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Review Article

The Impact of Cigarette Smoking on Sperm Chromatin Structure and its Consequences on IVF and ICSI Outcomes

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Cigarette Smoking Contents

There have been a number of reviews indicating that our epigenomes may be sensitive to "environmental" influences which can be broadly defined to include diet, toxins, stressors, and psychosocial influences [1,2,3].

A variety of environmental chemicals of concern for human health have been investigated for their potential effect on DNA methylation and other epigenetic effects [4].

Cigarette smoking is a modifiable risk factors for advers health outcome and a major cause of morbidity and mortality [5].

Various groups reported the negative impact of smoking on sperm pararameters, concomitant with increased seminal reactive oxygen species [6,7].

In fact, cigarette smoke is a complex mixture of chemical compounds containing about 4000 hazardous materials; out of which 400 are toxic chemicals, about 40 are malignant, and more than 55 are carcinogens [8,9,10].

An analysing study have quantified the cadmium (1.2 to 90.3 nanograms [ng] per cigarette), lead (0 to 41.4 ng/microgram [µg], and mercury (0.25 to 4.3ng/µg) in mainstream smoke.

The toxic effects of the heavy metals lead, mercury, and cadmium on reproduction and development are well known and widely reviewed in both clinical and animal studies [11,12].

Studies of men report an adverse relationship between levels of lead in blood and levels in sperm, in addition to adverse pregnancy outcome in their partners [13,14].

Male and female reproductive effects from metal toxicity are well documented including effects on fertility, menstrual cycle function and adverse pregnancy outcome [15,16].

In addition, many of these chemicals are poisonous substancessuch as nicotine and its metabolite cotinine [17]. These harmfull substances such as nicotine, alkaloids, nitrosamine, cotinine and hydroxycotinine are important in the production of reactive oxygen species [18,19].

Cigarette smoking is a responsible for a high concentration of reactive oxygen species (ROS), nitric oxide (NO), peroxynitrite, and free radicals of organic compounds.

ROS include a variety of oxygen containing substance with high reactivity with other biomolecules. ROS include both free radicals (containing one or more unpaired electrons) , such as superoxide anion (O_2-), hydroxyl (-OH), peroxyl (ROS_2.), and hydroperoxyl (HO_2.) and nonradical species that are either easily converted into radicals or are oxiditzing agents, such as hydrogen peroxide (H_2O_2) and other peroxides (ROOH). Under physiological condition, ROS are produced in a controlled manner and play important roles as secondary messenger in many intracellular signalling pathways [20,21]. In sperm, ROS have been shown to have an important participation in the regulation of all the functional parameters, including motility, capacity, sperm –zona pellucida interaction, acrosome reaction and sperm –oocyte fusion [22,23].

It has been demonstrated that free radicals can oxidize lipids, amino acids and carbohydrates as well as causing DNA mutations [24].

It was shown by many authors that when the levels of ROS rise above the body's antioxidant defense system, oxidative stress (OS) occurs. In this circumstance, the elevated levels of ROS damage cells, tissues, and organs [25,26].

A possible explanation for these finding could be the increased leukocytes thatinduced oxidative stress (OS) on developing or mature sperm, and inadequate scavenging antioxidant enzymes in the seminal fluid of smoker men [27].

Metabolites of cigarette smoke components may induce an inflammatory reaction in the male genital tracts with subsequent release of chemical mediators of inflammation such as interleukines -6 and interleukin-8, which can recruit and activate leucocytes [28].

The activated leucocytes can produce high levels of reactive oxygen species (ROS) in semen which may affects the antioxidants capacity and results in oxidative stress [29].

Chromatin Condensation of Spermatozoa

The appropriate packaging of the sperm chromatin is believed to be essential for male fertility.

Higher order chromatin structure withinthe nucleus may play an extremely important role in regulation of gene expression and in mediating other cellular functions [30,31].

Mammalian sperm DNA is the tightest compacted eukaryotic

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DNA [32]. Normal sperm nuclear composition is essential to maintain sperm DNA integrity [33]. Besides, sperm chromatin is very tightly compacted by virtue of the unique associations between the DNA and sperm nuclear proteins [34].

Mature sperm nuclei is highly compacted and measure a volume 40 time less than that of normal somatic nuclei [32], and the DNA in mammalian sperm is tightly compacted into linear arrays organized as loop domains [32].

