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INVITED REVIEW

Male Fertility

The hazardous effects of tobacco smoking on male fertility

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The substantial harmful effects of tobacco smoking on fertility and reproduction have become apparent but are not generally appreciated. Tobacco smoke contains more than 4000 kinds of constituents, including nicotine, tar, carbonic monoxide, polycyclic aromatic hydrocarbons, and heavy metals. Because of the complexity of tobacco smoke components, the toxicological mechanism is notably complicated. Most studies have reported reduced semen quality, reproductive hormone system dysfunction and impaired spermatogenesis, sperm maturation, and spermatozoa function in smokers compared with nonsmokers. Underlying these effects, elevated oxidative stress, DNA damage, and cell apoptosis may play important roles collaboratively in the overall effect of tobacco smoking on male fertility. In this review, we strive to focus on both the phenotype of and the molecular mechanism underlying these harmful effects, although current studies regarding the mechanism remain insufficient.

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INTRODUCTION

Tobacco consumption is directly responsible for nearly 6 million deaths annually, and a further 600 000 people die each year from exposure to second-hand smoke (SHS).¹ Tobacco is killing 1 in 10 adults worldwide and its quantity of consumption is increasing globally especially in developing countries according to WHO statistical data.² Many studies published during the past years indicated that tobacco smoking is one of the greatest risk factors of more than 60% noncommunicable diseases.³ Among all the negatives associated with tobacco consumption are decreased in both male and female fertility. In this review, we sought to assess the association between tobacco smoking and male fertility. The studies of the association between tobacco smoking and male fertility included in this review were identified by searching the database PubMed and Google Scholar for manuscripts published between 1980 and 2014 and by reviewing articles cited by other investigators. Keywords used to assess exposure, outcome, and estimates for the concerned associations were: tobacco smoking, male fertility, semen, sperm, and spermatogenesis.

HAZARDOUS SUBSTANCE IN TOBACCO SMOKE

Tobacco smoke contains more than 4000 kinds of constituents including nicotine, tar, carbonic monoxide, polycyclic aromatic hydrocarbons, radioactive substances, heavy metals, etc. Here, we strive to elaborate three of these substances which are closely related to male fertility according to the researches in recent years.

Nicotine

As a main hazardous substance and lethal toxin in tobacco, nicotine was detected in both the serum and semen of smokers, and the level of this substance showed a positive correlation with the dose of smoking exposure.⁴ Cotinine is a metabolite of nicotine and is also an alkaloid found in tobacco. *In vitro* studies have indicated that the seminal

cotinine and trans-3'-hydroxycotinine levels were negatively correlated to total sperm motility.⁵ The toxicological effects of nicotine were proven to be dose-dependent. *In vitro* study found that neither nicotine nor cotinine in low concentration (≤ 1 mmol l⁻¹) were conclusively harmful to sperm motility, but a higher nicotine concentration (≥ 1 mmol l⁻¹) decreased the sperm motion and viability parameters significantly.⁶ Condorelli *et al.*⁷ reported that nicotine suppressed sperm progressive motility in a concentration-dependent manner starting from the relative low concentration (1 ng ml⁻¹) *in vitro*. At higher concentration (100 ng ml⁻¹), nicotine reduced the percentage of viable spermatozoa and promoted spermatozoa apoptosis with DNA fragmentation or altered the chromatin compactness. This study indicated that nicotine may be considered as a toxic component of tobacco smoke that directly impairs male reproductive functions. Other studies revealed that nicotine could induce mouse Leydig cell apoptosis and inhibit androgen biosynthesis in rat Leydig cell,⁸ suggesting the possibility that nicotine may impaired male reproductive hormone system.

