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Review Article

Effects of Nicotine on Sperm Health and Semen Quality: A Review based on Animal Studies

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ABSTRACT

Various studies have revealed the toxic effects of nicotine, an alkaloid compound of tobacco, on semen quality and sperm physiology; however, these studies collectively are still unsummarized. Here, I systematically discuss and summarize the nicotine exposure, pharmacology and its insults on semen quality and sperm physiology. I searched the Web of Science, PubMed, and Scopus databases for only English language articles or abstracts from September 1969 to March 2019 (inclusive) using the key words "Nicotine" and "tobacco" versus "sperm". In conclusion, the mainstream published work demonstrates the negative effects of nicotine on semen quality: first, by increasing sperm count, and by enhancing sperm motility and reducing sperm DNA damage, though there are a few in clinical studies that have deliberated some adverse effects. The detrimental effects of Nicotine on semen quality may be due to decreased functionality of reproductive organs, increased levels of oxidative damage to sperm, reduced amount of energy produced by spermatozoa. However, additional research, mainly clinical, is still needed to confirm these

Keywords: Nicotine; sperm; testicular damage; semen quality

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Introduction:

The WHO reports that approximately 8% of couples are infertile worldwide (WHO, 2008) and about 50% of these infertility cases are related to male fertility factors. There is a significant relationship between smoking and infertility in men. Smoking exposes the body to more than 4000 chemicals among which nicotine is one of the main pharmacological, addictive substances which mimics most of the deleterious effects of cigarette smoke. Various studies on animal and human suggest that nicotine has an overall negative impact on conventional semen quality and semen parameters of smokers such as decreased sperm count, motility of the sperms, and overall increase in the number of abnormal sperms and therefore reduce reproductive outcome. It also is reported that chronic nicotine exposure reduces fertilizing potential by lowering spermatogenic cells, sperm count and motility, in dose- and time-dependent manner.

Nicotine and its metabolite cotinine act more complicated in male reproduction system. Chronic nicotine exposure has been positively correlated to lower plasma testosterone

concentration. It has now been well established that nicotine-induced reductions of sperm production, maturation and fertilizing potential in rats were accompanied by a decrease in testosterone.

However, the mechanism by which nicotine causes male infertility is poorly understood. Some studies have revealed that nicotine can damage the male reproductive system through various means including oxidative stress, which is a primary factor in the pathogenesis of male infertility. There has been strong positive correlation among nicotine exposure and development of testicular and seminal oxidative stress. In fact, chronic exposure of nicotine, which act as both oxidant which act as both oxidant and pro-oxidant impairs homeostasis of the testicular oxidant and antioxidant enzymes.

Origin of Nicotine:

Nicotine (NIC) is the main hazardous component of cigarette smoke. It is a naturally occurring volatile-alkaloid, primarily found in plant of the Solanaceae family, which includes tobacco, potato, tomato, green pepper, and eggplant. German Physicians Wilhelm Heinrich and Karl Ludig Reimann first

extracted nicotine from tobacco (*Nicotiana tabacum*). The major alkaloid is nicotine in commercial tobaccos which accounts for about 95 % of the total alkaloid content [1]

Physical and Chemical Properties:

Nicotine is an amine, which is composed of pyridine and pyrrolidine rings. Nicotine, 3-(1-methyl-2-pyrrolidinyl)pyridine, is a volatile alkaloid with a molecular weight of 162.23. It is volatile base, colourless, water soluble and forms hydrosoluble salts [2]

Chemical formula: C₁₀H₁₄N₂

IUPAC name: 3-[2-(N-methylpyrrolidinyl)] pyridine

Appearance: oily, colourless hygroscopic liquid, with characteristic odour, turns brown on exposure to air

Boiling point (decomposes): 247 °C

Density: 1.01 g cm⁻³

Solubility in water: miscible

Vapour pressure at 20 °C: 0.006 kPa

Octanol/water partition coefficient as log Pow: 1.2

Exposure to Nicotine:

Global Scenario:

Nicotine consumed with tobacco is probably the second most used drug in the world after caffeine from coffee and tea. It plays a central role in tobacco use and has been used by humans for thousands of years[3]. It is reported to be one of the major hazardous components of tobacco which mimics most of the deleterious effects of cigarette smoke (Kavitharaj and Vijayamal, 1999). It has been used in many forms but often and more recently, at least in the Western world, almost only in smoked forms. Tobacco/nicotine is consumed regularly across all countries, cultures and almost all religions. Thirty-one (31%) percent of men and 6% of women are tobacco users globally 2012[4]. Attributable burden due to tobacco smoking including second hand smoking was 6.3 million deaths and 156 million disability-adjusted life year globally.

