Smoking and male fertility: a contemporary review

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Abstract

The turn of the past century has witnessed a remarkable increase in public awareness of the potential hazards of many environmental factors to general health. Extensive research efforts have focused on the effect of smoking on male reproduction. Smoking has been proved to cause deleterious effects on male fertility at various levels, starting from the early stages of spermatogenesis to the post-natal period. We review the current literature on these adverse effects on male fecundity extending our focus beyond the basic semen analysis to sperm DNA damage and the implications of this damage on pregnancy outcomes.

Key words: smoking, male fertility, sperm quality, offspring welfare.

Introduction

Despite the growing knowledge about the detrimental health hazards caused by smoking, 35% of reproductive-aged American men still smoke cigarettes on a daily basis [1]. While the general population is acutely aware of the role of smoking in lung and heart diseases, the adverse effect of smoking on male reproductive health is less well known. During the past two decades, the whole perception of smoking as having an insignificant effect on male fertility has changed. A growing body of evidence reveals that smoking leads to declines in semen parameters such as sperm concentration, viability, forward motility and morphology [2-9]. Other studies show a decline in sperm penetration ability and fertilization rates [10, 11]. Defects in these parameters not only affect normal fecundity but also lower assisted reproduction success rates [12-14]. Smoking has also been associated with an increase in seminal leukocytes and reactive oxygen species (ROS) levels to an extent exceeding the antioxidant capacity of seminal plasma, leaving the sperm vulnerable to oxidative damage. Others have documented alterations in the sperm DNA integrity which may ultimately threaten the health of future offspring [15-22]. This raises serious concerns not only about fertility but also, the welfare of offspring that may inherit this damaged genetic material.

In this review, we will focus on the effects of smoking on male reproduction. In addition to the effects of tobacco smoking on conventional semen parameters and reproductive hormonal levels, we will address current evidence on the adverse effect of smoking on seminal fluid antioxidant capacity and sperm genetic material. The implications of these detrimental effects on fertilization and pregnancy rates as well as the health of the offspring will be reviewed.

Smoking and semen parameters

Starting in the early nineties, the concept of smoking having a negligible effect on male fertility was challenged by emerging studies revealing the detrimental effect of smoking on male reproduction. One of the earliest studies showed a significant difference in sperm motility and morphology when comparing smokers to non-smokers [5]. Vine's meta-analysis in 1994, reported that smokers had a 17% lower sperm density than non-smokers [7]. During the same year, Chia reported 618 males undergoing initial screening for infertility, noting a strong correlation between the smoking duration and poor volume and morphology of sperm, with an increase in headpiece defects [2]. This was one of the first studies revealing the dose-dependent effect of smoking on semen. The level of cotinine, a tobacco metabolite, was inversly proportional to semen density and motility. Controlling for potential confounding variables, such as seasonal variation for semen sample collection or associated alcohol and caffeine consumption, had no effect on these correlations, emphasizing the pure effect of smoking on male fertility [8]. Subsequent large population studies demonstrated similar dose-dependent detrimental effects on all four semen parameters combined (density, viability, morphology and motility) [4, 6, 9]. A cohort study comparing 655 smokers to 1131 non-smokers revealed declines of over 15% in sperm density, 16% in motility and 10% in normal sperm morphology [3]. In contrast to this global decrement in semen parameters, other studies demonstrate isolated abnormalities associated with smoking, to include negative effects on sperm motility and morphology, especially head defects and cytoplasmic droplets [23, 24]. Retention of sperm cytoplasm was a unique finding in sperm of smokers compared to non-smokers [23, 25]. Cytoplasmic droplets may be a critical morphological obstacle in the pathway of normal sperm function. Alteration of progressive motility of sperm was another consequence of smoking irrespective of tobacco dose [26], while seminal volume was the only affected semen variable in another study [27]. A cross-sectional analysis of seven separate studies for the association between occupational or environmental exposure and semen quality, revealed that heavy smokers had 19% lower sperm density than non-smokers in addition to depressed testosterone levels [28]. The amount of cigarette smoke exposure may determine the type of insult to sperm. After stratifying smokers according to their level of tobacco consumption, sperm motility seemed to be the first susceptible parameter to injury in light smokers followed by morphology in heavy smokers [29]. In addition to the significant decline in sperm concentration in heavy smokers, increased numbers of immature spermatozoa were also noted [30].

