have such a wide range of metabolic consequences that it is impossible to attribute their effect on fertility to OS.

Antioxidants, lifestyle modification and integrated treatment approaches may be viable options to improve fecundity. Currently, there is a gap in the literature with few studies reporting the OS-mediated effects of multiple lifestyle factors on female fertility – an issue this research aims to address. This review is beneficial for both the women in reproductive age facing subfertility and their physicians, who wish to improve fertility potential. Infertility affects 10–15% of couples [1,2], and half of all infertility cases are attributed to female factors [3]. Therefore, understanding the negative impact of lifestyle and OS on female fertility is critical.
Free radicals, antioxidants & oxidative stress

Free radicals are reactive, unstable molecules that may be generated as the result of normal metabolic processes, or may come from exogenous sources. Free radicals can be classified as either reactive nitrogen species or reactive oxygen species (ROS). The three main types of ROS are superoxide (O$_2^•$-), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH•). The superoxide radical is dismutated and converted into H$_2$O$_2$. H$_2$O$_2$ can also be formed directly by oxidase enzymes. The hydroxyl radical is generated by the conversion of H$_2$O$_2$ or superoxide by a metal ion catalyst.

Antioxidants are protective mechanisms that scavenge excess ROS and neutralize these radicals into molecules of water. These molecules maintain ROS at physiologically compatible levels. There are two main types of antioxidants. Enzymatic antioxidants or natural antioxidants include superoxide dismutase (SOD), catalase and glutathione (GSH). Non-enzymatic antioxidants or synthetic antioxidants include vitamins C and E, selenium, zinc, taurine, hypotaurine, glutathione, β-carotene and carotene. These nutrients are not actually ‘often’ obtained in dietary supplements. There is a great geographic and cultural variation in supplement access and use, and this variation is often correlated with lifestyle factors and healthy lifestyle in general. In the USA, female smokers are more likely to take multivitamins than non-smokers. Thus, there are both endogenous and exogenous contributors to the antioxidant milieu.

Normally, there exists a delicate balance between levels of ROS and antioxidants. However, OS is a condition that arises when the production of ROS exceeds the scavenging capabilities of the antioxidants. This imbalance can be caused by an increase in ROS or a decrease in antioxidant activity. OS is detrimental to the entire system, because high levels of ROS can damage biological molecules such as lipids, proteins and DNA, resulting in damage and dysfunction of the cell. ROS are produced by the mitochondria during glucose metabolism and the ROS, that is generated, can cause alterations of the mitochondria and depletion of ATP. Although an overabundance of these species can result in pathology, ROS are necessary for a variety of physiologic functions in the body, including those of the female reproductive system [4].

Redox pathways in the female reproductive system

Each month within the ovarian follicles, a group of oocytes begins to develop. Meiosis I resumes only in the dominant oocyte. This stage in oocyte development is characterized by an increase in ROS; antioxidants retard this process. Antioxidants, however, are beneficial to meiosis II [5]. Within the pre-ovulatory follicles, ROS stimulates programmed cell death, although this is offset by the actions of GSH and follicular stimulating hormone (FSH) in the dominant follicle [5]. Additionally, the ROS produced by the follicles contributes to the induction of ovulation. Ovulation is induced by the LH surge, and results in the release of a secondary oocyte from the ovary. Post-LH surge inflammatory precursors are necessary for the process of ovulation, although an excessive amount leads to the production of ROS [6]. Following ovulation, the corpus luteum develops from the remnants of an ovarian follicle. Within the corpus luteum, the mitochondrial electron transport chain has been found to leak electrons, leading to the generation of the superoxide radical, when these electrons combine with oxygen. In order to cope with the increase in ROS within the corpus luteum, levels of SOD also increase. The corpus luteum will regress if no pregnancy takes place, and will persist if a pregnancy does occur. Pregnancy is considered to be a state of OS. During the first trimester of pregnancy, an inflammatory response is produced as a result of leukocyte activation, and the production of superoxide radicals increases. OS is supported by increases in lipid peroxides, free radicals and vitamin E in the placental mitochondria as the pregnancy progresses [7,8].

Reactive oxygen species in oocytes & embryos

Levels of OS are related to oocyte and embryo quality, programmed cell death, permanent meiosis arrest, age-related defects and chromosomal abnormalities. The excessive production of ROS in oocytes and embryos can affect mitochondrial function, and lead to OS-induced arrest of cell division and cell death [9]. In 2010, Liu et al. found that H$_2$O$_2$ treatment induced programmed cell death in mouse zygotes, and that mitochondrial damage caused by OS was associated with cell cycle arrest [9]. Studies in mouse and rat embryos have demonstrated that the antioxidant defense system develops gradually with increasing embryonic age, peaking at the neonatal period [10]. Because the antioxidant system remains immature during the early development of the embryo, and since high concentrations of ROS are produced during the early developmental stages, this leaves the embryo susceptible to oxidative damage to DNA, proteins and lipid membranes. There is a role for OS in impairing mitochondrial function and the production of ATP during embryogenesis. Cell division requires the contraction of non-muscular myosin II, the energy needed for this contraction is produced by the mitochondria. OS may reduce the ATP production and render cell division problematic. This process can have severe consequences for organogenesis and may result in embryonic damage.

An elevated concentration of homocysteine has been reported in follicular fluid after ovarian hyperstimulation [11]. There are reported evidence that elevated homocysteine can cause epigenetic changes in the embryos. There is a potential that elevated homocysteine levels will induce epigenetic changes and correlate with poor embryo quality [12].

There are several endogenous mechanisms in place to guard developing oocytes and embryos from OS-induced damage. Many aspects of development in the gamete and embryo naturally occur in low oxygen environments, which may help to protect from free radical damage by ROS [13]. Developing follicles and embryos are also protected from toxicity and OS by antioxidant molecules, although as discussed previously, there is a lag time before these antioxidants become sufficiently active. Internal sources of protection within the embryo include...
antioxidant enzymes such as SOD and γ-glutamylcysteine synthetase [14]. Human follicular fluid contains antioxidant proteins such as SOD, GST and paraxonase [15]. Follicular fluid also includes non-enzymatic antioxidants such as hypotaurine and ascorbic acid which can act as external sources of protection against ROS for the embryo [14]. Higher concentrations of melatonin, a direct free-radical scavenger, have been found in follicular fluid as compared with serum, suggesting that this molecule may also protect maturing oocytes and developing embryos from OS [16]. In fact, exogenous melatonin administration has been found to reduce oxidative damage to the follicle and to improve fertilization rates [16]. Treatment with exogenous antioxidants and other substances can also improve oocyte and embryo quality. The addition of polyethylene glycol-catalase to embryos having been treated with H₂O₂ attenuated the ROS-induced apoptosis and arrest, resulting in increased development [17]. Vitamins C and E, carotenoids and folic acid may be effective in abating both embryonic and maternal OS, and thus reducing congenital anomalies, although further clinical investigation is needed [10].

Role of oxidative stress in female infertility

Infertility is defined as the inability to achieve a pregnancy within 12 months of unprotected intercourse. It is characterized as primary if there is no previous conception, and as secondary if at least one member of the couple has conceived previously. Infertility affects approximately 10–15% of couples [1,2], and about half of all infertility cases are attributed to female factors [3]. OS, evidenced by an increase in ROS and decrease in antioxidant capacity, has been hypothesized to play a role in the etiology of infertility, including endometriosis, polycystic ovary syndrome, idiopathic infertility and miscarriages. OS may also contribute to infertility in males.