High order of chromatin packaging is required for normal sperm function, various authors have also pointed out that tight chromatin packaging has protective function against endogenous and exogenous agent such as nucleases, free radicals or mutagenes.

Also, the function of sperm is to safely transport the haploid paternal genome to the egg containing the maternal genome. The subsequent fertilization leads to transmission of a new unique diploid genome to the next generation. Before the sperm can set out on its adventurous journey, remarkable arrangements need to be made during the post-meiotic stages of spermatogenesis [35].

Haploid spermatids undergo extensive morphological changes, including a striking reorganization and compaction of their chromatin. Thereby, the nucleosomal, histone-based structure is nearly completely substituted by a protamine-based structure. This replacement is likely facilitated by incorporation of histone variants, post-translational histone modifications, chromatin-remodeling complexes, as well as transient DNA strand breaks [35].

During the process of sperm chromatin remodeling, any abnormalities in each steps including DNA organization levels, histone-protamine replacement, and disulfide bond formation may cause DNA and chromatin damages.Sperm chromatin structure and DNA integrity are known to have a crucial influence on the fertilizing process [36,37] and on individual fertility capabilities [36,38]. Besides, the integrity of sperm DNA is crucial for the correct transmission of genetic information to future generations.

Many studies have shown negative correlation between the defetcts of sperm chromatin integrity and male fertility potential.

Indeed, a correlation between chromatin condensation and the occurrence of DNA nicks has been reported in mouse and human spermatozoa [39,40].

Sperm chromatin quality correlates with pregnancy outcome in *in vitro* fertilization [41,42].

Sperm chromatin abnormalities have been studied extensively in the past two decade as a cause of male infertility [43]. Abnormalities in the sperm chromatin organization, characterized both by damaged DNA and incompletely remodelled chromatin in mature sperm cells, may be indicative of male infertility regardless of normal semen parameters [44,45].

Histones

Histones are a group of evolutionary conserved proteins that play a critical role in the packaging of DNA into chromatin. There are four classes of core histones (H2A, H2B, H3,H4) and a linkerhistone (H1).

The regulatory function of histones is mainly achieved through their covalent modification, primarily at amino acid residues in their N-terminal tail region [46]. Chromatin modelling is accompanied by changes in the shape, conversion of negatively supercoiled nucleosomal DNA into nonsupercoiled state [47]. It is mediated by drastic change at the most fundamental level of DNA packaging where a nucleosomal architecture shifts to a toroidal structure [48].

This change implemented bysperm nuclear basic proteins (SNBs) that include variants of histone subunits, transition proteins, and protamines [49]. The first new proteins to appear are four histone variants that replace some or the majority oftheir somatic H2B, H3, H2A, and H1 histone counterparts [50]. Also, the histone in sperm involves a group of proteins that are compositionally and structurally similar to the core (H2A, H2B, H3, and H4) and linker (H1/H5) somatic histone. They are enriched in both lysine and arginine.

The major functions of canonical histones are genome packaging and gene regulation. The non-canonical histones (histone variants) play a role in a wide range of processes, such as transcription initiation and DNA repair by establishing a distinct chromosomal domain to carry out a specialized function [51].

Male germ cells have an unusually high number of histone variants in comparison to somatic cells.Several histone variants are exclusively expressed in male germ cells, so they are testis –specific histone variants [52].

Incorporation of one of the testis –specific histone variants is thought to form nucleosome with lower stability than those containing canonical histone [53]. The testis specific histones include H1 variants [54], H2A and H2B and H2BFWT variants [55] and H3 variants [56]. The predominant isoform is histone H2B [57]. The exact role of these histone H2B variants is largely unknown. However, the temporal accumulation of histone variants during spermatogenesis indicates their potential involvement in meiosis, spermiogenesis, and fertilization [58]. Specifically, H2BFWT may be associated with telomeres, a finding that suggests a putative role in early chromatin remodeling at fertilization [50].

Histone H4 belongs to the most slowly evolving proteins in eukaryotes, and variants has been described only in a few species [59]. In mammals, no histone H4 variant is known. Hyperacetylation of H4 is the normal mark at the onset of the histone to protamine change [60].