Endocrine effects of smoking

An endocrine effect of smoking on fertility has also been suggested although study results have been conflicting. Field examined 1241 randomly selected middle-aged U.S. men, controlling for age and body mass index. Smokers had higher levels of a variety of androgens including 18% higher dihydroepiandrosterone (DHEA), 33% higher androstenedione, 9% higher total testosterone, 14% higher dihydrotestosterone (DHT) and 8% higher sex hormone-binding globulin (SHBG) [31]. Others reported significant increases in estrone and estradiol levels among smokers, while testosterone levels remained constant [32]. Testosterone levels were significantly lower in a group of men that were followed after reducing or quitting smoking in comparison to those who continued smoking [33]. Moreover, a positive dose-response relationship was observed between smoking and mean concentrations of testosterone, luteinizing hormone (LH), and LH to free testosterone ratios [28]. Serum follicle-stimulating hormone (FSH) levels were 17% higher among non-smoking men. Again this negative difference among smokers seemed to be dose dependant as smokers who smoked more than 10 cigarettes daily displayed a 37% lower level of FSH than those who smoked less [34].

Although the majority of studies suggest a positive correlation between smoking and the levels of male reproductive hormones, others show either an inverse relationship or no relationship at all. No statistically significant differences in serum testosterone, LH or FSH levels were observed in animals exposed to tobacco [35].

The exact role of endocrine disturbances on smoking related infertility remains to be established. Methodological flaws such as small sample size, lack of standardized hormonal assays and failure to consider confounding factors, all contribute to conflicting conclusions [36]. Larger prospective trials are required to clarify the impact of smoking on the hormonal milieu.

Smoking and oxidative stress

Reactive oxygen species (ROS) are free radicals characterized by a high ability to oxidatively modify biomolecules. Reactive oxygen species play an important physiological role in the function of sperm, however with high levels, their effect on sperm converts from beneficial to detrimental [37]. High levels of ROS exceed the normal antioxidant capacity of seminal plasma resulting in oxidative damage to spermatozoal plasma membranes and genetic material [38]. Smoking significantly increases leukocytes in ejaculates of smokers [10, 24, 33, 39] leading to increased levels of seminal

ROS produced by these leukocytes. A prospective study comparing a group of infertile smokers to two other groups of infertile and healthy non-smokers demonstrated a 48% increase in seminal leukocyte concentration, 107% increase in ROS levels and a 10-point decrease in ROS-Total antioxidant capacity (TAC) scores among the smoking group [39]. Medium, heavy and long term smokers also showed substantial decreases in levels of natural anti-oxidants found in seminal plasma including zinc, copper and superoxide dismutase [9, 40]. Ascorbic acid levels decreased significantly in the seminal plasma of smokers vs. non-smokers which correlated to a similarly significant decline in the semen parameters of smokers [6]. In attempts to prove the hypothesis of the oxidant-anti-oxidant imbalance, several scientists studied the effect of seminal plasma obtained from smokers, presumed to contain disturbed anti-oxidant systems, on spermatozoa of non-smokers. This combination resulted in impairment of sperm viability and motility [41, 42]. These recent studies provide an explanation for the significant alteration in sperm viability that was previously demonstrated almost a decade before when a similar experiment was conducted [43]. Accordingly, this conclusion could have clinical implications in assisted reproductive technologies (ARTs) if sperm from smokers were to be reconstituted in culture media providing supplementary anti-oxidant systems.

Smoking and genetic damage

In addition to the high content of oxidants in cigarette smoke, smoke is a rich source of mutagens and carcinogens which ultimately lead to DNA damage [16]. These compounds pass through the blood-testis barrier altering not only bulk sperm parameters but also the sperm DNA integrity [20]. DNA damage at the gamete level represents a serious potential risk for transmission of mutations to offspring. This concern has paralleled the increased utilization of assisted reproductive technologies (ARTs), especially intracytoplasmic sperm injection (ICSI) which bypass the natural mechanisms of sperm selection potentially amplifying the potential of using DNA damaged sperm as a result of smoking.