Endometriosis

Endometriosis is a chronic disorder that is characterized by the growth and implantation of endometrial cells and stroma in areas of the body, outside of the uterus, most often on the pelvic peritoneum or ovaries. It is often associated with pelvic pain and infertility. Endometriosis affects 6–10% of reproductive-aged women [18] and over 30% of infertile women [19]. It has been suggested that OS is involved in the etiopathology of the disease. Several studies have demonstrated an increased presence of biomarkers for OS in the women with endometriosis. Levels of SOD and GSH peroxidase in the peritoneal fluid were lowest among patients with endometriosis. Women with endometriosis also had higher concentrations of lipid peroxidase [20]. Stromal cells from women with endometriosis had increased superoxide and H₂O₂ production, as well increased SOD activity as compared with stromal cells from controls [21]. This suggests that an increase in ROS production in the endometriotic cells is compensated by a subsequent increase in antioxidant activity. Women with endometriosis also have higher serum levels of the heat shock protein HSP70b, suggesting that the OS associated with endometriosis is systemic, rather than confined to the peritoneal cavity [22].

Endometriosis is associated with decreased uterine receptivity, tubal dysfunction due to adhesions or scarring and poor oocyte quality, which may lead to reduced implantation rates [23]. These factors may be the underlying causes of the infertility in patients with endometriosis, and OS may be responsible for these negative effects. Ovarian cortex tissue surrounding endometriotic lesions contains higher levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) than ovarian tissue surrounding other benign ovarian cysts [24]. Infertile women with endometriosis were found to have lower follicular fluid levels of vitamin C, lower plasma levels of SOD and higher plasma levels of vitamin E [25]. However, this study did not account for dietary differences between women with and without endometriosis. Since markers of the OS are present both in the ovarian cortex where the follicles are located, and in the follicular fluid itself. This suggests that the OS associated with endometriosis may be responsible for the decrease in oocyte quality and subsequent decrease in implantation found in these patients. Changes in the endometrial epithelium in women with endometriosis may also decrease uterine receptivity and lead to reduced implantation rates. It has been hypothesized that free radicals mediate these changes in the endometrium [26,27]. Ota et al., in 2000 found that there are phase-dependent changes in the expression of glutathione peroxidase (GPxs), an enzyme that reduces ROS to water or alcohol, throughout the menstrual cycle in normal eutopic endometrial tissue. This variation in expression of GPx was not observed in the eutopic endometrium of women with endometriosis, suggesting that the endometrial tissue of women with endometriosis is more susceptible to OS.

Lifestyle factors such as intake of specific fatty acids and markers such as adipokines have been investigated [28]. These factors are known to affect inflammation and OS and influence risk and behavior of endometriosis in humans and animal models.

OS has been identified in women with endometriosis, and this underlies the cause of infertility among women with endometriosis. Majority of the study reports have investigated cases of women with endometriosis where OS and related factors have been measured post-diagnosis. The design of these studies temporally makes it impossible to tease these levels from consequences of disease to imply a causal relationship. In the future, two types of studies are needed—one where OS is measured prior to symptom onset or at least soon after symptom onset but prior to diagnosis of endometriosis, and another where it is evaluated if induced endometriosis in animal models or ectopic endometrium in cellular models confers advancement of OS.

Polycystic ovary syndrome

PCOS is one of the most common hormonal disorders among women of reproductive age, with a prevalence of approximately 4.6% [29]. PCOS is characterized by polycystic
ovaries (enlarged ovaries containing multiple, small cysts around the perimeter), hyperandrogenism and ovulatory dysfunction [30]. Menstrual irregularities, such as amenorrhea or menorrhagia, are also common in PCOS patients [30]. Patients with PCOS also experience a higher rate of failed implantation after inducing ovulation, as well as an increased risk of spontaneous abortion once pregnancy is achieved. This suggests that infertility associated with PCOS is due to ovulatory dysfunction and an altered endometrial environment [31]. The exact cause of PCOS remains unknown. However, OS may play a role in its etiology.

Several studies have found increased biomarkers of OS in PCOS patients. Serum levels of malondialdehyde (MDA)-modified proteins, a maker of oxidative protein status, were higher in patients with PCOS than in controls [32]. Kuscu et al. found that young, non-obese women with PCOS had significantly higher levels of MDA and SOD, and suggest that PCOS is itself responsible for the presence of OS in these patients [33]. PCOS patients were also found to exhibit mitochondrial dysfunction, manifested as a decrease in oxygen consumption by the mitochondria, an increase in ROS production and a decrease in serum levels of GSH antioxidants. This characteristic augmentation of ROS and reduction in antioxidant status suggests that OS is present in women with PCOS [34]. OS has also been linked to the development of insulin resistance and hyperandrogenism, by way of a proinflammatory state, in women with this disorder [35]. Therefore, OS may play a role in the development of PCOS itself, as well as in the development of its associated symptoms. However, PCOS is a complex disorder because many women with PCOS are also overweight or obese. Therefore it is difficult to tease apart if it is PCOS itself, or the comorbid obesity which contributes most significantly to the infertility associated with the disorder. Additional research is greatly needed in order to elucidate this relationship.

Sabuncu et al. compared PCOS patients (n = 27) with mean BMI 31.4 and mean age of 26.7 with BMI- and age-matched control group. They demonstrated that higher level of erythrocyte MDA was seen in PCOS patients (mean value 70.9 µmol/mol Hb) compared to controls (p = 0.009) [36]. The significantly higher levels of MDA in PCOS patients than the control were also found by Palacio and colleagues [32]. Protein oxidation status was often assessed with a colorimetric assay that measures protein carbonyl (PC) content, after reacting the patient’s serum with dinitrophenylhydrazine. Fenkci et al., demonstrated that the PC level was significantly higher in PCOS patients with normal BMI compared to the control (18.01 ± 0.80 vs 14.19 ± 0.40 nmol/l, p = 0.001) [37]. This observation of higher protein oxidation suggested that free radical damage proteins in PCOS patients. Furthermore, protein carbonyls were shown to have a positive correlation with fasting insulin. This suggested a strong association between insulin resistance and protein oxidation in PCOS.

This characteristic augmentation of ROS and reduction in antioxidant status suggests that OS is present in women with PCOS. OS has also been linked to the development of insulin resistance and hyperandrogenism, by the way of a proinflammatory state, in women with this disorder. The role of OS in the pathogenesis of PCOS is not fully understood and the evidence is conflicting. The current evidence points toward an association between the oxidative microenvironment of the ovarian tissue and ovarian steroidogenesis and follicular development. Whether the OS is the cause or the result of the metabolic disturbances encountered in PCOS, remains to be elucidated. However it is recognized that there is a strong relationship between hyperinsulinemia, hyperlipidemia and OS.

**Unexplained infertility**

Idiopathic infertility affects 10–20% of infertile couples. Although the pathophysiology of unexplained infertility is unknown, OS may play a role in its development. Patients with unexplained infertility have lower total antioxidant status in peritoneal fluid than fertile women and those with tubal infertility [38]. Higher levels of MDA, a biomarker of lipid peroxidation, have been found in women with idiopathic infertility [39]. Significant higher levels of ROS were also present in the processed peritoneal fluid of patients with idiopathic infertility [8]. Women experiencing unexplained infertility have lower levels of antioxidants and higher levels of ROS, implicating OS as a possible mechanism for the development of this disorder. Although the administration of antioxidants such as N-acetyl cysteine (NAC), an antioxidant and precursor to GSH, may combat increases in ROS production and control systemic OS, NAC has not been demonstrated as an effective treatment for unexplained infertility [40]. Future well designed, with strict randomization of patients to intervention versus placebo and adequately powered randomized, controlled trials need to investigate antioxidant supplementation and free-radical focused lifestyle modifications in cases of recurrent spontaneous abortion.

