



Smoking and Male Infertility: An Evidence-Based Review

Avi Harlev^{1,2}, Ashok Agarwal¹, Sezgin Ozgur Gunes^{1,3}, Amit Shetty¹, Stefan Simon du Plessis^{1,4}

¹American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA, ²Fertility and In Vitro Fertilization Unit, Department of Obstetrics and Gynecology, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beersheba, Israel, ³Department of Medical Biology, Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey, ⁴Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa

Many studies have reported that the contents of cigarette smoke negatively affect sperm parameters, seminal plasma, and various other fertility factors. Nevertheless, the actual effect of smoking on male fertility is not clear. The effect of smoking on semen parameters is based on the well-established biological finding that smoking increases the presence of reactive oxygen species, thereby resulting in oxidative stress (OS). OS has devastating effects on sperm parameters, such as viability and morphology, and impairs sperm function, hence reducing male fertility. However, not all studies have come to the same conclusions. This review sheds light upon the arguable association between smoking and male fertility and also assesses the impact of non-smoking routes of tobacco consumption on male infertility. It also highlights the evidence that links smoking with male infertility, including newly emerging genetic and epigenetic data, and discusses the clinical implications thereof.

Key Words: Erectile dysfunction; Infertility, male; Smoking; Spermatogenesis; Spermatozoa

INTRODUCTION

Despite the well-known detrimental impacts of smoking on health in general and on the male reproductive system in particular [1], smoking remains a worldwide phenomenon. According to the World Health Organization (WHO), 30% of all 15 years and older men, smoke [2]. Men of reproductive age (20~39 years of age) make up approximately 46% of smokers [3]. Although the prevalence of male smokers, based on nationally representative sources from 187 countries, decreased from 41.2% in 1980 to 31.1% in 2012, the actual number of everyday

smokers increased from 721 million in 1980 to 967 million in 2012 [4].

The WHO reports that approximately 8% of couples worldwide and 10% to 15% of those in industrialized nations experience infertility [5], and that male factor infertility is involved in 30% to 35% of all cases [6]. In order to identify possible associations between smoking and male infertility, numerous studies have been conducted, with some reporting contradictory results. While many studies have reported a negative impact of smoking on semen analysis parameters and male infertility [7-10], others have found no such effects, and in some cases, have even

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Correspondence to: Ashok Agarwal

American Center for Reproductive Medicine, Cleveland Clinic Lerner College of Medicine, 9980 Cleveland, OH 44195, USA,
Tel: +1-216-444-9485, Fax: +1-216-445-6049, E-mail: agarwaa@ccf.org

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found positive effects on sperm motility [8,11] and the extent of nuclear DNA damage in sperm [12]. Other inconsistent and conflicting data regarding the influence of smoking on male infertility have been reported [12-14]. Moreover, even studies reporting an effect of smoking on semen parameters have not clearly demonstrated any effect of smoking on male fertility [15-17].

These opposing and confusing results are not surprising. Three possible explanations for the emergence of contradictory results can be hypothesized. First, several metrics are used to assess the influence of smoking on male fertility, such as semen parameters, spermatozoa function, histologic alterations, and others. The use of different assessments could result in inconsistent findings among studies. Second, the mechanisms of how smoking may influence male fertility have not been securely established. Third, it is challenging to directly compare data among the studies due to the difficulty of adjusting for confounders such as exposure to alcohol use, medical illnesses, toxins, and socioeconomic status.

In this review, we discuss the content of cigarettes and detail the effect of various forms of tobacco exposure on male infertility. Next, we characterize the effect of smoking on different aspects of the male reproductive system, focusing on both the physiological and pathological impacts of smoking. Subsequently, this study summarizes the possible mechanisms through which smoking causes genetic and epigenetic alternations in male reproductive function, a topic that has not been thoroughly reviewed previously. Finally, we discuss how this area of research should advance to meet the clinical needs of infertile smokers.

SMOKING: AN OVERVIEW

1. Content of cigarette smoke

Cigarette smoke is composed of gases, vaporized liquid, and particles. Nearly 4,000 compounds are released through the chemical processes of hydrogenation, pyrolysis, oxidation, decarboxylation, and dehydration. The smoke release is biphasic (gaseous and particulate). In the gaseous phase, carbon monoxide is released [18], and in the particulate phase, nicotine and tar are released. Cigarette smoke contains several toxic chemicals, muta-

genic substances, and carcinogens, including nicotine and its metabolites, cotinine, radioactive polonium, benzo(a)pyrene, dimethylbenzanthracene, naphthalene, methylnaphthalene, polycyclic aromatic hydrocarbons (PAHs), and cadmium [19,20].

The primary psychoactive component of tobacco is nicotine. Nicotine is the active element responsible for tobacco addiction. The majority of nicotine in humans is metabolized to cotinine, and cotinine is further metabolized to trans-3'-hydroxycotinine (3HC) [21]. In cigarette smokers, seminal and serum levels of cotinine and 3HC appear to be similar, whereas seminal nicotine levels are generally higher than serum levels [22]. While total sperm motility is negatively correlated with seminal cotinine and 3HC levels, forward sperm motility is correlated with seminal cotinine levels [22].

The major active components of smoke that influence semen parameters are the heavy metals cadmium and lead. The negative effect of cadmium on sperm parameters has been previously shown in animal studies [23]. Elevated seminal cadmium in smokers has been observed if > 20 cigarettes/day are consumed, and cadmium levels in the blood have been found show a statistically significant positive correlation with cigarette-years and a statistically significant negative correlation with sperm density [24]. Additionally, lead levels in seminal plasma have been shown to be higher in infertile smokers than in fertile men and infertile non-smokers [25]. Moreover, negative associations between seminal lead and cadmium concentrations and sperm concentration, motility, and morphological abnormalities in abnormal spermatozoa have been observed [26].