Early studies proved that smokers had higher levels of oxidative DNA damage than non-smokers [44]. Well designed studies regard the history of tobacco consumption as a subjective measure due to the diversity in nicotine concentration within different cigarette brands and the actual amount of smoke inhaled according to personal habits. To overcome this methodological flaw, many studies measure body concentrations of nicotine or its main metabolites and use these objective parameters. 8-Hydroxydeoxyguanosine (8-OHdG), one of the major forms of oxidative DNA damage, was positively correlated to the level of the major degradation product of nicotine, cotinine [45].

DNA damage can be structural or numerical in nature. DNA fragmentation is one of the prominent forms of structural sperm DNA damage demonstrated by recent studies comparing sperm of smokers to non-smokers. This form of alteration may result in sperm DNA mutations, increasing the rates of miscarriages and predisposing offspring to a greater hazard of congenital defects, childhood cancer and infertility [14, 16, 19] (Figure 1). Sepaniak and his group utilized the TUNEL assay in a prospective trial revealing that smokers had a significantly higher DNA fragmentation level within their spermatozoa than non-smokers [19]. Another type of structural damage is the covalent-bonding of sperm DNA to smoking-induced carcinogens resulting in the increased level of bulky DNA adducts in the sperm of smokers. Benzo(a)pyrene diol epoxide (BPDE) has been one of the most studied tobacco derived carcinogens over the past three decades. Healthy male smokers were found to have a 21% positive staining reaction for BPDE-DNA adducts in their sperm compared to only 4% in non-smokers, thus having a significantly higher risk of transmitting prezygotic DNA damage [22]. Smokers had a 1.7-fold increase in levels of sperm DNA adducts compared to non-smokers, suggesting sperm DNA-adduct level to be a significant potential biomarker in male infertility research [15]. Aneuploidy represents another form of smoking-induced chromatin damage. Fluorescence *in situ* hybridization (FISH) based tests have shown smokers to have elevated frequencies of sperm chromatin aneuploidy. After controlling for confounding factors such as age, alcohol and caffeine intake and abstinence period, disomy X remained a statistically significant numerical abnormality in sperm of smokers [46]. In a subsequent study, increased sperm Y disomy was noted in teenage smokers [18]. In a Chinese study, which controlled for other life style factors, disomy 13 was significantly higher in sperm of smokers [21]. These numerical alterations in sperm DNA elevate the potential of fathering children with aneuploidy syndromes.

Smoking, fertilization and pregnancy rates

After reviewing the various forms of damage to semen quality as a consequence of smoking, it becomes possible to infer the ultimate effect of smoking on fertility and pregnancy. From an evidence-based view point, current studies have proven the inverse association between smoking and rates of fertilization and pregnancy [47, 48]. Early studies examined the effect of tobacco on sperm fertilization capacity using sperm function