**Recurrent pregnancy loss**

Recurrent pregnancy loss affects 1–3% of women, and is characterized by a history of three or more consecutive pregnancy losses. In half of all recurrent pregnancy loss cases, there is no identifiable cause. However, impaired antioxidant defenses and augmented production of ROS have been associated with unexplained recurrent spontaneous abortion [41]. Patients experiencing unexplained recurrent pregnancy loss had significantly lower blood concentrations of GSH and SOD, as well as higher levels of MDA and nitric oxide (NO) than controls, reflecting an increase in OS [41]. This increase in OS may contribute to the pathogenesis of unexplained recurrent abortions.

Simsek et al. also demonstrated the involvement of OS in the development of habitual abortion [42]. Women with habitual abortion were found to have lower blood levels of the antioxidants vitamin A, vitamin E, GSH and β-carotene, and higher blood levels of lipid peroxidation and alkaline phosphatase than healthy controls. It was also found that in women with recurrent abortion, an increase in serum lipid peroxidase levels occurred in the time immediately preceding the
pregnancy loss, and a decrease in serum concentrations of lipid peroxidase occurred immediately following the abortion [43], suggesting that OS is the mechanism by which this syndrome develops.

Augmented levels of TNF-α, an inflammatory cytokine, have also been linked to recurrent pregnancy loss [41]. NAC has been shown to attenuate elevated levels of TNF-α in serum and amniotic fluid [44], suggesting that the control of OS with the administration of antioxidants could be protective against pregnancy loss in these women. Future is well designed, with strict randomization of patients to intervention versus placebo and adequately powered randomized, controlled trials need to investigate antioxidant supplementation and free-radical focused lifestyle modifications in cases of recurrent spontaneous abortion.

The link between lifestyle factors, oxidative stress & fertility

**Obesity**

The NIH has reported that 35.5% of women in the USA are obese. Increased BMI is associated with a variety of health complications, including those of the reproductive system. A pre-pregnancy BMI >24 is associated with a higher risk of complications, including those of the reproductive system. The NIH has reported that 35.5% of women in the USA are obese [51]. This suggests that obesity-associated miscarriage is independent of embryonic aneuploidy, and may instead result from an impaired uterine environment in these women. Therefore, obesity may also negatively affect the endometrium, potentially resulting in poor implantation rates and miscarriage.

OS, due to an increase in ROS, has been suggested as a potential mechanism responsible for the pathogenesis of obesity [52]. Several studies have demonstrated the association between obesity and increased OS. After the consumption of a high-fat meal, blood levels of MDA and ROS were maintained and levels of xanthine oxidase activity, H2O2 and triglycerides continued to increase in obese women, while blood levels of these substances decreased in women who were not obese [52]. Increasing body weight was correlated with decreasing levels of SOD and increasing levels of catalase and MDA in erythrocytes [53]. This simultaneous decrease in antioxidants and increase in peroxidation products suggests that OS is present in overweight women. Urinary creatinine-indexed levels of 8-epi-PGF2α, a marker for systemic OS, were also positively associated with BMI as well as with diabetes and smoking [54]. In addition to BMI, 8-epi-PGF2α was also positively correlated with waist circumference, peripheral DXA-derived regional fat mass accumulations and leptin levels [55]. This suggests that these parameters associated with obesity and central adiposity are important in the development of systemic OS in women. Increased OS may be the mechanism by which fertility and pregnancy outcomes are impaired in obese women. A study in mice demonstrated that maternal obesity was associated with increased rates of ROS production, decreased concentrations of GSH and mitochondrial alterations in oocytes and zygotes as compared to lean female mice [56]. Blastocyst development was also significantly decreased in obese mice, suggesting that OS impairs the mitochondrial functioning in the oocytes and embryos of obese women which subsequently inhibiting blastocyst maturation and implantation [56]. Ultimately, the generation of OS as a result of obesity and central adiposity seems to affect every aspect of the female reproductive tract, suggesting OS may be a possible mechanism by which infertility develops among overweight and obese women.

**Underweight & eating disorders**

The average lifetime prevalence rate for anorexia nervosa, a disorder characterized by extreme thinness, or emaciation [201], is 0.9% in adult women [57]. The disorder of infertility is one of the presentation of anorexia nervosa that may develop over time. Women who are underweight or have an eating disorder have a longer time to conception [58] and an increased risk of spontaneous abortion [59]. In a sample of patients undergoing intrauterine insemination treatments at a fertility center, 20.7% of these infertile women had a past or current diagnosis of an eating disorder [60]. Since this statistic is five-times the national average lifetime prevalence rate in the USA, this suggests that infertility is a serious complication of eating disorders.

Menstrual abnormalities are a potential complication of disordered eating and underweight. Stewart et al. reported that...
58.3% infertile women with abnormal menstruation had been diagnosed with an eating disorder [61]. Anorexia nervosa is associated with severe limitation of food intake and excessive exercise [201]. Reductions in metabolic fuel caused by caloric restriction or increased energy expenditure can result in neuroendocrine alterations that lead to amenorrhea and anovulation [62]. Women with anorexia nervosa had lower blood levels of prolactin, estrone, estradiol, progesterone, testosterone, androstenedione and LH and a significantly higher FSH response to GnRH [63]. Women with anorexia nervosa, 73.5% of whom were underweight and also had lower levels of estrogen, progesterone and gonadotropins [64]. Lower levels of these hormones are associated with a small, hypertropic uterus and small, amorphous and inactive ovaries, which may contribute to menstrual and ovulatory dysfunction [65]. Rich-Edwards et al. estimated that 12% of ovulatory infertility is caused by being underweight [46]. Maternal underweight may also be detrimental to fetal health. Underweight is associated with an increased risk of preterm birth [66]. A recent systematic review and meta-analysis of 78 studies reported that underweight women had an increased risk of preterm birth, both spontaneous (adjusted relative risk [RR]; 1.32; 95% CI: 1.10–1.57) and induced (adjusted RR: 1.21; 95% CI: 1.07–1.36), in addition to an increased risk for a low birth weight infant (adjusted RR: 1.64; 95% CI: 1.38–1.94) as compared to healthy controls [67]. Women with eating disorders also had a higher rate of intrathecal growth restriction and significantly higher rates of low birth weight infants and cesarean sections than healthy mothers [68]. This correlation between underweight and poor fetal and infant health could be caused directly, by a lack of nutrients leading to insufficient fetal growth, or indirectly, by other factors that are associated with maternal underweight, such as smoking, susceptibility to illness or generalized debility.

Similarly to obesity, low BMI is also associated with increased levels of OS. Patients with low BMI (<18.5 kg/m²) had higher plasma concentrations of 8-OHdG and higher serum concentrations of MDA-modified low-density lipoprotein—both markers of OS—than did patients within a normal weight range [69]. A study of the antioxidant status in female adolescents with anorexia nervosa found that erythrocyte tocopherol concentration and SOD activity were significantly decreased (p < .02 and p < .001, respectively) in women with anorexia, as compared with controls [70]. This finding suggests that oxidative damage is occurring in anorexia nervosa, due to insufficient intake of micronutrients and OS as a result of low antioxidant capacity [70]. The oxidative damage present in women who are underweight or who have been diagnosed with an eating disorder could negatively affect the reproductive system and may be a mechanism by which infertility develops.