2. Means of smoking and routes of nicotine exposure

Several types of smoke are produced when a cigarette is smoked (Fig. 1). Mainstream smoke is the actual mouth-to-cigarette inhalation of cigarette smoke into the smoker's lungs. Exhaled mainstream smoke is the smoke that escapes into the environment after it is drawn through the cigarette, filtered by the smoker's lungs, and exhaled. Sidestream smoke arises from the burning end of the cigarette and enters directly the environment directly [7]. Firsthand or active smoking includes inhaled and exhaled mainstream smoke, as well as sidestream smoke. This type

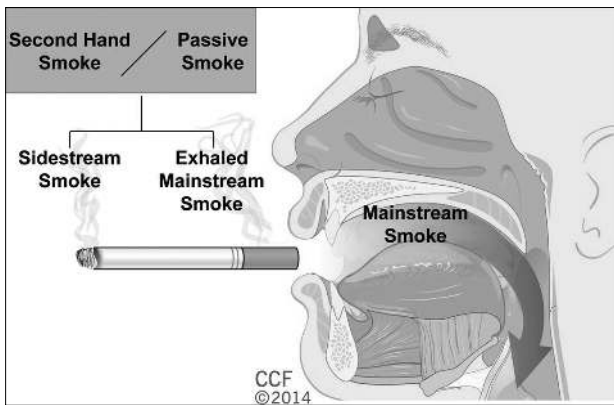


Fig. 1. Types of smoke produced when a cigarette is smoked.

of smoking may affect sperm quality, most notably sperm concentration, motility, and morphology [27-30].

Passive or secondhand smoking mainly refers to the involuntary inhalation of tobacco smoke present in the air. Most of this smoke comes from the cigarette's burning end, while about 10% originates from exhaled mainstream smoke [31]. Approximately 50% of non-smokers are passive smokers. Serum cotinine, a biomarker of tobacco smoke exposure, can be detected in approximately 50% of the non-smoking USA population. Secondhand smoke contains thousands of compounds, many of which are known to be harmful to humans [32]. The negative effects of sidestream and passive smoke are fairly well understood, and these types of smoke can negatively affect reproduction in non-smokers with excessive exposure to cigarette smoke [17].

Comparing the effect of mainstream and sidestream smoke on mouse male germ cells, Polyzos et al [33] showed that sidestream smoke reduced sperm motility, whereas mainstream smoke increased sperm DNA fragmentation. Both mainstream and sidestream smoke adversely affected sperm chromatin integrity and reduced fertilization rates. They concluded that male exposure to secondhand smoke prior to fertilization is likely to have negative results on the reproduction process.

The findings of epidemiological studies of people exposed to secondhand smoke can be difficult to evaluate due to the difficulties in determining the amount of secondhand smoke exposure, confounding exposures (the occasional cigarette or environmental exposure), and the lack of parallel but fully unexposed control groups [34].

For instance, maternal cigarette smoke exposure is often a confounding variable that makes analyzing the effects of paternal secondhand smoke exposure on fertilization and early embryonic development particularly challenging. However, a number of toxicants are at higher levels in sidestream smoke than in mainstream smoke and have been shown to effect sperm motility, including reactive oxygen species (ROS) such as superoxide and hydrogen peroxide [35-38], as well as cadmium [37,38].

Electronic cigarettes have a liquid-filled container that holds concentrated flavors, humectant (*i.e.*, propylene glycol, vegetable glycerin, and/or polyethylene glycol 400), and variable concentrations of nicotine [39]. Several analyses have detected toxicants and carcinogens similar to those found in cigarette smoke, although usually at much lower levels [40,41]. The safety of electronic cigarettes has not yet been well established [42]. A recent systematic literature review [43] concluded that the current data do not warrant health concerns, at least according to the standards used to guarantee workplace safety. However, electronic cigarette use exposes users to aerosol that contains toxicants, making further research necessary.

Chewing tobacco is one of the most common methods of smokeless tobacco intake, with approximately 6 million to 22 million users of smokeless tobacco products in the USA alone [44]. Sales of smokeless tobacco have increased over the past decade [45,46]. The nicotine distribution features of smokeless tobacco make it both addictive and a feasible substitute for cigarette smoking for many consumers, since it delivers a higher nicotine dose than other tobacco cessation products [47]. Chewing tobacco is markedly less harmful than smoking, but it is not harmless. Its nicotine absorbance is equal to that from cigarettes, but with a less toxic effect [48]. Chewing tobacco increases the risk of multiple oral premalignant lesions [49,50], respiratory tract cancers [51], and cardiovascular disease [48]. Moreover, chewing tobacco affects semen parameters in a dose-dependent manner. Sperm concentration, percentage of motility, morphology, and viability have been found to be significantly reduced in tobacco chewers [52,53]. Structural defects in the sperm head and cytoplasmic residues were correlated with the intensive use of chewing tobacco, but anomalies in the midpiece and tail were not observed [53].

Nicotine patches and nicotine gum are commonly used as nicotine replacement therapy for those who want to stop smoking. Since orally ingested nicotine is metabolized first in the liver, the bioavailability of swallowed nicotine medication is reduced. Skin transdermal patches and nicotine intake through the oral mucosa (chewing gum, sublingual tablets, etc.) avoid this reduction in bioavailability [54]. Although nicotine is absorbed transdermally, no major side effects or complications have been reported with these products [55-57]. We found no well-conducted studies dealing with the influence of nicotine patches or gum on fertility.

EFFECTS AND POSSIBLE MECHANISM OF SMOKING ON MALE INFERTILITY

In studies investigating the influence of cigarette smoke on semen quality, most researchers have studied semen parameters based on the basic WHO recommendations for semen analysis, which include volume, count, concentration, motility, and morphology.