Indian Scenario:

India is the second largest consumer of tobacco where tobacco is used as smoked and smokeless tobacco (SLT). It has been reported that 28.6% of the Indian population consume tobacco in any form, 10.7% smoke, and 21.4% use SLT. Use of smokeless tobacco is documented in 120 countries, among them India has the largest number of SLT users in the world. Nearly one million people die every year in India due to tobacco use. Tobacco-related morbidity and mortality are high in the productive age group of 24 to 69 years. If, present trend continues, tobacco-associated mortality in India is estimated to be 1.5 million by 2020. India is a low- and middle-income country (LMIC) where tobacco related diseases increase financial burden and thus become a cause and consequence of poverty.

Nicotine exposure occurs in any or multiple of the following conditions:

- Personal exposure such as usage of any tobacco-related product such as cigarettes, cigars, snuff and chewing tobacco (First hand smoking).
- So called 'second hand smoking' or passive smoking refers to the involuntary inhalation of tobacco by somebody else's who are present in close vicinity of first hand smokers.

- Occupational exposure during tobacco extraction or during making tobacco related product e.g cigarette.
- Environmental exposure as nicotine has been used as an insecticide in the past.
- Dietary exposure during consumption of tobacco containing edible foods like potato, green tomato etc. High level of nicotine in fresh potato and tomato products was reported by Siegmund et al. (1999)[5].

Forms of Nicotine used:

Followings are some means by which humans exposed to nicotine:

Cigarettes

A cigarette is a roll of tobacco. The most commonly thought of form of tobacco when you hear the word "smoking." Cigarettes include tobacco leaves, nicotine, and many cancer-causing chemicals. The smoke from a cigarette is inhaled into the lungs. Indians smoked about 6.2 cigarettes per day. Each cigarette contains considerable amount of it (10-14mg) and it is variable with brand[6]

Cigars

Cigars are another form of tobacco, they are tightly rolled, cured tobacco leaves in a leaf wrapper. Although cigars are usually not inhaled into the lungs, they still contain harmful, cancer-causing ingredients. Also, a cigar will have anywhere between 100 and 200 mg of nicotine while an average cigarette contains 12 mg. They can also lead to a nicotine addiction.

Bidis

Bidis hail from India. They are hand-rolled and the tobacco is covered in a tendu or a temburni leaf that is native to Asia. bidis are accounted for the largest proportion of smoked tobacco consumed in India especially among the lower socio-economic group, they consume bidis is 8 to 10 times more than cigarette smoking. They contain 3 times more nicotine and 5 times more amount of tar than the regular cigarette.

Electronic Cigarettes (e-cigarette)

Electronic cigarettes or E-cigarettes is a newer kind of tobacco. This device, looking like a cigarette, cigar, or pen, have a liquid-filled container that holds variable concentrations of nicotine, flavouring, and other chemicals which may be harmful, and uses batteries to heat and create a vapour. The amount of nicotine in individual e-cigarette varies, although no tobacco smoke is produced. It is not yet known whether e-cigarettes are safe or not[7].

Hookahs

A hookah is a different form of tobacco — but is still just as dangerous. It is used to smoke flavored tobacco, which contains nicotine and cancer-causing chemicals. It is heated, then cooled by passing through a bowl of water, and the smoke is then inhaled through a mouthpiece. It can lead to addiction, and is not safer than smoking cigarettes.

Chewing tobacco/ smokeless tobacco (SLT)

Chewing tobacco is one of the most common methods of smokeless tobacco intake. This comes in the form of loose tobacco leaves, plugs, or twists. 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 [8].

It can come sweetened and flavored, and is placed in the mouth between the cheek and lower lip. It can also be chewed. Although it is commonly thought that chewing is not as harmful, like the others, it contains nicotine and cancer-causing chemicals, and can also cause other health problems.

Nicotine patches

Nicotine patches and nicotine gum are commonly used as nicotine replacement therapy (NRT) 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 [9].

Nicotine is also present in certain edible plants, especially plants from the family *Solanaceae* (potatoes, tomatoes) in the range of 2–7 microgram per kg of dry weight.

Insecticides

Nicotine has also been used as an insecticide since 17th century. However, after World War II, its use has declined owing to the availability of cheaper, more potent pesticides that are less harmful to mammals. The environment Protection Agency of United States has banned use of nicotine as a pesticide from 1st January 2014. India, one of the largest producer and exporter of nicotine sulphate, has progressively banned its use as agricultural pesticide. [APIB. Banned Pesticides. Available from: http://megapib.nic.in/Int_pest_bannedPest.htm. [Last updated on 2002 Mar 25; Last accessed on 2014 Sep 27].