**Exercise**

The overall health benefits of exercise often outweigh the risks. Exercise has been shown to have protective effects on infertility in some populations, such as in women who are overweight or obese. However, excessive physical activity is associated with menstrual and ovulatory disturbance and impaired follicle development, which may result in decreased fertility in women who participate in vigorous exercise or in female athletes. Menstrual abnormalities are often seen both in female athletes and in women who participate in intense physical activity. Ovarian function is especially vulnerable to chronic low energy balance and extremely low amounts of body fat [64]. The female athlete triad refers to the relationship between energy balance, menstrual function and bone density, and may manifest itself as functional hypothalamic amenorrhea [71]. Women with a high drive for thinness had significantly lower daily dietary energy intakes than controls. 73.9% of the women with high drive for thinness had severe menstrual disturbances, such as amenorrhea and oligomenorrhea, as opposed to 38% of controls with menstrual abnormalities [72]. Russell et al. found that women undergoing strenuous exercise and experiencing oligomenorrhea had lower serum levels of LH, prolactin and estradiol-17β [73]. These hormonal imbalances are often due to disturbances of hypothalamic-pituitary-ovarian axis, causing a suppression of pulsatile gonadotropin-releasing hormone (GnRH) secretions, which controls the secretion of LH and FSH and ultimately resulting in hypoestrogenism and anovulation [74].

Several studies suggest that there is a positive correlation between amount of exercise and risk of infertility. Increases in the frequency, intensity and duration of physical activity were also correlated with increased subfertility in women. Women who exercised to exhaustion had 2.3-times the odds of infertility as compared to women who participated in mild physical activity [75]. However, some researchers have reported a lower risk of ovulatory infertility with increasingly vigorous exercise [46,76]. Ultimately, hormonal disturbance and low energy availability are few of the likely causes of infertility related to excessive exercise. Moderate amounts of physical activity have been shown to decrease levels of OS, as it decreases systemic oxidation and increases the expression of antioxidants. However, strenuous exercise may have a deleterious effect. Intense exercise increases oxygen consumption, thereby upsetting the balance between pro-oxidants and antioxidants within cells [77]. A large body of literature has reported an increase in lipid peroxidation products following exercise, indicating that oxidative damage is occurring. Blood levels of protein carbonyls, lipid hydroperoxides and total antioxidants significantly increase (p < 0.05) after both exhaustive aerobic and nonaerobic isometric types of exercise [78]. Exercising in a warm environment (33°C) was also associated with an OS response, evidenced by significant elevations (p < 0.001) in blood levels of lipid hydroperoxides, as well as in antioxidant capacity [79]. Animal studies have found that total plasma thiols (TPT) significantly decreased (p = 0.022) and plasma xanthine oxidase (XOD) activity significantly increased (p = 0.039) following exhaustive swimming exercise in guinea pigs, indicating that exhaustive exercise
generates OS, and that XOD is a significant source of these ROS [80]. Additionally, plasma antioxidant levels were diminished following exhaustive exercise, although the finding was not significant (0.755) [80]. Nutritional supplements and exogenous antioxidants, including L-carnitine [81], coenzyme Q10 (CoQ10) [82] and dark chocolate [83], are protective against the OS induced by excessive exercise.

Cigarette smoke

Approximately 30% of reproductive-aged women in the USA are smokers [84]. Cigarette smoke is composed of over 4000 chemicals. Smoking is associated with a number of potential health complications in women, including infertility. Female smokers have a significantly increased frequency of infertility as compared to women who are non-smokers [85]. The negative effects of smoking on fertility involve almost every aspect of the female reproductive system [86].

Cigarette smoke can be detrimental to ovarian function as well as oocyte and embryo quality. Benzo(a)pyrene (BaP) is a chemical component of cigarette smoke that has well-documented effects on fertility, including premature ovarian failure and a longer time to achieve pregnancy. Sobinoff et al. found that, in ovariies exposed to BaP, there was an increase in primordial follicle activation, in addition to atresia of the developing follicles [87]. Oocytes retrieved from these women displayed a decrease in sperm-egg fusion and an increased concentration of mitochondrial ROS and lipid peroxidation [87]. These results implicate OS as a potential mechanism by which cigarette smoke affects follicular development within the ovary and results in subfertility. Cigarette smoke is also associated with a diminished ovarian reserve, which may be another factor contributing to infertility in women who smoke [88]. Mice exposed to cigarette smoke or cigarette smoke condensate (CSC) for 4 weeks were found to exhibit reduced development of blastocysts in vitro, as a result of increased egg fragmentation and delayed fertilization. Additionally, the rate of abnormal embryos significantly increased in the mice exposed to cigarette smoke or condensate, and there was a dose-dependent relationship between exposure time and embryo apoptosis [89]. A decline in embryo quality could affect implantation, leading to impaired conception in women who smoke. It is also hypothesized that cigarette smoke alters uterine receptivity, which may lead to infertility. Oocyte donation is a model most often used to ascertain the role of uterine factors in reproductive outcome. A retrospective study by Soares et al., in which embryo quality was controlled, found that, among women receiving donor oocytes, non-smokers had a significantly higher pregnancy rate (52.2%) than women who smoked 10 or more cigarettes per day (34.1%) [90]. These results suggest that cigarette smoke may negatively affect the uterine tissue, resulting in lower pregnancy rates in women who smoke.

Impaired functioning of the fallopian tube could also contribute to infertility problems among women who smoke [91]. The oviduct has an important role in transportation of ovulated oocytes as well as in fertilization. A study in rats found that when they were exposed to either mainstream or sidestream smoke, 70–75% of embryos were retained in the oviduct, as compared to 35% retention rates in controls [92]. The contraction rate of muscles in the oviducts significantly slowed within 15 min of exposure to smoke, suggesting that cigarette smoke retards transport of embryos within the fallopian tube by slowing contractions of the muscle [92]. As evidenced, the adverse effects of cigarette smoke on ovarian, uterine and fallopian tube functioning, as well as on embryo quality are likely responsible for the decreased fertility, observed in women who smoke.

Cigarette smoking is associated with increases in OS and thus an increased risk of many health complications. Exposure to cigarette smoke extract is associated with the generation of the hydroxyl radical, leading to plasma membrane damage, as well as the production of H₂O₂ and O₂, which results in cell apoptosis [93]. Smokers have been found to have lower blood serum concentrations of vitamin C, carotenoids and selenium, as well as lower erythrocyte GSH peroxidase activity and increased erythrocyte CuZn-SOD activity, as compared to non-smokers [94]. As cigarette smoke has been demonstrated to both increase ROS and decrease antioxidant capacity, OS may develop in people who smoke.

Additionally, several studies have linked cigarette smoke-induced OS specifically to deficits in reproductive health. Paszkowski et al. found that as exposure to cigarette smoke increased, follicular fluid levels of final peroxidation products increased significantly (p < 0.001), and follicular fluid levels of cotinine, a biomarker for exposure to tobacco, were significantly correlated with these values (r = 0.471; p < 0.001) [95]. Furthermore, as cotinine concentrations increased, follicular fluid total antioxidant potential significantly decreased (p = 0.004) [95]. The impaired folliculogenesis observed in female smokers may be a result of the OS induced by this shifted balance between pro-oxidants and antioxidants in the follicular fluid. A study using a mouse model found that the eggs of mice have been exposed to cigarette smoke or CSC, had an increased level of fragmentation, and that the fragmented eggs exhibited an increase in ROS [89]. Cigarette smoking also leads to the development of the tar adducts in the oocytes and potentially impaired oocyte and embryo quality. Some of this damage can be repaired by the oocyte itself and the repairing capacity of the oocyte is critical for carrying out damage control [96].