1. Smoking and sperm parameters: concentration, motility, and morphology

Table 1 [58-80] summarizes the results of studies evaluating the effect of smoking on semen parameters. Although many studies did observe a negative influence of smoking on semen quality, many other studies did not show a significant effect on one or more basic sperm parameters.

Künzle et al [10] prospectively studied 2,105 participants, of whom 839 persons (39.9%) were smokers and 1,266 persons (60.1%) were non-smokers. Sperm concentration, motility, and morphology were significantly affected in smokers. Nevertheless, sperm vitality, ejaculate volume, and progressive motility concentrations were slightly but not significantly reduced among smokers. In a large cross-sectional study involving the general healthy population, Ramlau-Hansen et al [80] observed a significant reduction in sperm concentration, total sperm count, and motility in smokers. In a meta-analysis, Vine et al [81] reported that the sperm concentration in smokers was approximately 13% lower than in non-smokers on average. Among normal healthy males, a 24% lower

sperm concentration was observed in smokers in comparison to non-smokers [81].

In order to investigate the effects of smoking on sperm parameters, we searched the literature for studies that compared differences between smokers and non-smokers, since simply comparing data from smokers with WHO reference values cannot provide a true reflection of the changes caused by smoking. A total of 38 relevant studies were found, five of which were excluded based on methodological concerns and sample size. Twenty-eight of the remaining 33 studies were prospective and five were retrospective studies (Table 1).

When stratified according to study population, 13 studies were performed on a healthy population and 20 were performed on infertile men. Due to the diversity of the studies and of the study populations, it was challenging to extrapolate the data to draw definitive conclusions. When all studies comparing smokers to non-smokers were considered, 36.4% reported a significant reduction in motility and concentration, and 41.4% showed a decrease in normal morphology. When the studies were stratified based on the studied population, it was observed that motility, concentration, and morphology were affected in 23.1%, 38.5%, and 44.4% of the healthy population and in 40%, 30%, and 42.1% of the infertile population studies, respectively, when smokers were compared to non-smokers (Fig. 2).

A number of studies in our analysis reported adverse effects of smoking on sperm parameters. However, a large number of studies showed no significant effect, resulting in a lack of conclusive evidence. To date, only a single meta-analysis has been performed on the effects of smoking on semen parameters, but that study mainly focused on sperm concentration. The authors showed that exposure to more than 10 cigarettes per day reduced sperm concentration by 13% to 17% [81]. The latest American Society for Reproductive Medicine Committee opinion [82] states that smoking can affect basic semen parameters in a dose-dependent manner. However, the values still mostly remain within the normal reference ranges. Therefore, future studies should investigate the effect of cigarette smoking on sperm function at a molecular level.

Table 1. Studies reporting a negative effect of smoking on semen parameters

Study No.	Author	Year	Study type	No. of participant	No. of smoker	No. of non-smoker	Study group	Association between semen parameters and smoking		
								Motility	Concentration	Morphology
1	Osser et al [58]	1992	Prospective	350	186	164	Infertile smokers vs. infertile non-smokers	No	No	No
2	Künzle et al [10]	2003	Prospective	1,786	655	1,131	Infertile smokers vs. infertile non-smokers	Yes	Yes	Yes
3	Chia et al [59]	1998	Prospective	243	152	91	Healthy population	No	No	No
4	Dikshit et al [60]	1987	Prospective	626	219	288	Infertile smokers vs. infertile non-smokers	No	No	No
5	Dunphy et al [61]	1991	Prospective	330	135	195	Infertile smokers vs. infertile non-smokers	No	No	No
6	Gerhard et al [62]	1992	Prospective	225	136	89	Infertile smokers vs. infertile non-smokers	No	No	No
7	Hassa et al [63]	2006	Prospective	223	126	97	Infertile smokers vs. infertile non-smokers	No	No	No
8	Vogt et al [14]	1986	Prospective	333	150	183	Healthy population	No	No	No
9	Colagar et al [19]	2007	Prospective	101	53	48	Infertile smokers vs. infertile non-smokers and fertile smokers compared to infertile smokers	Yes	Yes	Yes
10	Jeng et al [64]	2014	Prospective	192	103	89	Healthy population	No	No	Yes
11	Lewin et al [8]	1991	Prospective	675	293	382	Healthy population	No	Yes	NA
12	Liu et al [65]	2010	Prospective	147	68	79	Infertile smokers vs. infertile non-smokers	Yes	Yes	Yes
13	Mak et al [66]	2000	Prospective	87	18	69	Infertile smokers vs. infertile non-smokers vs. fertile non-smokers	No	No	No
14	Merino et al [30]	1998	Prospective	358	197	161	Infertile smokers vs. infertile non-smokers	Yes	Yes	Yes
15	Ochedalski et al [67]	1994	Prospective	70	40	30	Infertile smokers vs. infertile non-smokers	Yes	Yes	No
16	Oldereid et al [68]	1989	Prospective	350	147	203	Infertile smokers vs. infertile non-smokers	No	No	No
17	Richthoff et al [20]	2008	Prospective	302	217	85	Healthy population	No	No	NA
18	Saleh et al [2]	2002	Prospective	65	20	32	Infertile smokers vs. infertile non-smokers	No	No	No
19	Sepaniak et al [69]	2006	Prospective	108	51	57	Infertile smokers vs. infertile non-smokers	No	No	No
20	Sergerie et al [12]	2000	Prospective	97	69	28	Healthy population	No	No	No
21	Trummer et al [3]	2002	Prospective	1,104	478	517	Infertile smokers vs. infertile non-smokers	No	No	No
22	Zavos et al [70]	1998	Prospective	40	20	20	Healthy population	Yes	Yes	Yes