Pharmacology of Nicotine

Absorption:

Nicotine absorption can occur through the lung, oral cavity, urinary bladder, gastrointestinal tract and skin[10]. It's absorption in human body is highly dependent on route of exposure and pH. In acidic environments, it is in the ionized state and does not rapidly cross biological membranes. So nicotine is poorly absorbed from the stomach due to the acidity of the gastric fluid, but is well absorbed in the small intestine, which has a more alkaline pH and a large surface area[10].

At a high (alkaline) pH, nicotine remain as the non-ionized state, which passes more easily through lipoprotein membranes than the ionized (charged) state and be readily absorbed across the epithelium of the lung, the oral mucosa, and the nose, and through the skin.

Bioavailability:

The bioavailability of nicotine is route dependent with the highest about 90% when inhaled (e.g. cigarettes) and approximately 60% when used orally because of the huge surface area of the alveoli and dissolution of nicotine at physiological pH (approximately 7.4) and also because nicotine reaches the systemic circulation before passing through the liver where it is metabolized (first-pass metabolism) [11].

After absorption, It distributes extensively to body tissues, including the liver, kidney, spleen, lung, and brain and also accumulates in gastric juice and saliva, breast milk, skeletal muscle, and fetal serum and amniotic fluid [12].

Metabolism of nicotine:

Nicotine has a half-life of about 2 hours in the human body, during this time 70–80% absorbed nicotine is metabolized in the liver, mainly by CYP2A6, UDP-glucuronosyl transferase,

and flavin-containing monooxygenase to cotinine and other metabolites[12] The metabolism takes place in two phases:

In phase I, microsomal oxidation of nicotine takes place via multiple pathway and lead formation of various metabolites like cotinine, nornicotine, demethyl cotinine, trans-3-hydroxy-cotinine and δ -(3-pyridyl)- γ -methylaminobutyric acid, N-oxidation and N-methylation of nicotine, among which cotinine is the major metabolite and has a half-life of about 20 hours in the body.

In phase II metabolism, there is N- and O-glucuronidation of nicotine and its metabolites[10]

Lethal Dose:

In experimental animals, the dose of nicotine which is lethal to 50 % of the animals (LD50) varies widely, depending on the route of administration and the species used. (i.v.) The intraperitoneal (i.p.) LD50 values for nicotine in mice is 5.9 mg/kg and in rats is 14.6 mg/kg [13].

In humans probable oral lethal dose is less than 5 mg/kg for a 70 kg person. It may be assumed that ingestion of 40 mg to 60 mg of nicotine is lethal to humans. (US-EPA. Chemical profile: Nicotine. US Environmental Protection Agency; 1987.)

Mode of Action:

As mentioned earlier that 70 to 80% nicotine from inhaled smoke are absorbed and then metabolized in liver to six major metabolites. The fact that nicotine and its water-soluble metabolite cotinine are detectable in the seminal plasma of smokers and passive smokers exposed to environmental tobacco smoke[15]. Nicotine concentrations of between 70 μ g/l (0.00043mM) and 300 μ g/l (0.00185mM) are commonly found in the semen of casual (1-10 cigarettes/day) and habitual smokers (>30 cigarettes/day) respectively [14].

This finding suggests that nicotine and other harmful components of tobacco smoke would pass through the blood–testis barrier[15] and activate nicotinic acetylcholine receptors (nAChRs) that result in various gonadotoxic effects on testes and male reproductive organs [16]

Nicotine binds to a class of ionotropic acetylcholine receptors, the “nicotinic receptors” (nAChRs) present in spermatozoa, made up of five subunits. In human spermatozoa, only 8 out of 16 nAChR sub units (namely α 1, α 2, α 3, α 4, α 5, α 6, α 7, α 9, α 10, β 1, β 2, β 3, β 4, γ , δ) are expressed. The different subunits form a large number of receptor isoforms, allowing a variety of different responses for each tissue.[16]

The mechanism by which nicotine exerts its toxic effects on testes is not clear. However various studies suggested that nicotine damages sperm function by interacting with nAChR on the cell surface of human spermatozoa. Over expression of α 7 subunit is involved in the process of apoptosis, chromatin/DNA integrity, mitochondrial function and other sperm parameters [16].