Alcohol

The CDC reports that 43% of adult women in the USA are regular drinkers, defined as having consumed 12 or more alcoholic drinks within 1 year (CDC, 2012). Alcohol consumption may be a predictor for and cause of infertility problems in women. Many early studies concluded that alcohol had a negative impact on female fertility. Infertile women in Finland consumed significantly more alcohol and reported experiencing...
more hangovers during a period of 12 months than did fertile controls [97]. Tolstrup et al. also found that alcohol intake was significantly correlated with the incidence of infertility problems among Danish women aged 30 or older, although it did not predict infertility among younger women [98]. The odds ratio (OR) of conception also decreased as a woman’s alcohol consumption increased from one to five drinks per week (OR: 0.61; 95% CI: 0.40–0.93) to more than 10 drinks each week (OR: 0.34; 95% CI: 0.22–0.52; p = 0.03), as compared with women who did not consume alcohol [99]. Moderate and heavy alcohol intake has been correlated with increased infertility associated with both ovulatory factor and endometriosis [100], although this result has been inconsistent. More recent literature suggests that there may be no significant relationship between alcohol and infertility risk. For instance, Chavarro and colleagues found that the incidence of ovulatory infertility was unrelated to alcohol consumption (RR: 1.11; 95% CI: 0.76–1.64; p = 0.78) [101].

Maternal alcohol use may also be associated with spontaneous abortion. In a case-controlled study by Rasch in 2003, it was found that women who consumed five or more units of alcohol each week had a risk of spontaneous abortion five-times greater than that seen among women who did not drink alcohol [102]. However, Tolstrup et al., found no association between maternal alcohol consumption and increased risk of miscarriage [103]. Furthermore, moderate and high levels of alcohol consumption were not found to correlate with a delayed time to conception, as compared with women who did not drink alcohol [104]. In fact, women who did not consume alcohol experienced a longer time to conception than women who did drink (OR: 1.2; 95% CI: 1.1–1.3). Although some studies conclude that any amount of alcohol consumption is detrimental to fertility in women, the evidence is largely inconclusive.

The regular consumption of substances of abuse, including alcohol, has been linked to the generation of OS within the body. Although a smaller percentage of adult women than men reported using alcohol within the past month (49.4 and 62.3%, respectively), women may be more susceptible than men to alcohol-related problems at lower levels of the substance, due to differences in genetic and biological factors [202]. A recent study in rats found that the livers and esophagi of animals fed with a high concentration of ethanol and an irregular, vitamin-deprived diet contained higher levels of 8-oxoguanine, a common DNA lesion resulting from oxidative damage, than did rats fed with a low level of ethanol and a regular diet [105]. Chronic red wine consumption is also associated with a significant increase in urinary levels of 8-iso-PGF(2α), a marker of oxidative lipid damage, as compared with the consumption of de-alcoholized red wine (p = 0.006) [106]. Furthermore, any amount of maternal alcohol use during pregnancy caused a significant decrease in plasma GSH concentrations post-partum (p < 0.05), while alcohol consumption greater than three drinks per occasion was also associated with significant oxidation of the plasma GSH redox potential (p < 0.05) [107].

### Caffeine

Caffeine is the most widely consumed psychotropic drug in the world. Caffeine consumption is suspected to have negative effects on conception and pregnancy. Several studies have demonstrated the relationship between caffeine, infertility, and miscarriage. Wilcox et al., found that women who consumed more caffeine than the equivalent of one cup of coffee, had half the fecundability as compared to women drinking less caffeine [108]. There was also a dose-dependent relationship between caffeine consumption and pregnancy rates in these women. High levels of caffeine intake may also delay the time to conception. Women consuming >500 mg of caffeine per day had a significantly increased risk for subfecundity in their first pregnancy, and had an 11% increase in time to conception [109].

Not only the time to conception is affected by caffeine, but spontaneous abortion is also associated with caffeine [110]. Women aged between 20–29 years who consumed 75–900 mg of caffeine per day had an increased risk of spontaneous abortion compared to women whose pre-pregnancy caffeine intake was below 75 mg/day [105]. One study examining fetal karyotypes determined that women who consumed caffeine were more likely to abort a fetus with a normal karyotype in the first trimester than those women who were taking in little or no caffeine [110]. Several studies report negative outcomes associated with caffeine consumption during the first trimester such as increased chance of stillbirth and miscarriage [111]. One study conducted by Wisborg et al., in 2003 reported that women who drank four to seven cups of coffee a day had almost an 80% increased chance of stillbirth, and that for women who drank at least eight cups a day had almost a 300% increased chance of stillbirth [112]. However, a meta-analysis of 15 epidemiological studies concluded that, despite the fact that many researchers have reported positive correlations between caffeine consumption and spontaneous abortion, this evidence is equivocal at best, given that biases are likely present in the data that may lead to overestimation of any associations [113]. Furthermore, the current evidence is limited by confounding variables such as smoking and by measurement error, and thus the hypothesis that caffeine consumption negatively impacts fertility is not supported [114].

There is little agreement among researchers on the relationship between caffeine and OS. Caffeine is actually thought to exhibit antioxidant-like properties. Several recent studies have concluded that, by attenuating OS, caffeine may be protective against pathologies including alcoholic liver injury [115], galactose cataracts [116] and the neurodegeneration associated with Alzheimer’s [117]. Other research, however, provides evidence that caffeine may in fact induce OS. Azam et al., demonstrated that, in addition to its ability to suppress the production of hydroxyl radicals, caffeine and its catalytic products induce oxidative DNA damage when in the presence of copper ions [118]. Therefore, caffeine may possess both antioxidant and pro-oxidant capabilities. However, based on the current literature it seems unlikely that the effect caffeine may have on fertility is due to an oxidative mechanism.
Recreational drug use

Between the years 2007 and 2009, 11.4% of adult women reported using an illicit drug [202]. However, research on the effects of drug use on fertility is limited, due to ethical concerns and constraints that prevent randomized controlled human trials. Studies therefore are limited to epidemiologic or retrospective research. A potential confounding factor in these studies, however, is the reporting and confirmation of drug use by the individuals being studied. Nevertheless, there is a body of evidence that suggests that recreational drug use is detrimental to fertility and reproductive success.

Marijuana is the most used illegal drug among women of reproductive age. The use of cannabis and cannabinoid derivatives can have a negative impact on fertility and pregnancy outcome. The cannabinoid receptor CB1, which responds to the major psychoactive component of marijuana, Δ⁹-tetrahydrocannabinol (THC), has been localized in the female reproductive tract—specifically, in the ovary and uterine endometrium [199]. These cannabinoid receptors have been postulated to play a significant role in the maintenance and regulation of early pregnancy; thus, any disruptions to the functioning of the CB1 receptor could have a negative impact on reproduction. Marijuana smoke can also act on estrogen receptors [120], and THC has been shown to shorten the menstrual cycle [199], potentially disrupting fertility. Cannabis use has been associated with increased risks of infertility among women. A study by Mueller et al., in 1990 found that women who smoked marijuana had a slightly elevated risk of ovulatory abnormality-induced infertility (RR: 1.7; 95% CI: 1.0–3.0) as compared to pair-matched controls, and that risk was greatest among women who smoked marijuana in the year preceding pregnancy (RR: 2.1; 95% CI: 1.1–4.0) [121]. Among the women with ovulatory abnormalities, 33% and 16% were oligo- and amenorrheic, respectively, and 34% had a luteal phase defect [121]. Marijuana use can also directly affect fetal health. Maternal cannabis use during pregnancy was associated with decreased fetal growth and lower birth weight [122]. In fact, fetuses exposed to cannabis in utero exhibited a reduction in growth of -14.44 g/week as pronounced than that associated with tobacco exposure [122].