Table 1. Continued

Study No.	Author	Year	Study type	No. of participant	No. of smoker	No. of non-smoker	Study group	Association between semen parameters and smoking		
								Motility	Concentration	Morphology
23	Zhang et al [71]	2000	Prospective	362	191	110	Infertile smokers vs. infertile non-smokers vs. fertile non-smokers	Yes	Yes	Yes
24	Kiziler et al [72]	2007	Prospective	71	26	22	Infertile smokers vs. infertile non-smokers vs. fertile non-smokers	Yes	Yes	Yes
25	Shaarawy et al [73]	1982	Prospective	40	20	20	Healthy population	No	Yes	Yes
26	Shen et al [74]	1997	Prospective	60	28	32	Healthy population	No	No	No
27	Yu et al [75]	2014	Prospective	322	147	175	Healthy population	No	No	NA
28	Taha et al [76]	2012	Prospective	160	80	80	Healthy population	Yes	Yes	Yes
29	Martini et al [77]	2004	Retrospective	3,546	372	3,174	Infertile smokers vs. infertile non-smokers	No	No	No
30	Meri et al [78]	2013	Retrospective	960	396	564	Infertile smokers vs. infertile non-smokers	Yes	No	Yes
31	Pasqualotto et al [79]	2006	Retrospective	889	367	522	Healthy population	No	No	No
32	Ramlau-Hansen et al [80]	2007	Retrospective	2,542	1,052	1,490	Healthy population	Yes	Yes	NA
33	Zhang et al [1]	2013	Retrospective	1,512	737	775	Infertile smokers vs. infertile non-smokers	Yes	No	Yes

All studies evaluated semen parameters according to the World Health Organization guidelines at the time the study was conducted.

NA: not applicable.

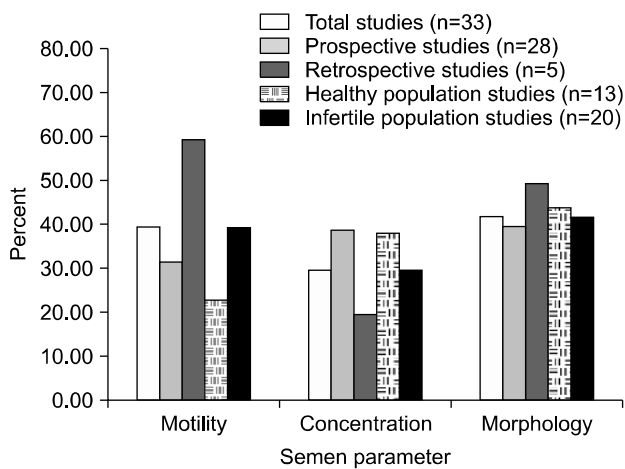


Fig. 2. Percentage of studies reporting a negative effect of smoking on semen parameters.

2. Smoking and male infertility: dose-response correlations

Despite the large number of studies performed using basic semen parameters, the available data do not conclusively indicate the impact of smoking on male fertility [17]. However, two conclusions may be drawn. First, a more significant association between smoking and basic sperm parameters may be observed in studies that investigated the general population than in studies involving infertile men [83]. Second, a dose-dependent relationship between the amount of cigarette consumption and negative effects on semen parameters was observed [80,84,85]. In a large cross-sectional study, Ramlau-Hansen et al [80] reported an inverse dose-based association between smoking and semen volume, total sperm

count, and the percentage of motile spermatozoa. The sperm concentration of heavy smokers was 19% lower than that of non-smokers. Therefore, smoking cessation, or at least a reduction in cigarette use, may be advised as a way of reducing toxin exposure [86].

3. Smoking and spermatozoa: biological function

The axoneme, a basic structural organ of motile cilia and flagella, is composed of microtubules. Variations in both the quantity and positioning of axonemal microtubules in smokers has been described [87]. In one study, ultrastructural assessment of the axoneme revealed aberrations in 99% of smokers and in 26% of non-smokers. These smoke-induced variations in the normal axoneme structure could impair flagellar movement, causing the sperm motility pathologies seen in smokers [88].

Another important biological activity is sperm motility. Creatine kinase is an enzyme expressed by cells, such as spermatozoa, that require large amounts of energy. It plays a major role in adenosine triphosphate (ATP) and adenosine diphosphate metabolism [89], and also provides an ATP buffering system. Ghaffari and Rostami [90] reported that creatine kinase activity in sperm was reduced in smokers, affecting sperm motility and overall fertility.

Acrosin is a proteolytic enzyme that is released by the sperm when it comes into contact with an ovum, degrading the zona pellucida of the oocyte and allowing the sperm to penetrate it [91]. Smokers have been found to display lower acrosin activity than non-smokers, even in the presence of normal semen parameters [91,92].

The effect of smoking on sperm function and ability to fertilize have been investigated *in vivo*, and a negative correlation was observed [93]. Furthermore, sperm penetration assays in cigarette smokers have shown a correlation with poor sperm function [28,94].

The functional impairment of sperm highlights the possibility of male subfertility in the presence of normal semen parameters, and also calls into question the ability of semen-based parameters to detect subfertility in infertile males who smoke.

4. Oxidative stress and leukocytospermia

Oxidative stress (OS) develops from an imbalance between ROS and natural antioxidant defenses. Such an im-

balance may be caused by an elevation in ROS, a decrease in total antioxidant capacity (TAC), or both.

Cigarette smoking has been correlated with increased seminal OS markers, as shown by a prominent increase in ROS levels [95] and a decrease in ROS-TAC measures [96]. Spermatozoa are particularly prone to damage caused by excessive ROS due to the large amount of polyunsaturated fatty acids in the plasma membrane that are substrates for ROS [97] and the low concentrations of scavenging enzymes in their cytoplasm [98].