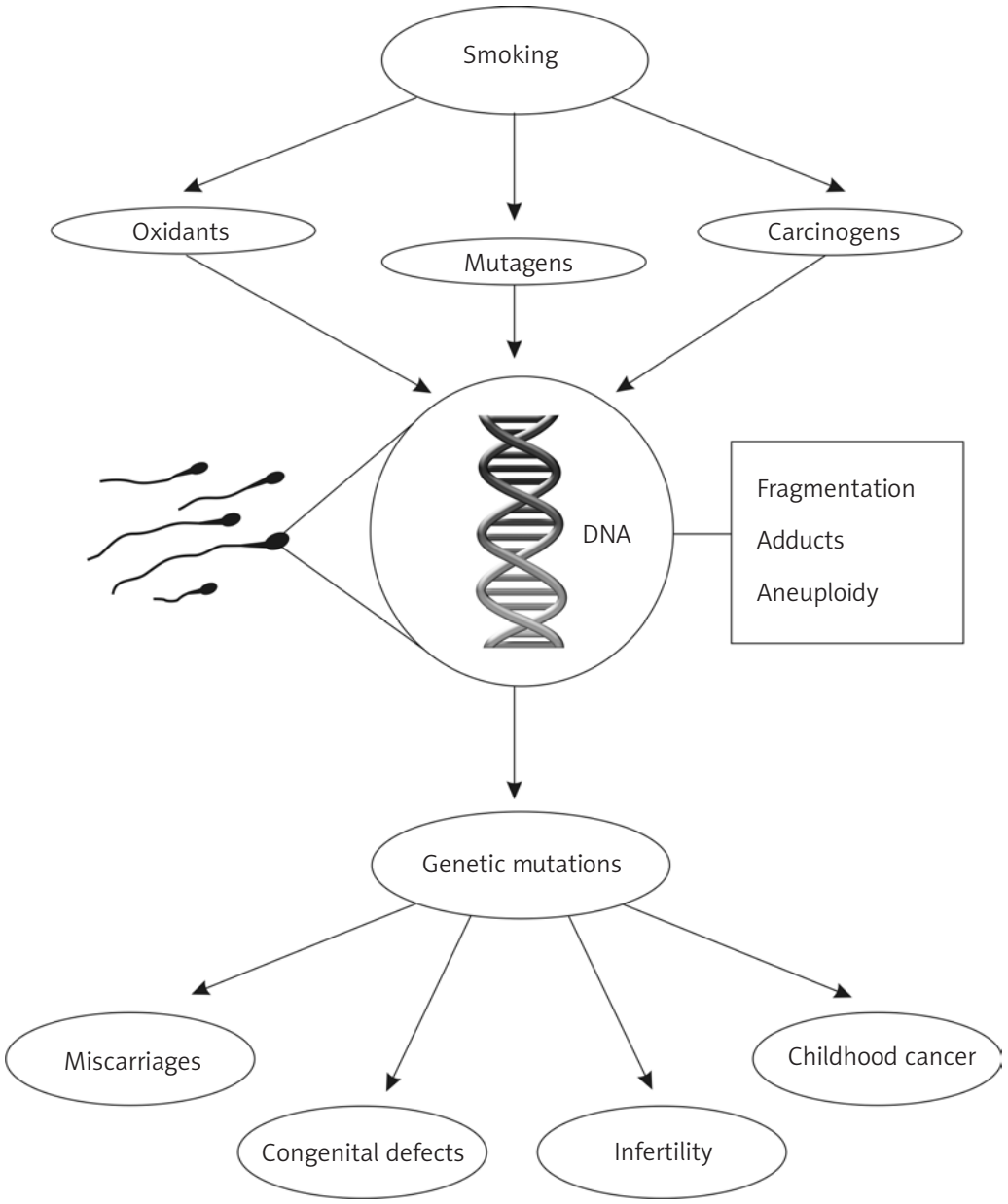

Figure 1. Schematic representation of the pathogenetic link between smoking and potential manifestations of sperm DNA damage

tests including the hamster oocyte sperm penetration assay (SPA), hypo-osmotic swelling test and sperm acrosin profile. A study on 164 men displayed a significant decline in SPA scores with a mean of 2.5 in smokers vs. 8 in non-smokers [10]. Serum nicotine and cotinine levels were inversely proportional to all three sperm function tests when measured in smokers vs. non-smokers. Furthermore, sperm function tests improved in smokers who quit smoking [11].

Studies comparing the cumulative pregnancy rates and outcomes of smokers to non-smokers have raised similar concerns. The first study to record that paternal smoking had a detrimental effect on pregnancy outcome concluded that smokers had a 2.4% decrease in the chance of achieving a 12-week pregnancy. This decrease correlated with their age which served as an indirect measure for the duration of smoking [12]. Smokers carried a relative risk of 2.41 for not achieving conception and 3.76 for not reaching a live birth through *in-vitro* fertilization (IVF) and gamete intra-Fallopian transfer (GIFT) compared to non-smokers. A history of smoking for over five years almost doubled the relative risk of failing to cause a pregnancy [13]. Smoking by the male partner appeared to be a predictor of IVF and ICSI failure in addition to female age and number of embryos transferred. Smokers had an odds ratio of 2.65 and

2.95 for IVF and ICSI failures respectively as compared to non-smokers [14]. Animal studies revealed similar declines in IVF success in males exposed to cigarette smoke. Oocyte fertilization and cleavage rates together with blastocyst development rates were significantly lower when cigarette smoke exposed rats were compared to controls which may explain the failure of embryo implantation observed in human studies [35].