Marijuana is associated with the induction of OS. Endothelial cells exposed to marijuana smoke containing 3.95% THC had 80% increase in levels of ROS, as well as an 81% decrease in GSH levels as compared with controls [123]. Acute exposure to marijuana smoke containing any level of THC also induced necrotic cell death in the endothelial cells [123]. However, more recent studies have found that cannabinoids have antioxidant properties, and may in fact reduce the OS caused by other illicit drugs, such as 3,4-methylenedioxyamphetamine (MDMA) [124].

Cocaine is another recreational drug associated with infertility and the generation of ROS. Cocaine use has been found to significantly increase a woman’s risk of developing tubal factor infertility (RR: 11.1; 95% CI: 1.7–70.8) [121]. Furthermore, a study by Thyer et al., in nonhuman primates found that cocaine administration led to a decrease in ovarian responsiveness to exogenous gonadotropins, suggesting that cocaine has a direct effect on the ovary and transiently disrupts ovulatory function [125]. Increased levels of ROS were found in both the frontal cortex and striatum of rats treated with either an acute injection or chronic injections of 20 mg/kg of cocaine over a period of 10 days; this increase in ROS was accompanied by an increase in SOD and GPx [126]. Cocaine-induced oxidative damage has also been reported in heart, liver and kidney tissues [127]. Impaired mitochondrial function, per-oxidation of phospholipid membranes and depletion of antioxidant defenses are the proposed mechanisms by which cocaine-induced OS may lead to cell damage and apoptosis [127,128]. Although the current evidence is suggestive of a negative impact on fertility due to cocaine use, further research is required for better understanding of its effects.

Psychological stress

The relationship between stress and infertility is complex and cyclical. Infertility problems may themselves cause significant levels of stress [129]. Although stress from social and job-related sources may lead to subfertility in women, the personal distress caused by infertility itself may perpetuate this cycle. Women are more likely to report fertility-related, personal and social stress than men [129], suggesting that stress may have a more significant effect on females. Domar et al., reported that approximately 37% of infertile women show depressive symptoms [130].

Cortisol, a hormone associated with stress, is suspected to suppress the secretion of gonadotropins, leading to impairments in the development of follicles and in ovulation. This is expected to contribute to the subfertility associated with psychological stress. A study in sheep determined that the infusion of concentrations of cortisol comparable to those generated in conditions of stress, attenuated or blocked expected increases in serum estradiol as well as the LH surge [131]. This suggests that cortisol is responsible for a delay in follicular maturation and ovulation. Salivary α-amylase levels were also negatively associated with probability of conception in women between the age of 18 and 40 years, suggesting that stress may contribute to female subfertility [132].

Job-related stress is another potential risk factor for infertility problems in women. An Italian study found that female traffic police officers exposed to urban psycho-social stressors had increased plasma concentrations of LH in both the follicular and luteal phases of the ovarian cycle, which could lead to reproductive health disorders [133]. Furthermore, women who work 32 or more hours/ per week have a significantly longer time to pregnancy than women who work between 16 and 32 h per week [134].

The role of emotional stress and anxiety in pathology is not well understood or thoroughly researched. However, a small body of evidence suggests that the induction of OS may be the mechanism by which psychological stressors affect overall health. In men, increased levels of mental stress are associated with higher seminal plasma levels of the SOD as compared to...
periods of non-stress, which suggests that antioxidant defenses increase during stressful conditions in order to counterbalance an increase in the production of ROS [135]. Furthermore, researchers found significantly higher levels of 8-OHdG in liver nuclear DNA of rats as compared to controls following the rats’ second, third and fourth exposures to psychologically stressful stimuli (p < 0.01), suggesting that psychological stress may be responsible for oxidative DNA damage [136].

A potential limitation of the current research regarding stress is the difficulty of accurately measuring levels of stress. Studies on stress may implement a number of different techniques for quantifying stress levels, including self-report, concentrations of biomarkers such as cortisol or salivary amylase or stimulation response, limiting the generalizability and reproducibility of the results. Future research should focus on developing a standardized protocol for quantifying levels of stress, in order to enhance the current understanding of the relationship between stress and health. There is also a difficulty of quantifying stress through self-report, biologic marker or stimulation response, limiting the reproducibility and generalizability of much of this field and requiring future developments in methods to enhance understanding of these relationships.

Environmental & occupational exposures
Chlorinated organic compounds such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) are environmental contaminants that have adverse effects on fertility. Many chemicals and pesticides are endocrine disrupting agents. High blood concentrations of organochlorine compounds have been found to cause menstrual abnormalities, spontaneous abortions and longer times to conception in women [137]. Prenatal exposure to organochlorines can have several negative effects on fetal development and neonatal health. A study by Herrero-Mercado et al., found that concentrations of organochlorine pesticides increased from maternal adipose tissue, to maternal blood serum, to umbilical cord blood serum, suggesting that maternal adipose tissue releases organochlorine pesticides into the blood, and that these pesticides are transferred to the fetus via the umbilical cord blood [138]. Neonicotinates that were exposed to organochlorine compounds, such as DDT and PCBs, in utero had reduced birth size parameters, including decreased birth weights, lengths and head circumferences [139]. Furthermore, many cosmetics and personal care products for women contain endocrine-disrupting chemicals such as phthalates and bisphenol A (BPA). Parlett, Calafat and Swan found that women who used perfume had a 2.92-times higher urinary concentration of monoethyl phthalate than in other women (95% CI: 2.20–3.89) [140]. In a study of 56 couples experiencing infertility, infertile women had significantly higher urinary concentrations of mono-ethylphthalate (p < 0.001) than in a control group of women who had previously given birth [141]. Despite a growing body of evidence, conclusions regarding the effects of pesticides and organochlorine compounds on female fertility and fetal health remain controversial, as many studies have been unable to show significant dose-response relationships and results are often inconsistent with previous observations [142].

Exposures to organochlorine compounds such as PCBs, HCB, DDT and DDE, as well as to pesticides and other environmental pollutants, are associated with increased levels of ROS and oxidative damage. PCBs and PCB metabolites have been hypothesized to increase OS, which may result in cytotoxicity and increase susceptibility to disease. Workers in electrical factories in China exposed to high levels of PCBs had significantly increased urine concentrations of 8-OHdG in samples collected post-work (24.55 +/- 5.96 μmol/mol creatinine), as compared with samples collected before work (6.40 +/- 1.64 μmol/mol creatinine; p < 0.05) [143]. Studies in MCF-10A human breast and RWPE-1 human prostate epithelial cells have demonstrated that continuous exposure to 3 μmol/mol PCBs induces apoptosis, as well as increases intracellular concentrations of both superoxide and H2O2; treatment with the antioxidant N-acetylcysteine up to 1 h after exposure may attenuate these effects [144]. A recent study by Pathak et al., determined that pre-term delivery was associated with significantly increased markers of OS, including increased levels of MDA and protein carbonyls and decreased GSH activity, as well as increased concentrations of organochlorine pesticide (OCP) residues in both maternal and cord blood, and that there was a significant positive correlation between OS and OCP residue levels [145]. Pesticides such as organophosphates are also associated with markers of OS. Agricultural workers exposed to organophosphate pesticides were found to have lower levels of SOD and GSH reductase activity as compared to controls [146]. Urban air pollution has also been linked to the induction of OS, as evidenced by increased levels of 8-isoprostane [147] and MDA [148].