Elevated seminal ROS levels in smokers could stem from either endogenous or exogenous sources. While the exogenic sources of ROS consist of environmental factors such as smoking [99], alcohol use [100], and air pollutants [101], leukocytes (neutrophils and macrophages) are the main endogenic source of ROS [102], although immature spermatozoa contribute as well [103]. A correlation between cigarette smoking and leukocytospermia has been well established [2,94]. As a major producer of ROS in seminal fluid, elevated leukocyte levels hinder fertility by exposing spermatozoa to OS [104], which in turn damages sperm DNA and the bilayer lipid membrane [105]. In addition, tobacco metabolites can generate an inflammatory response, triggering the activation and infiltration of leukocytes into the seminal plasma [106]. Sequentially, activated leukocytes elevate levels of ROS in the seminal fluid, which may overwhelm antioxidant defenses, resulting in OS [107]. An additional source of ROS in smokers is cigarette smoke itself, which contains high levels of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals [108].

Intracellular antioxidant enzymes function to reduce ROS levels. When these enzymes are not capable of protecting the plasma membrane, cell function is altered [109]. The seminal plasma supports and protects spermatozoa from pathological levels of ROS through free radical scavengers such as ascorbate, alpha-tocopherol, uric acid, and ROS-metabolizing enzymes such as superoxide dismutase (against superoxide), catalase (against hydrogen peroxide), and glutathione peroxidase (against glutathione peroxide) [110]. Ascorbic acid is an essential antioxidant in seminal fluid. Human seminal plasma contains up to 10 mg/dL of ascorbic acid, which is more than nine times the blood plasma concentration [111]. For instance,

male heavy smokers have been found to have a 20% to 40% reduction in serum ascorbic acid levels, but when they are given as supplements the sperm quality has been shown to improve [111,112]. Likewise, Zhang et al [71] compared seminal zinc, copper, and superoxide dismutase levels in medium and heavy long-term smokers and non-smokers, and found negative correlations between the amount and duration of cigarette smoking and semen quality as reflected by antioxidant levels.

DIRECT AND INDIRECT EFFECTS OF SMOKING ON THE MALE REPRODUCTIVE SYSTEM

1. Epididymis

The epididymis is the site of spermatozoa maturation where sperm develop their tails. However, the adequate maturation of sperm is known to be inhibited by various factors, including smoking [113]. Detached ciliary tufts have been detected in the semen of smokers [114]. It is thought that detached ciliary tufts are derived from the epithelium of the epididymal lining. Detached ciliary tufts may indicate a testicular pathology with an epididymal involvement as a consequence of smoking [114].

2. Varicocele

Varicocele is the abnormal dilation of the pampiniform plexus of the veins near the upper and lateral parts of the scrotum. This dilation is similar to that of varicose veins in the legs, which occurs due to valve insufficiency that prevents blood backflow. As a consequence, blood pools in the pampiniform plexus of veins, dilating the blood vessels and causing an elevated temperature in the groin. Hyperthermia has been proven to be harmful to the normal development of spermatozoa and may result in reduced male fertility [115,116].

The combination of smoking and varicocele has been found to be correlated with a 10 times greater incidence of oligozoospermia than observed in non-smoking men with varicocele and a five times greater than the incidence in men who smoked but did not have varicocele [117]. This impairment may be attributed to the increased catecholamine secretion from the adrenal medulla that results from smoking [118]. The secreted catecholamines reach the

testes by flowing down through the internal spermatic vein. The significant increase of OS due to smoking, varicocele, and hyperthermia of the scrotal region is a major contributor to dysfunction in the male reproductive system [79].

3. Erectile dysfunction

Both firsthand and secondhand smoking have been conclusively proven to be modifiable risk factors for erectile dysfunction [119-121]. In a systematic review that included four prospective cohort studies and four case-control studies, Cao et al [121] reported that smoking significantly increased the risk of erectile dysfunction. Smoking cessation significantly improved both physiological and sexual health in male smokers, regardless of their baseline level of erectile dysfunction [122,123].

4. Accessory glands and seminal plasma

The accessory sex glands of the male reproductive tract include the prostate gland, seminal vesicles, and bulbourethral glands. The role of these accessory glands is to secrete a fluid that helps maintain the normal homeostasis of spermatozoa once they leave the epididymis, where they have matured and gained motility. Seminal fluid contains enzymatic and non-enzymatic antioxidants as well as physiological ROS [124].

The function of the accessory glands in smokers has been studied through the evaluation of a number of glandular markers in the ejaculate, including N-acetyl amino sugar, total phosphate (a marker of the functionality seminal vesicles), zinc, acid phosphatase (a marker of prostate gland functionality), and alpha-1,4-glucosidase (a marker of epididymal function). It was found that smoking reduced both vesicular and prostatic parameters in smokers [125]. Exposing spermatozoa from non-smokers to the seminal plasma of smokers significantly reduced sperm motility and the acrosome reaction, as well as elevating malondialdehyde levels [126]. Conversely, exposing spermatozoa from smokers to non-smokers' seminal plasma led to a nonsignificant improvement in the functional parameters of the sperm. Hence, removing a smoker's seminal plasma and subsequently reconstituting the sample with physiological media could be of clinical significance in the various assisted reproductive technology programs

utilized for smokers [70,127].

5. Hypothalamic-pituitary-gonadal axis

Nicotine can alter the hypothalamic-pituitary axis by stimulating the release of growth hormone, cortisol, vasopressin, and oxytocin, which in turn inhibit luteinizing hormone (LH) and prolactin [128]. In a study assessing the effects of tobacco smoking on hormone levels, Ochedalski et al [129] reported that mean estradiol levels were higher and mean levels of LH, follicle-stimulating hormone (FSH), and prolactin were lower in smokers than in non-smokers, whereas the mean levels of testosterone and dehydroepiandrosterone did not differ. Similar results were reported in other studies investigating plasma or seminal fluid hormone levels [130]. Ramlau-Hansen et al [80] observed a positive dose-response relationship between smoking and testosterone, LH, and the ratio of LH to free testosterone. Moreover, FSH and inhibin B levels were also found to be increased in smokers. With regard to the physiology of the hypothalamic-pituitary-gonadal (HPG) axis, when FSH and LH levels increase, a resulting elevation of testosterone and inhibin B is anticipated, which in turn decreases FSH and LH levels by negative feedback. The results of this study led to the proposal that elements of tobacco smoke might interrupt the regular functioning of the HPG system, leading to Leydig cell failure in smokers [80].