The *in vivo* reproductive effects of nicotine have been assessed in many animal studies. In male reproductive system, nicotine (0.5 mg kg⁻¹) could decrease sperm motility and count while increase the percentage of sperm abnormality in rats.^{9,10} Oyeyipo *et al.*¹¹ focused on the alteration of the male reproductive hormones caused by nicotine exposure. Results showed that nicotine intake significantly decreased testosterone level in rats. The follicle-stimulating hormone (FSH) level decreased only in the high-dose group; however, the mean luteinizing hormone (LH) and prolactin (PRL) level increased compared with those found in the control group. A more comprehensive research assessed testicular gametogenesis, steroidogenesis, and expression of steroidogenic regulatory protein in nicotine treated rats. Activities of the testicular key androgenic enzymes, testosterone concentrations in intratesticular and plasma, and

plasma concentration of gonadotropin were significantly reduced. These reductions were accompanied by the alterations of testicular antioxidant status and dysfunction of spermatogenesis as indicated by a significant reduction in the number of different generations of germ cells at specific stages in spermatogenesis cycle.¹² Nicotine also caused degenerative changes in the seminiferous tubules, revealed by altered general tubular architecture, decreased thickness of the spermatogenic cell masses, Sertoli cell vacuolation, and thickened basal lamina.¹³ These results indicated comprehensive effects of nicotine on male reproduction which involved oxidative stress in the gonads¹⁴ and dysfunction of reproductive hormone system. In addition, Sofikitis *et al.*⁵ observed that spermatozoa exposed to cotinine demonstrated significantly lower outcomes in sperm motility assays, hypoosmotic swelling test, and zona-free hamster oocyte sperm penetration assay. These findings indicated a detrimental effect of nicotine on the function of sperm membrane and the ability of spermatozoa to undergo capacitation.

Heavy metals (cadmium and lead)

Cadmium (Cd) in soil enriched in tobacco and smoking is the primary source of Cd intake in the ordinary population.¹⁵ Cd exhibits an extremely long biological half-life in the human body (20–40 years) which makes it an accumulative toxin.¹⁶ Elevated blood Cd and seminal Cd in smokers were observed, and smoking habits (cigarettes per day) were significantly correlated with Cd level.¹⁷ Animal study clearly illustrated the reproductive toxicity caused by Cd exposure including reduction of sperm cell numbers and sperm motility with increases in DNA fragmentation and sperm abnormality.¹⁸ This result is basically consistent with those of epidemiological studies before.¹⁷

Higher levels of lead (Pb) were also observed in serum and semen of both fertile and infertile smokers.^{19,20} In the animal study, Pb exposure decreased alkaline phosphatase and $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity with a dose effect indicating that Pb could impair sperm motility.²¹ The relationship between Pb exposure and male fertility in general population remains vague, but some indications have been described.²² Moderate exposure to Cd and Pb was not proved to impair male reproductive endocrine function but attenuated human semen quality significantly.²² This finding indicated that heavy metals from tobacco may impair male fertility though more direct ways which will be discussed later.

Benzo(a)pyrene

Benzo(a)pyrene is found in smoke resulting from tobacco combustion, it mainly presents in tobacco tar and acts as a mutagen and carcinogen.²³ The major diol epoxide (DE-I) binds covalently to DNA strand and forms adducts called benzo(a)pyrene diol epoxide-DNA. These adducts are permutation lesions in Guanosine nucleotides which could be potential sources of carcinogenic damage. It is reported that BPDE-DNA adducts in spermatozoa were elevated by tobacco consumption.²⁴ An *in vitro* study showed that benzo(a)pyrene ($\geq 25 \mu\text{g ml}^{-1}$) significantly reduced the percentage of acrosome halo formation.²⁵ These findings indicated that benzo(a)pyrene could be considered as a major etiology of DNA damage in smokers.

SMOKING AND TESTIS FUNCTIONS

The primary functions of the testis are spermatogenesis and production of androgenic hormones. The androgenic hormones secreted by testis play important roles in maintaining the spermatogenic niche and the spermatogenesis process.²⁶ Thus, we will discuss the effects of smoking on reproductive hormones and the spermatogenesis process here.