Adverse Effects on Male Reproductive System:

Effects of nicotine on sperm count /concentration (Oligozoospermia)

According to WHO, male factor' infertility is generally involved an alteration in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyses, collected between 1 and 4 weeks apart [17].

The *in vivo* reproductive effects of nicotine have been assessed in many animal studies and most of them have reported nicotine adversely affect semen quality by reducing sperm concentration, alter morphology and by lowering sperm motility. Oligozoospermia, a medical condition characterized by low sperm count and quality, is responsible for 90% of male infertility[18]. Several *in vivo* studies have indicated a significant reduction in the semen volume and sperm concentration in nicotine treated animals[19-21]. Nicotine and its metabolite cotinine easily enter into the blood circulation of testis and can have a direct cytotoxic effect on spermatozoa by damaging DNA[22].

Effects of nicotine on sperm motility (Asthenozoospermia)

Asthenozoospermia or reduced spermatozoa motility, may be an early indicator of reduced semen quality in smokers. Nicotine proved to be a potential oxidant which can affect spermatogenesis. Damage of membrane structure of the spermatozoa caused by nicotine is a major cause of the impaired motility [22-23]. The seminal fluid of both smokers

and passive smokers was found to contain nicotine at a concentration of 100ng/ml and 10ng/ml respectively which was able to suppress sperm motility by about 90% and may cause asthenozoospermia in smokers and non smokers[15,18 24]. *In vitro* studies also have indicated that the seminal nicotine, cotinine and trans-3'-hydroxycotinine levels were negatively correlated to total sperm motility [15, 18, 25].

Effects of nicotine on sperm morphology (Teratozoospermia)

The incidence of teratozoospermia, or increased abnormal spermatozoal morphology, was also significantly higher in heavy smokers than in non-smokers [28]. Chronic smoke/nicotine exposure has been positively correlated to increased abnormal morphology both in animals and humans studies[29]. Sepaniak et al. (2006) have reported that nicotine and its metabolite cotinine easily enter into the blood circulation of testis and can have a direct cytotoxic effect on spermatozoa by damaging DNA.

Table: Toxic Effects of Nicotine on Male Reproductive system

Toxic Effects	Target	References
Decrease Testicular weight & degeneration of tissues	Germ cells, Sertoli/ Leydig cell	22,26 27
Decrease Sperm parameters: Sperm count, Sperm motility, sperm morphology	Sertoli/ Leydig cell Function/spermatozoa	15,18 21-23
Spermatogenesis	Leydig cell/ Sertoli cell	19, 22,
Decrease Testosterone secretion	Leydig cell	22,23

Nicotine induces Oxidative stress: A possible mechanism of reproductive toxicity

A possible mechanism underlying adverse nicotine-induced reproductive health outcome. However, the mechanism by which nicotine causes male infertility is poorly understood. There is considerable evidence from animal studies that exposure to nicotine can damage the male reproductive system through various means including oxidative stress, which may be a potential mechanism underlying deleterious nicotine-induced reproductive outcomes[23]. Indeed, adult animals exposed to nicotine exhibited increased ROS production and oxidative damage in the testes, which was associated with testicular damage (e.g., degeneration of seminiferous tubules, germ cell exfoliation, loss of Leydig cells, and disrupted spermatogenesis) and decreased sperm counts[19,22,26-27].

Nicotine increases ROS production by breaking the mitochondrial respiratory chain. A large number of experiments on rodent's(rats/mice) reflected that nicotine increased the production of ROS [H₂O₂ and hydroxylradicals (OH⁻)] which has been associated with the induction of testicular oxidative stress[21-23].

Further studies also confirmed that nicotine reduces testicular /seminal antioxidant enzymes e.g CAT, SOD and glutathione reductase activity[19,21-23, 28] and this imbalance of ROS generation and oxidant enzymes lead to oxidative stress which may be a con-current mechanism of NT testicular toxicity and infertility.

Conclusion:

Thus far, the mainstream published studies (*in vivo*, or *in vitro*) present the negative effects of nicotine on semen quality in primarily decreasing sperm count and secondarily reducing sperm motility and increasing sperm DNA damage, though there are still a few in clinical studies that have deliberated some adverse/blunted effects.

However, further research-primarily clinical, is still necessary to confirm these adverse effects. At present, our laboratory is running an experimental study (*in vivo*) that uses different bio analytical methods, such as flow cytometry, to determine the mechanism of toxic action nicotine on reproductive system of male rats and its amelioration by vitamin B12 complex.

Conflict of Interest

The author declares that he has no conflict of interest

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