However, in a study evaluating hormone levels in 889 fertile men divided into mild, moderate, and heavy smokers, no significant differences in FSH, LH, or serum total testosterone levels were observed [79].

6. Testicular endocrine and spermatogenic function

Direct toxic testicular damage in smokers may interrupt testicular endocrine and spermatogenic function. The two major roles of the testis are spermatogenesis and the hormonal secretion of androgens. Contradictory results have been found regarding the influence of smoking on the final products of the HPG axis, mainly androgens. Some evidence indicates that smoking influences the secretory function of Sertoli and Leydig cells in the testis, thereby causing the impaired sperm quality seen in smokers.

The vascular blood supply in the spermatic cord is rela-

tively insufficient, and smoking is known to compromise oxygen delivery [131]. The process of spermatogenesis has a high metabolic demand. However, the testis is physiologically vulnerable to hypoxia. Oxygen insufficiency, which can be caused by smoking, is therefore suspected to negatively affect testicular function.

SMOKING-RELATED GENETIC, EPIGENETIC AND MOLECULAR ALTERATIONS: RELATIONSHIP WITH MALE INFERTILITY

1. Smoking-induced DNA damage in sperm

Tobacco smoke contains harmful compounds and generates reactive intermediates, such as ROS and reactive nitrogen species, which can induce multiple genetic and epigenetic changes. Through the interaction of these intermediates, exposure to tobacco smoke can directly or indirectly cause the formation of DNA and protein adducts, mutations, chromosomal abnormalities, micronucleus formation, sister chromatid exchange (SCE), and promoter methylation (Fig. 3). Interestingly, even tissues that are not directly exposed to tobacco smoke show elevated levels of DNA adducts due to indirect systemic exposure. Furthermore, tobacco smoke and its contents lead to oxidative DNA damage [132,133].

PAHs are formed during the combustion of tobacco and target endothelial cells. Well-known examples of PAHs include benzo[a]pyrene and benzo[a]pyrene diol epoxide. These PAHs cause the formation of DNA adducts at the N2 position of guanine and can be detected in both somatic cells and spermatozoa [133-135]. Some studies have found that higher levels of DNA adduct formation and DNA damage were associated with sperm parameters. In a small subfertile group, DNA adducts were found to be inversely related, to a statistically nonsignificant extent, with both sperm concentration and motility [136,137]. Another study showed that these adducts were transmitted to the zygote and that they were not always repaired by the ovum [132].

No consensus exists about whether smoking affects spermatozoa DNA. Gallagher et al [138] found no significant variation in DNA adduct levels in sperm from heavy smokers (≥ 20 cigarettes/d), light smokers (< 20 cigarettes/d), and non-smokers. However, a more recent