Reproductive hormones

Tobacco smoking may cause sub-fertility by influencing the level of

reproductive hormones,²⁷ but the specific effects remain uncertain. With regard to androgens secreted from Leydig cell in the testis, some studies have indicated increased levels of total testosterone and androstenedione in smokers.²⁸ Meanwhile, other studies put forward different views that the mean levels of testosterone (T) and dehydroepiandrosterone did not differ in smokers.²⁹ Another research team evaluated the hormonal levels of fertile men, which were divided into heavy, moderate and mild smokers and found nonsignificant difference in the testosterone level.²⁹ Moreover, estrone,³⁰ 17 beta-estradiol and Sertoli cell secreted inhibin²⁸ were also up-regulated in smokers according to some researches. Because of the relative small sample size and the widespread confounding factors in these observational studies, animal studies may provide some more convincing results. These studies implied that chronic cigarette smoking increases hepatic metabolism of testosterone and may lead to a reduced testosterone level in serum. In conclusion, secretory dysfunction of Sertoli and Leydig cells in the testis may be the cause of the impaired sperm quality observed in smokers.

Follicle-stimulating hormone and LH are synthesized in the pituitary gland and act as important regulating hormones in Sertoli cell and Leydig cell function. Ochedalski *et al.*³¹ reported that the 17 beta-estradiol (E) level was elevated and the levels of FSH, LH, and PRL were lower in smokers. Blanco-Muñoz *et al.*²⁸ observed the male reproductive hormone profile in a group of 136 flower growers and concluded that current smokers who consumed 5 or more cigarettes per day showed significantly higher levels of LH compared with nonsmokers.

Spermatogenesis

An impaired reproductive hormone system bound to affect spermatogenesis process. The testis is considered to be physiologically vulnerable to hypoxia because of its high metabolic requirements owing to continuous spermatogenesis process and its relative insufficient vascular supply in the spermatic cord.³² Therefore, tobacco smoking compromised oxygen delivery³³ and was suspected to have a detrimental effect on testicle functions. Animal study detected several ultrastructural effects of smoking on the testis. The basal lamina of the seminiferous tubule was significantly irregular and thickened in rat testis after daily tobacco smoke exposure.³⁴ We assume that histological detriment in testis bound to affect spermatogenesis. And more intuitively, chronic cigarette smoke was also found to induce apoptosis in mouse testis,³⁵ and the number of germ cell, Leydig cell and Sertoli cell reflected concurrent reductions.³⁶ Xu *et al.*³⁷ investigated 31 differentially expressed proteins extracted from the testes of mice exposed daily to cigarette smoke using matrix-assisted laser desorption/ionization time of flight mass spectrometric (MALDI-TOF-MS) analysis. Most proteins are involved in cell signal pathway networks, including Pkc (s), ERK1/2, Akt, and nuclear factor- κB , which were known to be crucial in spermatogenesis process. They also found four CpGs near Pebp1 transcriptional start site were hypermethylated in the testis of the smoking group. This result suggests a research direction involving modified DNA methylation patterns due to tobacco smoking.

SMOKING AND EPIDIDYMIS FUNCTIONS

During the transit in the epididymis, sperm undergoes maturation processes necessary to acquire normal functions. Detached ciliary tufts (DCTs) in semen originated from the epididymal epithelium and were considered as a biochemical evidence of epididymal dysfunction.³⁸ It is reported that the DCTs observed in the semen were associated with a high incidence of tobacco smoking.³⁸ This finding may suggest the epididymal involvement as a result of the reproductive pathology caused by tobacco smoking. Smoking exposure was also found to attenuate the activity of sorbitol dehydrogenase while increase the activity of lactate

dehydrogenase in rat epididymis.³⁴ The changes of these two enzymes reflected the inhibitory effect of smoking on sperm maturation. Zhu *et al.*³⁹ utilized 2-D gel electrophoresis and MALDI-TOF-MS analysis to screen the different expressed proteins in mice epididymis treated with tobacco smoking and identified 27 proteins which show significant alterations in protein profile. These proteins participated in energy metabolism, protein process, and oxidative stress process. This result implied the mechanism of impairments in epididymis due to tobacco smoking.