Methylparaben is present in almost all cosmetics. This substance acts as a strong xeno-estrogen and considered as an endocrine disruptor. It is important to highlight the adverse impact of this substance, which is also present in surface waters and in refined natural fibers, such as cotton and silk used in ready-made clothes. Studies have reported that there is diminished ovarian reserve associated with exposure to the paraben [149].

Impact of lifestyle on assisted reproductive techniques
The outcome of IVF and intracytoplasmic sperm injection (ICSI) treatments may be negatively affected by several of the lifestyle factors previously discussed. Obesity has one of the most severe effects on IVF treatment. Within a single cycle of IVF, the pregnancy rate for obese women has been shown to be half of that for women within a normal weight range [150]. In a retrospective study of 6500 IVF-ICSI cycles, obese women had significantly lower embryo implantation rates, lower pregnancy rates and live birth rates than women with a normal weight. In fact, there was a significant decrease in pregnancy rate (OR: 0.984; 95% CI: 0.972–0.997; p = .016) and live birth rate (OR: 0.981; 95% CI: 0.967–0.995; p = .009) with
each increasing unit of BMI [151]. Increases in pregnancy complications after conception with assisted reproductive techniques (ART) were also associated with maternal overweight and obesity [152]. Overweight and obese women also had increased risks of early pregnancy loss in IVF and ICSI [153], and very early preterm birth in IVF treatments [154]. Using data from the society for assisted reproductive technology clinic online reporting system (SART CORS), Luke et al., evaluated a total of 45,163 ART embryo transfers. They found that increasing obesity was significantly correlated with failure to achieve a pregnancy using autologous oocytes (p < 0.0001), as was failure to achieve a live birth, although there was no significant correlation when donor oocytes were used [155]. Furthermore, Syncross et al., found no difference in implantation or pregnancy rate between obese and healthy women who were using donated oocytes, suggesting that endometrial receptivity is not impaired in obese women [156]. In a sample of 1721 women undergoing IVF, women with BMIs >35 were also found to have fewer normally fertilized autologous oocytes than women of a normal weight (p < .03), even when adjusting for age [157]. Although weight loss may seem to be a logical treatment option to consider before undergoing ART, weight loss may not confer any significant benefit to ART outcome. Chavarro et al. reported in a sample of 170 women, although short-term weight loss was associated with a higher number of metaphase II oocytes retrieved, it was unrelated to successful pregnancy and live birth rates [158].

Underweight and low energy balances caused by excessive exercise have been shown to have a slight, but significant negative impact on ART outcome. Underweight women in China had a lower rate of clinical pregnancy with IVF or ICSI treatment (31.1%) as compared to healthy weight controls (37.1%), although there were no differences in the amount of ovarian stimulation required between these groups [159]. Of women who were able to conceive within a cycle of IVF-ICSI, underweight women had an increased risk of miscarrying, and this risk was even greater if hormonally substituted frozen-thawed embryo cycles were utilized [160]. Underweight women also had fewer developed embryos available in each IVF or ICSI cycle [161]. Exercising for 4 or more hours per week was associated with a 40% decrease in live birth rate, a threefold increase in cancellation of cycles and a twofold increase in implantation failure and pregnancy loss with the first cycle of IVF, as compared to women undergoing IVF who did not exercise [162]. These results demonstrate that maternal weight is an important factor influencing the outcome of assisted reproductive techniques.

Cigarette smoking among infertile women seeking ART can also be detrimental to the outcome of IVF and ICSI procedures. A meta-analysis with nine selected studies found that among women undergoing IVF treatments, there was a significant reduction in fertility in smokers (OR: 0.66; 95% CI: 0.49–0.88) [85]. Another recent meta-analysis of 21 studies found that women who smoked had significantly lower odds of both live birth (OR: 0.54; 95% CI: 0.30–0.99) and clinical pregnancy (OR: 0.56; 95% CI: 0.43–0.73) per cycle. These women also had significantly higher odds of both spontaneous miscarriage (OR: 2.65; 95% CI: 1.33–5.30) and ectopic pregnancy (OR: 15.69; 95% CI: 2.87–85.76) with each cycle of ART [163].

Substances of abuse such as alcohol, recreational drugs and caffeine have been demonstrated to negatively impact IVF. Women who consumed four or more alcoholic drinks per week had a 16% decrease in live birth rate with IVF, as compared to women who consumed fewer than four drinks each week [164]. Drug abuse, such as with marijuana and cocaine, can be potentially detrimental to ART outcomes. Women who had smoked marijuana within the year preceding IVF or gamete intrafallopian transfer (GIFT) treatment had 25% fewer oocytes retrieved, as well as smaller infants at birth [165]. As stated previously, research in this area is limited, and no information regarding the effects on cocaine on ART have been published to date. Caffeine may also be harmful to assisted reproductive processes. Overall caffeine consumption was negatively correlated with the number of oocytes retrieved for IVF treatments, while coffee intake was associated with pregnancy loss and tea intake was associated with a decrease in embryo quality [166]. Despite these correlations, no association between caffeine consumption and pregnancy rate with IVF was found [166]. Although evidence that substance abuse affects ART outcome is limited, these findings suggest that alcohol, illicit drugs and caffeine may have a negative impact on assisted conception.

Several studies have demonstrated that psychological stress has a significant, if only slight, impact on conception with IVF and ICSI. The IVF and ICSI treatments are often the sources of stress themselves. The incidence of depressive or anxious symptoms was 18.5% in infertile women undergoing IVF treatment [167]. Baseline levels of psychological stress are negatively associated with clinical pregnancy rate in women undergoing IVF [168]. Both infertility-specific stress, including social, sexual and relationship concern, need for parenthood and rejection of a childless lifestyle; and nonspecific anxiety, including feelings of tension, nervousness, worry and perception of the environment as threatening, were negatively associated with IVF outcome [169]. An et al. in 2011 also found that in women undergoing IVF or ICSI, State Anxiety Inventory scores were positively correlated with norepinephrine and cortisol concentrations in serum and follicular fluid, and that elevated norepinephrine and cortisol concentration were associated with failure to become pregnant [170]. This finding was supported by Li et al., who found that an increased level of norepinephrine in follicular fluid was associated with a decrease in the percentage of good embryos [168]. A recent meta-analysis of 31 prospective studies also found a slight but significant correlation between stress, defined as perceived stress, job-related stress or minor life events; distress, defined as the presence of anxiety or depression; and clinical pregnancy rate in women undergoing ART procedures [171]. These studies suggest that psychological stress, whether it originates as a result of IVF and ICSI treatments...
or from another source, can have a negative impact on the outcome of assisted reproduction.

Environmental exposures can negatively affect conception and pregnancy with ART, and over 50% of women attending IVF clinics have detectable levels of organic chemicals in their sera and follicular fluids [172]. Chlorinated organic compounds such as PCBs, HCB, DDT and DDE are environmental contaminants that can have adverse effects on IVF and ICSI outcomes. A prospective study by Meeker et al., found that maternal blood serum levels of PCBs were significantly and dose-dependently correlated with elevated odds of implantation failure and decreased odds of live birth among 720 women undergoing IVF-ICSI [173]. Serum concentrations of HCB were also demonstrated to be significantly, dose-dependently correlated with decreased implantation rates among women undergoing IVF, although no significant associations were found between DDT and implantation rate [174]. Serum and follicular fluid levels of DDE were negatively correlated with fertilization rate [172].