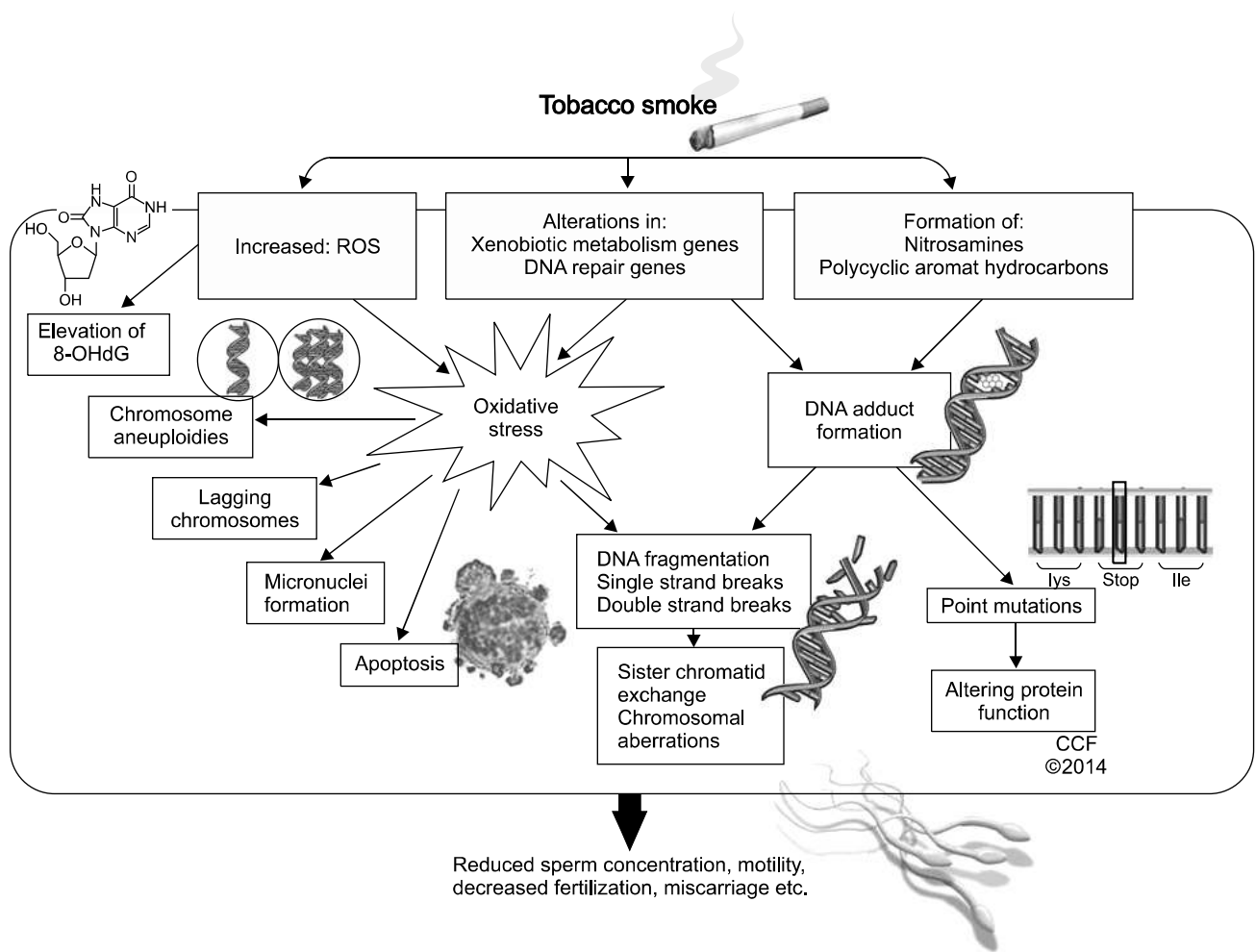


Fig. 3. Likely mechanisms through which smoking affects sperm parameters and quality, including genetic and epigenetic alternations as well as oxidative stress formation. ROS: reactive oxygen species, 8-OHdG: 8-hydroxyguanosine.

study found significantly higher levels of DNA adducts in the spermatozoa of smokers [134]. 8-oxo-2'-deoxyguanosine, a mutagenic base modification of DNA that occurs upon exposure to carcinogens such as tobacco smoke, was also shown to be present at a significantly higher level in the DNA of spermatozoa from smokers than in the DNA of spermatozoa from age-matched non-smokers (Fig. 3) [74].

As summarized by Begum [135], several studies have reported phenotypic and molecular abnormalities in cell lines treated with cigarette smoke condensate, including lagging chromosomes, sticky chromosomes during anaphase and telophase, micronucleus formation, and an increased frequency of SCEs [135]. A micronucleus is the formation of a small nucleus as a result of lagging whole chromosome or chromosomal fragments during anaphase

in both meiosis and mitosis. DNA damage, as well as spindle and kinetochore defects, may lead to micronucleus formation in the germ line. It is also widely recognized that a higher incidence of chromosomal instability, as assessed using micronuclei as a marker, is present in the infertile population [139]. Lähdetie [140] analyzed micronuclei in Golgi-phase or cap-phase spermatids found in semen samples from 62 subfertile men. Smokers had a 1.7-fold increase in micronuclei formation compared to non-smokers, although the difference was not statistically significant and was not related to the number of cigarettes smoked daily. These findings are supported by those of another study that did not find an association between smoking habits and the frequency of micronuclei in immature seminal germ cells [141].

SCE is the exchange of genetic material between two pa-

rental sister chromatids during replication. During these exchanges, which are caused by DNA damage, both DNA strands break, allowing whole DNA duplexes to cross between the chromatids. Two studies have reported an association between infertility and SCE [142,143]. Since SCE can be caused by cigarette smoke, it is therefore possible that cigarette smoke is responsible for genetic causes of infertility via this mechanism.

It is apparent that all smokers are not infertile, which suggests that genetic variations or polymorphisms in DNA repair, apoptosis, and xenobiotic metabolism genes among smokers may increase susceptibility to infertility. Recently published studies have reported a link between genetic variants of xenobiotic metabolism genes and infertility in smokers. Glutathione S-transferases (GSTs) are isoenzymes involved in the detoxification of both endogenous and exogenous compounds. Yarosh et al [144] found a statistically significant relationship between the *GSTM1*+/*GSTT1* del genotypes and the *GST* gene *GSTP1* 105IV/*GSTT1* polymorphism and smoking in infertile men. The *GSTM1*, *GSTT1*, and *GSTP1* genes are collectively involved in the development of idiopathic male infertility, and their phenotypic effects on the risk of disease are potentiated by cigarette smoking [144]. N-acetyltransferase (NAT2) is a xenobiotic metabolism enzyme that plays a role in the detoxification of toxicants of tobacco, such as hydrazines, aromatic amines, and arylamines. Hypoacetylated genotypes of *NAT2* have been shown to be associated with a link between cigarette smoking and infertility, presumably by increasing ROS production and OS (odds ratio [OR]=1.71, 95% confidence interval [CI]=1.02~2.87, $p=0.042$) [145].

The polymorphism 590G>A of *NAT2* can therefore be used as a novel genetic marker for susceptibility to idiopathic male infertility, but the risk is potentiated by exposure to various environmental oxidants, including cigarette smoke.

Cytochrome P450, a family 1, subfamily A polypeptide 1 (*CYP1A1*) is another xenobiotic metabolizing enzyme that participates in the metabolism of PAHs. In another study by Yarosh et al. [146], an increased risk of idiopathic male infertility was reported in male smokers who were carriers of the 462Ile/Val genotype of the *CYP1A1* gene (OR=1.91; 95% CI=1.01~3.59), while non-smokers did

not show an increased risk of infertility [146].

In another study, amniocytes grown in nicotine-containing media showed numerical and structural abnormalities in their autosomal chromosomes, including chromosomes 8, 15, 20, 21, and 22 [147]. In addition, the accumulation of such smoking-induced molecular alterations may increase aneuploidy levels in spermatozoa. Numerical chromosomal alterations in both paternal and maternal gametes are a direct cause of infertility and/or spontaneous abortions. Nondisjunction and anaphase lagging in paternal meiosis are also responsible for about 5% to 10% of autosomal aneuploidies, approximately half of Klinefelter syndrome cases (47,XXY), nearly 70% of Turner syndrome cases (45,X), and all cases of 47,XYY syndrome [148]. A recent study in a small cohort showed a significant increase in disomy frequency of chromosomes 3 and XY in the spermatozoa of smokers. Interestingly, these findings were independent of the number of cigarettes smoked [149]. These studies make it quite evident that the male meiotic process is affected by genotoxic damage from tobacco use.