Effect of tobacco smoking on semen quality

The relationship between tobacco smoking and semen quality remains controversial even after 30 years research. **Table 1** shows some representative epidemiological researches involved in the relationship between tobacco smoking and semen quality. Most studies reported reductions in the conventional semen parameters including semen volume, sperm density, motility, viability, and normal morphology in smoking population. Specifically, all of the previous studies indicated that smoking had a negative effect on sperm quality in fertile population. However, the conclusions in the infertile and hypo-fertile population appeared confusing. Some studies⁴⁰⁻⁴² argued a conclusion as which are found in the fertile population, meanwhile Mak *et al.*⁴³ found no difference between the semen quality of infertile smokers and infertile none-smokers. A similar finding was also reported in hypofertile population.⁴⁴ Notably, the researches with negative conclusions all observed nonsignificantly ($P > 0.05$) reduced semen quality. These results may be due to the already lower semen quality in infertile and subfertile population. Because of the direct relationship between semen quality and male fertility, the above-mentioned epidemiological studies provided a clear indication about the reduced male fertility caused by tobacco consumption.

SMOKING AND SPERMATOZOA FUNCTIONS

Among all the conventional parameters, sperm motility is the most relevant indicator of fertilization. Sperm creatine kinase (CK) is an energy reservoir for the rapid buffering and regeneration of adenosine triphosphate and plays an important role in sperm motility. The reduced CK activity in sperm caused by smoking may potentially impair sperm energy homeostasis and is associated with lower sperm motility.⁶¹ As a key protein participates in fertilization, acrosin is released from the acrosome of spermatozoa as a consequence of the acrosome reaction. Smokers exhibited lower acrosin activity,⁶² and this is correlated with sperm density and motility. Moreover, the inducibility of the acrosome reaction was significantly lower in semen samples from smokers.⁶³ Zalata *et al.*⁶³ reported that acrosin activity was diminished by oxidative stress, indicating the possible mechanism underlying the effect of smoking on acrosin activity. An *in vitro* study revealed the inhibitory effects of tobacco smoking on capacitation-associated protein phosphorylation in human spermatozoa.⁶⁴ These changes in posttranslational modifications may be one of the mechanisms through which exposure to tobacco can negatively affect sperm functions and cause fertility problems. In addition to the decreased semen quality, acrosin activity and protein phosphorylation, tobacco smoking has been proved to have impairments to other functions of spermatozoa such as expression of miRNAs and histone-to-protamine transition.⁶⁵⁻⁶⁷

Ultra-structural abnormalities in spermatozoa

Structure determines function. Diversifications in the arrangement and quantity of axonemal microtubules and axonemal abnormalities have long been observed in heavy smokers.⁶⁸ Electron microscopy revealed coiling of tail filaments within the plasma membrane and the percentage of coiled spermatozoa was significantly correlated with heavy smoking.⁶⁹ The fertility index measured by sperm morphology which was assessed