Smoking and pregnancy outcomes

With the potential for smoking to induce sperm DNA damage as well as the widespread utilization of assisted reproductive techniques particularly ICSI which bypasses natural sperm selection, a theoretical risk of inducing pregnancies with genetically compromised sperm exists. This could raise the potential for adverse reproductive and developmental sequelae such as spontaneous abortions, congenital anomalies or cancers of childhood.

Despite the well documented impact of maternal smoking on pregnancy, there has been inconclusive support for the correlation between paternal smoking and the rate of spontaneous abortions [48, 49]. One study showed that paternal smoking was not associated with elevations in spontaneous abortion when controlling for confounding female factors such as age, alcohol and caffeine consumption, and prior fetal loss [50]. A prospective study on 526 newly married couples, exhibited a 0.93 adjusted odds ratio for clinical pregnancy if the husband smoked less than 20 cigarettes per day and 0.78 if the husband's daily consumption was 20 cigarettes or more, compared to a smoke free couple. Similarly, in comparison to the non-smoking group, the lighter smoking group showed a 1.04 odds ratio for early pregnancy loss as opposed to 1.81 for the heavy smokers [51].

A large body of literature has focused on the risk of developing childhood cancers due to paternal smoking. There have been repeated trials suggesting a link between paternal smoking and childhood cancers, such as rhabdomyosarcoma, brain tumors, neuroblastoma, retinoblastoma, lymphomas, leukemias, sarcomas or all cancers combined [36]. Nevertheless to date, studies in this area have been conflicting thus failing to reach solid support for this correlation. A case-control study on 642 childhood cancer cases documented a significantly elevated risk of childhood cancers with both acute leukemia and lymphoma on top of the list of cancers associated with preconception cigarette smoking. Paternal smoking more than five years prior to conception, resulted in an odds ratio of 3.8 for acute leukemia, 4.5 for lymphoma, 2.7 for brain tumors and 1.7 for the rest of the cancers combined [52]. This hypothesis was later supported by another study that found a significant positive correlation between paternal smoking and the risk of childhood cancer when comparing 555 cases of children with cancer to the same number of children from the general population [53]. Recently, the Northern California Childhood Leukemia Study noted an association between paternal smoking and childhood leukemia especially when accompanied by maternal postnatal smoking [54]. Although these studies raise concern about associated risks of childhood cancer with paternal smoking, other case-control studies contradict this conclusion and show no association [55-59]. This question remains to be answered by large longitudinal studies.

There is a paucity of research asserting a paternal link between congenital anomalies in offspring and the smoking habits of their fathers. In one controlled study, no increased risk of anomalies was noted in 5 year old children with a history of fathers that smoked [60]. Zhang and his group documented a relative risk of 1.2 for birth defects associated with paternal smoking, noting a two fold increase in incidence of anencephalus, spina bifida and feet deformities. Moreover, the risk of multiple malformations seemed to be higher than isolated defects [61]. An increased incidence of birthweight reduction and limb deformities has been reported in offspring of fathers who smoked [62, 63].

Due to the paucity of data on these associations, definitive inferences remain difficult to draw. However, the deleterious effect of smoking on sperm DNA raises at least theoretical concerns on DNA integrity in the offspring of smokers.

Conclusions

While the general population is acutely aware of the role of smoking in lung and heart diseases, the adverse effect of smoking on the male reproductive health is less well known. Recent studies have raised concerns that smoking has a significant effect on male fertility, both in basic semen parameters and DNA integrity. Smoking leads to a significant decline in semen parameters that not only determine natural male fecundity but also success rates with assisted reproduction. Furthermore, smoking has been associated with an increase in oxidative stress within the seminal environment leaving sperm vulnerable to oxidative damage. Damage due to smoking has even extended beyond a basic seminal profile through sperm DNA fragmentation, chromosomal aneuploidy and DNA adducts.

All these studies draw attention to the paternal component of fertility impairment emphasizing the intense demand for further research on male lifestyle factors and their contribution to fecundity. This will hopefully enable health care providers to answer critical questions not only about the fertility of smokers but more importantly, the welfare of their offspring.

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