2. Smoking-induced epigenetic alterations in infertile men

Epigenetic changes alter gene expression without changing the DNA sequence, and can be transmitted through both mitotic and meiotic cell divisions [150]. Several epigenetic mechanisms have been defined, including DNA methylation, histone modifications, and chromatin remodeling. However, the activity of non-coding RNA and miRNA are not considered to be an epigenetic process [151].

Epigenetic and DNA methylation studies have indicated that smoking induces methylation in the genome in the majority of loci studied [152-155]. A recent genome-wide DNA methylation study reported alterations in the methylation profile of 95 sites in smokers, and furthermore suggested a possible association between methylation status and infertility [156].

In somatic cells and oocytes, DNA is packaged by proteins known as histones. Protamines are proteins smaller than histones that enable the further condensation of DNA in spermatozoa. During the advanced stages of spermatogenesis, histones are replaced by protamines. Protamina-

tion is an epigenetic regulatory process specific to spermatozoa [75]. The protamination of sperm chromatin facilitates compaction of the nucleus, protecting the paternal genome during its passage and delivery to the oocyte. Moreover, the stability of this nuclear structure protects the sperm genome from oxidation and other detrimental changes in the female reproductive tract [157].

Yu et al [75] reported that the histone abnormality rate in a Chinese population was lowest in normozoospermic non-smoking men and highest in heavy smokers with oligospermia. Similarly, a European population-based study showed a significant decrease in protamine 2 (P2) concentrations in smokers, which subsequently caused an abnormal elevation of the P1:P2 protein ratio [158]. Furthermore, OS and smoking markers such as ROS, malondialdehyde, 8-hydroxyguanosine (8-OHdG), and cotinine were significantly higher ($p < 0.010$) in smokers than in non-smokers and correlated significantly ($p < 0.050$) with P1:P2 ratios. ROS, 8-OHdG, and cotinine also correlated negatively with P2 levels.

1) miRNA/noncoding RNA

MicroRNAs (miRNAs) are non-coding short RNAs that regulate translation. A recent study showed that over 100 miRNAs can be found in spermatozoa. In addition, 28 miRNAs were found to be differentially expressed between smokers and non-smokers, and the expression of four of them (has-miR-146b-5p, has-miR-509-5p, has-miR-146d, and has-miR-652) was altered in infertile men. Moreover, the authors reported that these four miRNAs played a role in pathways involved in cell proliferation, differentiation, and apoptosis in spermatozoa as well as early embryogenesis [159]. Additionally, Yu et al [160] investigated the association between *IκBα* rs696 polymorphism and defective spermatogenesis in humans. Their study suggested that cigarette smoking-associated ROS may affect spermatogenesis via nuclear factor- κ B signaling and involve mechanisms such as interference with inflammatory signaling pathways in the reproductive system. These findings allow us to better understand the genetic mechanisms that impact male infertility.

Although the epigenetic mechanisms by which benzo[a]pyrene and nicotine-induced alterations can be vertically transmitted has not been resolved, smoking-induced

abnormal protamination may cause alterations in the methylation pattern of CpG in the promoter regions of DNA in the offspring of smokers [161].

THE EFFECT OF SMOKING CESSATION ON MALE INFERTILITY

Much like the relatively inconclusive effects of smoking on male infertility, definitive information on the effects of smoking cessation on male reproductive parameters is rather scanty as well. In an animal study, Oyeyipo et al [162] showed that exposure to a high dose of nicotine negatively impacted semen concentration, motility, and morphology, as well as the libido of rats, in a dose-dependent manner. Interestingly, an increase in morphologically normal spermatozoa and libido scores were observed in the nicotine recovery group. The authors concluded that nicotine cessation improved fertility in male rats [162].

A recent retrospective cross-sectional study of infertile couples compared 229 never-smokers, 56 past smokers, and 109 current smokers [163]. Lower testosterone levels were observed in both never-smokers and previous smokers than in current smokers. Other researchers have also found decreased testosterone levels in previous smokers [3,164]. In a study of the effect of smoking cessation on erectile dysfunction, Harte and Meston [165] examined physiological and subjective sexual arousal indices during and after a smoking cessation program. The authors reported that smoking cessation considerably improved both physiological and self-reported indices of sexual health, irrespective of baseline erectile impairment.

In conclusion, no conclusive data exist on the actual influence of smoking cessation on male infertility in human subjects beyond the finding that smoking cessation is likely to improve erectile dysfunction.

CONCLUSIONS

This review discussed the possible effects of smoking on male fertility. Although no unambiguous and clear-cut conclusions can be drawn, a few inferences can be made. First, smoking has a stronger measurable effect on semen quality and function in fertile men than in subfertile men. This may be attributed to impaired semen parameters and

spermatozoa function at the primary level in the infertile population. Critically evaluating the current data on the effect of smoking on semen parameters did not yield an unambiguous result, but fertility can be affected even in the presence of normal semen parameters. Secondly, OS and the resulting genetic and epigenetic changes that result from smoking may correlate directly with reduced sperm function and reduced fertility, thereby providing a possible mechanism for the effect of smoking on male fertility. This correlation should be further investigated. Third, dose-dependent correlations exist between smoking and semen quality and sperm function. Smoking cessation should certainly be advised to any male smoker, especially if he is trying to conceive with his partner. Healthcare providers should facilitate smoking cessation by education, monitoring, and constant support. The data on smoking and male fertility reinforce the preferred preventive approach of discouraging smoking and eliminating exposure to tobacco smoke among both males and females in general, and in particular, while trying to conceive.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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