Table 1: Relevant studies concerning smoking effect on semen quality

Conclusion(s)	Sample capacity	References
Smokers had lower sperm motility and a greater percentage of oval spermatozoa	1641F	Close <i>et al.</i> ⁴⁰ , 1990
Smokers had reduced sperm motility and higher changes in sperm morphology	1691F	Moskova and Popov ⁴² , 1993
Smoking affects sperm density and sperm morphology, especially the head-piece	6181F	Chia <i>et al.</i> ⁴¹ , 1994
Sperm motility, percentage of morphologically normal spermatozoa were significantly lower in smokers	49S 28NS	Sofikitis <i>et al.</i> ⁴⁵ , 1995
Tobacco consumption were associated negatively with semen quality (density, total count, and motility)	88	Vine <i>et al.</i> ⁴⁶ , 1996
Smokers showed elevated frequencies of sperm aneuploidy, reduced linearity of sperm motion, and more "round-headed" sperm	10S 15NS	Rubes <i>et al.</i> ⁴⁷ , 1998
Smokers resulted in a significant reduction in sperm viability and longevity	20S 20NS	Zavos <i>et al.</i> ⁴⁸ , 1998
Sperm density and sperm forward progress were lower in smokers	110S 191NS	Zhang <i>et al.</i> ⁴⁹ , 2000
Smokers had significantly more round cells in their ejaculates and the percentage of ejaculates with $>1 \times 10^6/\text{ml}$ leukocytes was higher in smokers	478S, IF 109ES, IF 517NS, IF	Trummer <i>et al.</i> ⁵⁰ , 2002
Cigarette smoking was associated with a significant decrease in sperm density, total sperm count and number of motile sperm	655S 1131NS	Künzle <i>et al.</i> ⁵¹ , 2003
Cigarette smoking tended to reduce semen volume	367S 522NS	Pasqualotto <i>et al.</i> ²⁹ , 2004
Tobacco smoking can reduce sperm concentration and percentage motile sperm	2542	Ramlau-Hansen <i>et al.</i> ⁵² , 2007
Heavy smoking is a inducing factor of teratozoospermia	100S 100NS	Gaur <i>et al.</i> ⁵³ , 2007
Smokers had low semen quality before and after swim-up compared with none-smokers	48S 53NS	Colagar <i>et al.</i> ⁵⁴ , 2007
Smokers had significant lower sperm motility vs. nonsmokers	61S 98NS	Kumosani <i>et al.</i> ⁵⁵ , 2008
Smoking has detrimental effects on sperm motility, viability, and it is directly correlated with cigarette quantity and smoking duration	80S 80NS	Taha <i>et al.</i> ⁵⁶ , 2012
No statistically significant effect of smoking habits on sperm density, motility, and morphologic features was detected	150S 37ES 52NS	Vogt <i>et al.</i> ⁵⁷ , 1986
No difference was found in sperm motility and morphology between smokers and none-smokers in hypofertile male	219S, HF 288NS, HF	Dikshit <i>et al.</i> ⁴⁴ , 1987
There was no significant association between smoking and any semen parameter	135S, IF 195NS, IF	Dunphy <i>et al.</i> ⁵⁸ , 1991
No statistically significant effect of cigarette smoking on sperm density, motility or morphologic features of sperm was detected	186S, IF 164NS, IF	Osser <i>et al.</i> ⁵⁹ , 1992
Semen volume, and sperm density, motility, and morphology were not significantly different between the two groups of infertile men	18S, IF 69NS, IF	Mak <i>et al.</i> ⁴³ , 2000
No significant difference in conventional parameters between smokers and nonsmokers	51S, IF 57NS, IF	Sepaniak <i>et al.</i> ⁶⁰ , 2006

S: smokers; NS: none-smokers; IF: infertility; HF: hypofertility

by transmission electron microscopy was significantly reduced in heavy smokers.⁷⁰ It seems that light or moderate smoking (≤ 20 cigarettes per day) is insufficient to induce ultra-structural sperm abnormalities according to the current researches.⁶⁹

Smoking and assisted reproduction outcomes

Assisted reproductive technology (ART) is reproductive technology used primarily for infertility (IF) treatments. Examples of ART include *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and gamete intra-fallopian transfer (GIFT). The effect of smoking on ART results in male was not reported or recorded in the vast majority of studies. Male smoking was considered as a deleterious factor in pregnancy outcome among IVF patients.⁷¹ Zitzmann *et al.*⁷² associated male smoking with lower success rate in 153 ICSI and 148 IVF couples. Significantly decreased live birth rates were also reported among 166 couples seeking pregnancy through ART.⁷³ Based on only one study, men who smoked for more than 5 years presented a higher risk of multiple births during IVF or GIFT.⁷⁴ The number of oocytes retrieved from patients decreased by 46% in case the respective partners used to smoke; such an effect was most probably due to the passive smoke inhaled by the woman.⁷⁴ We speculate that the negative ART outcomes due to male smoking are likely to be a combined result of both the direct effect upon spermatozoa function and the indirect effect of passive smoking of the female partners.

SMOKING AND VARICOCELE

Varicocele is an abnormal enlargement of the pampiniform venous plexus in the scrotum and causes a progressive decline in male fertility.⁷⁵ In population with varicocele, smokers had an incidence of oligozoospermia 10-fold greater than that of nonsmokers and was associated with altered semen quality, decreased sperm functional integrity, seminal oxidative stress,⁷⁶ increased sperm DNA fragmentation, lower zinc (Zn) level,⁷⁷ and increased sperm immaturity, necrosis and apoptosis percentages.⁷⁸ Varicocele is present in almost 40% of infertile males and was considered as a major cause of male IF⁷⁹ but the precise mechanisms remain uncertain. Hypotheses implicating testicular hypoxia due to venous stasis, reflux of renal/adrenal toxic metabolites, hypertension in the internal spermatic veins, hormonal dysfunction and temperature increase in testicle outline the etiology of varicocele.⁸⁰ Recent understanding of the role of oxidative stress in male reproduction led some postulation that oxidative stress was the possible cause of sperm dysfunction in patients with varicocele.⁸¹