Conclusions

There are cumulative evidence in the literature that lifestyle factors such as weight, exercise, cigarette smoking, alcohol and caffeine consumption, recreational drug use, stress and environmental exposures are detrimental to both natural and assisted fertility in women, and that these factors likely mediate their effects, at least in part, through an oxidative mechanism. Therefore, lifestyle modification may be the most effective way to counteract these negative effects, to improve fertility potential and to maximize a woman’s chances for a healthy and successful pregnancy. Women experiencing infertility may turn to assisted reproductive techniques in order to better their chances of achieving a pregnancy and having a child. However, the very causes of infertility in these women can also potentially be detrimental to ART procedure outcome. Therefore, it may be optimal to implement lifestyle changes before proceeding with assisted reproductive techniques, in order to attenuate the negative effects and maximize the potential for a positive outcome. However, the underlying cause of infertility can be treated and natural fertility potential can be improved, ART procedures may no longer even be necessary for these women. However, for many women experiencing infertility that turn to ART, age may be the most critical factor to address. In these cases, it may be best to proceed with ART, rather than to delay these procedures in order to first implement lifestyle changes.

Several studies have demonstrated the benefits that lifestyle modification, especially in regards to weight loss, may have in regards to female fertility. Dei et al. found that a greater change between current and premorbid BMI was a predictor of the resumption of menses in women with amenorrhea associated with low BMI [175]. A prospective study, which followed a group of obese (average BMI 38.7 ± 6.8), infertile and anovulatory women as they underwent a diet and exercise program, found that after a significant average weight loss of 6.3 ± 4.2 kg, 12 of the 13 subjects resumed ovulation and 11 were able to achieve pregnancy [176]. More extreme weight-loss procedures, such as bariatric surgery, may be detrimental to fertility in women. Although current research suggests that weight loss from bariatric surgery may improve ovulation, and thus spontaneous pregnancy rates in obese women, pregnancies in women who have undergone these procedures are often considered high risk [177]. Further research into the effects that other lifestyle modifications, such as smoking cessation, reduced alcohol and caffeine consumption and decreasing stress levels may have on fertility is greatly needed, as these fields remain relatively unexplored.

Another potential therapy for infertility caused by oxidative damage is antioxidants. Administration of antioxidants may significantly improve the imbalance between ROS and antioxidant defenses observed in infertile women [178]. A clinical trial of the nutraceutical Improve, which contains the antioxidants CoQ10, astaxanthin and anthocyanidines, essential omega-3 fatty acids, zinc and folic acid, in combination with fish oil for women found that the probability of conception and successful pregnancy was improved with supplementation of the nutraceutical in infertile women conceiving naturally or using ART [178]. It is recommended that treatment begin at least 6 weeks before the ART procedure and be continued for 2 weeks following IUI or IVF.

Current evidence is limited and lacking in well conducted RCT for assessing the impact of antioxidants in subfertility. There is a need for future well-designed randomized trials with adequate power to provide conclusive evidence. The trials need to follow strict randomization of patients to intervention or placebo and describe their allocation concealment methods. This will ensure that the future trials will be of the highest quality and has definite clinical implications.

Expert commentary

Lifestyle modification should be considered as a therapeutic option for women experiencing infertility. Radical surgical procedures, which may in fact be detrimental to fertility, should be avoided or considered only if attempts at lifestyle modification are unsuccessful. Antioxidants may be another alternative or complementary therapeutic approach. However, in some cases, such as when the age of the woman is of greatest concern, it may be best not to delay ART procedures to first implement lifestyle changes. Ultimately, although lifestyle modification appears to be potentially protective of fertility and a possible avenue for the treatment of infertility, future research must elucidate the duration of the benefits these lifestyle modifications may confer, as well as the time it may take from the implementation of modification to the recovery of reproductive function.

Five-year view

More research is needed into the potential benefits of lifestyle modification for the treatment of infertility in women. Although it seems evident that weight loss greatly improves
fertility potential among obese women, it remains to be seen if the negative reproductive effects caused by smoking, alcohol, caffeine, recreational drugs, stress and the environment are reversible and are able to be attenuated by ceasing or reducing the frequency of these behaviors and aspects of life. Furthermore, with our increasingly active society, and as everyday life stress, chronic depression and anxiety disorders become more prevalent, it is necessary for research to focus on determining the long-term implications that psychological factors may have on health, including fertility. The potential for antioxidants and nutritional supplements to have significant benefits in the attenuation of OS and the improvement of fertility and pregnancy outcome must be fully explored.

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### Key issues

- A number of conditions affecting fertility in women, including endometriosis, polycystic ovary syndrome, recurrent pregnancy loss and unexplained infertility are associated with a systemic increase in reactive oxygen species (ROS) or oxidative stress (OS).
- Obesity is linked to ovarian dysfunction, compromised oocyte and embryo quality and longer times to conception. Positive correlations have been found between increasing BMI and ROS, as well as negative correlations between BMI and antioxidants.
- Being underweight can be a risk factor for infertility. Women who have eating disorders do not take enough micronutrients and therefore have lower antioxidant levels. Women who are underweight tend to have higher levels of OS markers.
- Excessive exercise can negatively impact fertility by increasing oxygen consumption, and thereby increasing the ROS released by cells. Nutritional supplements and antioxidants may be protective against exercise-induced OS.
- Impaired ovarian function and decreased oocyte and embryo quality are often associated with women who smoke cigarettes. Smoking has been associated with a decrease in serum antioxidant levels as well as increases in ROS.
- Maternal alcohol consumption is associated with a longer time to pregnancy and elevated risk for spontaneous abortion, as well as with increased levels of markers of oxidative damage.
- Women who use recreational drugs and consume caffeine are more likely to develop infertility associated with a tubal factor or an ovulatory-abnormality, and have increased risks of spontaneous abortion and still birth, respectively. The substances have been found to possess both pro-oxidant and antioxidant capabilities.
- Psychological stress may be responsible for subfertility, potentially by disrupting the production of key hormones. Although to date there is little conclusive evidence, there may be a causal link between stress and an increase in oxidative DNA damage.
- Environmental and occupational exposure to chemicals is associated with menstrual abnormalities, spontaneous abortion and pre-term delivery. Levels of polychlorinated biphenyls, pesticides and urban air pollutants are correlated with markers of OS.
- The outcomes of IVF and intracytoplasmic sperm injection procedures may also be negatively affected by the oxidative damage associated with lifestyle factors such as weight, exercise, recreational and illicit substances, stress and environmental exposures.
- Lifestyle modification should be the first course of treatment to improve fertility potential. Antioxidants and other supplements may confer additional benefits by further combating ROS. Assisted reproductive technology should be considered only if less extreme, minimally invasive methods are unsuccessful.

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**An important review of the effects of oxidative stress on the embryo.**


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This study provides an observational evidence base on the association of caffeine intake with miscarriage and stillbirth.


Lifestyle factors & oxidative stress in female infertility

Review


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Fedorecsak P, Dale PO, Storeng R et al.


• The article investigates the challenges of treating infertility in women with high BMI.


• A significant meta-analysis on the adverse effects of smoking. The findings of the study can help in counseling patients.


• The article highlights the dietary approaches for enhancing natural and assisted fertility.

Websites