TOBACCO SMOKING AS A RISK FACTOR OF ERECTILE DYSFUNCTION

Many independent cohort studies implied a relationship between tobacco smoking and impotence. For instance, a higher percentage of current tobacco smokers were reported impotence than did nonsmokers⁸² according to a research focused on middle-aged men. Other researchers indicated that smoking is an independent risk factor of vasculogenic impotence⁸³ and arteriogenic impotence.⁸⁴ A recent systematic review of observational studies including four prospective cohort studies and four case-control studies suggested that current smoking increased the risk of erectile dysfunction significantly.⁸⁵ In summary, after 30 years research, tobacco smoking is scientifically recognized as a risk factor of erectile impotence.⁸⁶

ENVIRONMENTAL AND PRENATAL EXPOSURE TO TOBACCO SMOKE

Environmental tobacco smoke (ETS) is the inhalation of smoke, called SHS or passive smoking. Concerns around ETS have played a central role in the debate over the harms and regulation of tobacco products

because of its harm to by-standers especially woman and children.⁸⁷ Most of the studies involved the effects of ETS on fertility focused on females because of their susceptibility to ETS and the direct impairment to impregnation and embryonic development.⁸⁸

Intrauterine exposure to environmental toxic substances in pregnancy has the potential to cause adverse effects on the prenatal development of the embryo. Effects of prenatal exposure to tobacco smoke on semen quality in adult life have been investigated in only a few studies, and the results remain controversial. Ramlau-Hansen *et al.*⁸⁹ reported 38% lower total sperm count, 19% lower semen volume, and 17% lower sperm concentration in men exposed *in utero* to more than 19 cigarettes daily. Jensen *et al.*⁹⁰ observed a dose-dependent association between prenatal tobacco smoke exposure and lower sperm concentration with higher risk of oligozoospermia. Another cross-sectional study indicated that prenatal smoking lead to smaller testicles, lower total sperm counts and reduced spermatogenesis-related hormones.⁹¹ The similar findings were also reported in animal studies.^{92,93}

BIOCHEMICAL ABNORMALITIES UNDERLYING THE PHENOMENON

Seminal reactive oxygen species and antioxidants

Oxidative stress caused by smoking was long noticed by researchers. Kiziler *et al.*⁹⁴ reported that the malondialdehyde and protein carbonyls level were higher in smokers while the level of glutathione S-transferase and glutathione were higher in none-smokers. These indicators are all related to reactive oxygen species (ROS). ROS in seminal plasma generated from various sources that could either be endogenous or exogenous. The endogenous ROS mainly generated from leukocytes (neutrophils and macrophages) and immature spermatozoa while the exogenous sources include smoking, industrial compounds, alcohol *etc.*⁹⁵ Both seminal plasma and spermatozoa possess antioxidant systems capable of counteracting the harmful effects of ROS. The imbalance between total antioxidant capacity and ROS production in seminal fluid indicates oxidative stress and is strongly correlated with male IF.⁹⁶ Unsurprisingly, both fertile and infertile smokers show a higher seminal ROS level.⁹⁶

Sperm was considered to be particularly susceptible to oxidative damages due to the abundance of polyunsaturated fatty acids in the plasma membrane.⁹⁷ As an exogenous antioxidant in semen, ascorbic acid played a most important role in controlling the oxidative stress.⁹⁸ Serum ascorbic acid was found decreased significantly in heavy smoking group⁹⁹ and their sperm quality improved after ascorbic acid supplement. Furthermore, endogenous antioxidants such as superoxide dismutase and catalase were also lower in smokers.^{56,100} Seminal plasma Zn also plays an important role as antioxidant and antibacterial agent that protect the semen from heavy metals accumulation.²¹ Compared with fertile nonsmokers, fertile smokers were significantly associated with lower seminal Zn levels.⁵⁶ Kiziler *et al.*⁹⁴ utilized statistical methods to verify the correlation between semen oxidative stress and Cd/Pb exposure. In subfertile population, positive correlations were found between seminal Cd and seminal protein carbonyls. The seminal Pb and spermatozoa ROS levels were also positively correlated. While in fertile smokers, blood Cd and ROS level were positively correlated. Cd in tobacco smoke decreases antioxidant capacity by inhibiting antioxidant enzymes and displacing Zn in metalloenzymes thus decreasing their activity. In summary, tobacco smoking increases ROS production while decreases antioxidant capacity and these effects are mainly due to the Cd exposure in smokers.

A study investigated the genetic variants of erythroid 2-related factor 2 (*NRF2*) gene in 314 heavy smokers and its relationship with semen quality.¹⁰¹ The *NRF2* rs6721961 TT genotype was found more frequently in heavy smokers with low semen quality than in those with

higher semen quality. NRF2 is an antioxidative transcription activator that binds to antioxidant response elements in the promoter regions of target genes.¹⁰² These results indicated that exogenous or endogenous antioxidants may play an important role in rescuing the semen quality in smokers and oxidative stress can be considered as the core factor of the impaired male fertility owing to tobacco smoking.

DNA fragmentation

Spermatozoa chromatin is relatively tighter packaged due to the positively charged protamine instead of histone in somatic cells.⁶⁷ This extremely compact and stable structure diminishes the ability of DNA damage repairing. As a consequence, DNA fragments which correlated closely to sperm quality and viability¹⁰³ were detected in mature spermatozoa. Sepaniak *et al.*⁶⁰ showed that smokers' spermatozoa had a significantly higher level of DNA fragmentation than that of nonsmokers. Furthermore, a detrimental effect caused by smoking was detected by analysis of sperm DNA fragmentation after capacitation.¹⁰⁴ The level of bulky DNA adducts, which result from DNA damage, was also reported higher in healthy smokers.¹⁰⁵ DNA fragmentation can directly damage sperm function and may affect embryonic development of offspring. The sperm mitochondrial respiratory activity was also negatively affected by oxidative stress and DNA fragmentation.¹⁰⁶ This result may explain the decreased sperm motility caused by tobacco smoking. The most common origin of sperm DNA fragments is oxidative damage. Spermatozoa are especially vulnerable to oxidative stress because high ROS level can cause a breakdown of sperm phospholipids and fatty acids.⁴³ The relationship between tobacco smoking and the semen ROS level was discussed above, and the higher ROS level induced by tobacco smoking may result in DNA damage.

Many studies have shown that cell apoptosis was also involved in sperm DNA fragments formation.¹⁰³ A significant increase of apoptotic spermatozoa proportion in the ejaculates of smokers was observed.¹⁰⁷ Tawadrous *et al.*¹⁰⁸ tested some apoptosis markers in sperm from an infertile man and reported that sperm Smac/DIABLO, caspase-9 activities were correlated positively with the mean number of daily smoked cigarettes and smoking duration.

DISCUSSION AND CONCLUSIONS

Due to the fact that cigarette smoke contains more than 4000 harmful substances, there has long been concern that smoking could have adverse

effects on male reproduction. First and foremost, tobacco smoking leads to reduced semen quality including semen volume, sperm density, motility, viability, and normal morphology in smokers. Furthermore, reproductive hormone system disorders, dysfunction of spermatogenesis, sperm maturation process, and impaired spermatozoa function have also been observed in smokers. Despite the various harmful effects of smoking on male fertility, most male smokers are still fertile but have a higher risk of sub-fertility or IF. Studies of natural conception in couples with a smoking male partner fail to demonstrate a significant reduction in fecundity.¹⁰⁹ While other studies indicated that smoking is associated with lower fecundity rates, a higher risk of IVF failures and adverse reproductive outcomes.¹¹⁰ The process of fertilization involves a sperm fusing with an ovum, so we assume that male smokers who can generate normal spermatozoa may have the ability of producing offspring. The knowledge currently available indicates that the balance of ROS and antioxidants in semen plasma, sperm and testis plays an important role in maintaining the normal function of spermatozoa because of their high susceptibility to oxidative stress. Since sperm lipid and DNA are vulnerable to ROS, increased ROS level will lead to DNA fragmentation and apoptosis which will result in the impairment of sperm function leading to lower male fertility (**Figure 1**). Nicotine and its metabolite cotinine act more complicated in male reproduction system. The biological functions impaired by nicotine include testicular gametogenesis and steroidogenesis. Benzo(a)pyrene, a mutagen and carcinogen in tar, can bind covalently to DNA strand and forms adduct which may affect spermatozoa function and embryonic development of offspring. From another prospect, tobacco smoking aggravates the pathology lesions of varicocele which was considered as a major cause of male IF.

The assisted reproduction outcomes are also affected by tobacco smoking according to extensive clinical data. But the definitive conclusions remain controversial because of the relatively low susceptibility to smoking observed in the infertile population and the passive exposure of female partners to tobacco smoke. Parental tobacco exposure *in utero* was also reported to affect male fertility, and this result may be caused by direct exposure of male offspring during the embryonic developmental processes. The maternal effect of tobacco smoking prompts us the paternal effect which may involve epigenetical regulations such as microRNAs and alterations of DNA methylation and histone acetylation patterns. The reviewed articles

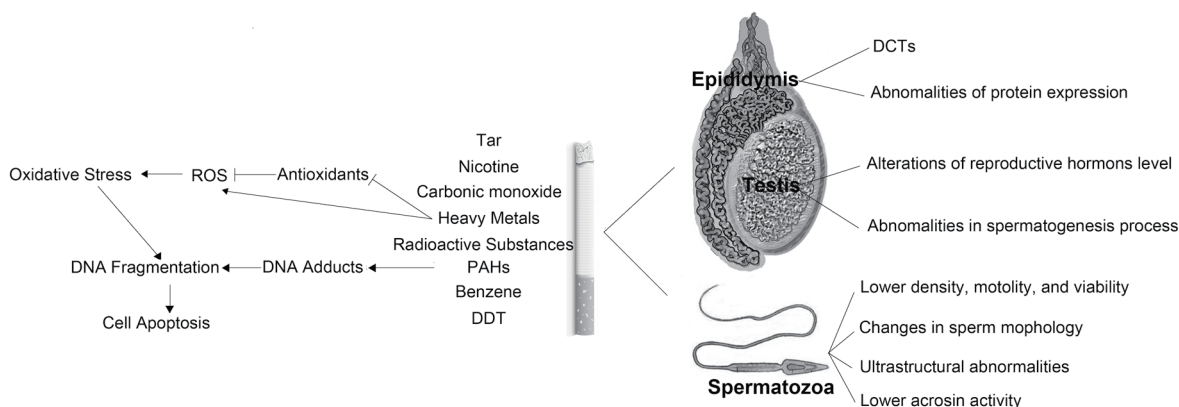


Figure 1: Schematic representation of the effects of tobacco smoking on male fertility. The figure identifies the main hazardous components of tobacco. Tobacco smoking leads to reduced semen quality including semen volume, sperm density, motility, viability, and normal morphology. Furthermore, reproductive hormone system disorders, dysfunction of spermatogenesis, sperm maturation process, and impaired spermatozoa function have also been observed in smokers. The left part of the figure indicates the underlying mechanism. Increased reactive oxygen species level caused by hazardous components of tobacco will lead to DNA fragmentation and apoptosis which will result in the impairment of sperm function leading to lower male fertility.

outlined comprehensively the hazardous effects of tobacco smoking on male fertility. There is insufficient insight into the underlying mechanisms of the observed toxicological effects because most of the current researches are descriptive. Further studies concerning the molecular mechanisms especially the epigenetic modifications involved in the pathogenic process of smoking are required. Because of the inheritability of epigenetic modifications, the trans-generational effects of tobacco smoking through paternal line are also worth expecting.

AUTHOR CONTRIBUTIONS

JBD carried out the literature studying and manuscript writing. ZXW carried out the conception and revision of the manuscript. ZDQ is responsible for the conception, revision, and approval.

COMPETING INTERESTS

The authors declare no competing interests.

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