During spermatogenesis, histone proteins are replaced with protamines [61,62], a characteristic previously thought to negate the ability of histone to transmit epigenetic information during the process of gametogenesis.

Because the protamines do not contain any modifiable tails, any epigenetic information carried on histones is unable to be passed through the male germ line [63]; however, it is unknown whether they play a role in passing on any epigenetic information to the resulting zygote [64,65].

However, it is now known that some histones are retained during protamine replacement [66], suggesting that modifications that are present on these retained histone proteins may help to mark the relevant chromosomal regions as those requiring the methylation marks that specify imprinting.

The degree of chromatin condensation can be assessed with the aid of acidic aniline blue staining, which discriminates between lysine-rich histones and arginine- and cystein-rich protamines [67]. This technique gives a specific positive reaction for lysine and reveals differences in basic nuclear protein composition of ejaculated human spermatozoa.

Spermatozoa that stained dark with aniline blue, reflects high levels of persistent histones in diminished maturity of ejaculated spermatozoa [68].

Ovari et al. [69] found an inverse correlation between the proportion of sperm with dark aniline blue staining and curvilinear sperm velocity.

However, it has been demonstrated that a significant percentage of spermatozoa in infertile men have a greater amount of residual histones than fertiles [70].

The transition proteins

The mammalian transition proteins TP1, TP2, and TP4 appears in the chromatin of mid-stage spermatids at the same time the majority of the histones are removed from the chromatin. TPs are required for normal chromation condensation, for reducing the number of DNA breaks andfor preventing the formation of secondary defects in spermatozoa and the eventual loss of genomicintegrity and sterility.

TP1 is a 6.2 Da, highly basic (about 20% each of arginine and lysine) protein with evenly distributed basic residues [71,72].

The best characterized of these proteins, transition protein 1 and transition protein 2, represent about 55% and 40% of spermatid total nuclear proteins, respectively [73]. Transition protein 1 is a small basic protein of 54 residues, rich in arginine, lysine and serine, TP2 is a 15,641Da basic (10% each of arginine and lysine) protein with distinct structural domains. Twice the size of transition protein 1, transition protein 2 is enriched in basic residues in its carboxy terminus and contains two putative zinc fingers in the amino-terminal region. After histone removal and before protamine deposition, transition proteins constitute 90% of all chromatin basic proteins.

With the appearance of TP1 and TP2,the chromatin begins to condense somewhat with condensation progressing in the nucleus from an apical to caudal direction [74,75].

Mice mutants for transition proteins 1 or 2 alone are able to produce offspring, although with reduced fertility, suggesting overlapping roles of these proteins and indicating that either the TPs were not essential or that the individual TPs complement each other [76,77]. Zhao et al. [78] using double knockout mouse model demonstrated that the absence of both TP1 and TP2 seriously compromise chromatin condensation leading to infertility whichsuggested that TP1 and TP2 are partially complemented by each other.

Protamine

The molecular structure of the human protamine–DNA complex is still poorly understood [79,80]. Protamines are highly basic proteins half the size of typical histone [81]. Protamines are small proteins (relative molecular mass 4,000–12,000) that are evolutionarily related to histone H1, but have significantly different biochemical properties [82]. Protamines have very low lysine content, and more than 50% of their residues are arginine, which is probably responsible for their high DNA-binding affinity. This can be attributed to the fact that arginine has a greater flexibility in the formation of hydrogen bonds with the DNA backbone owing to its complex guanidinium group [83]. Also, Arginines are represented as 55-79% of the amino-acid residues in protamines, permitting a strong DNA binding [84].

To achieve the highly compacted elongated nucleus, the chromatin is remodelled by a set of abundant transition proteins (TPs) subsequently replaced by the protamines (PRMs).

The PRMs bind DNA, neutralizing the phosphodiester backbone of the double helix [84] and allowing a tight compaction of the DNA as toroids [85]. Toroids are crosslinked by disulphide bonds formed by oxidation of sulfhydryl groups of cysteine present in the protamines [81,85].

Therefore, nuclear elongation halfway during spermiogenesis is accompanied by the transition of chromatin from a histone-based structure to a protamine-based structure. Nucleoprotamine is arranged into large toroidal subunits, each containing approximately 50 kbp of DNA [34]. An incomplete nucleosome to protamine remodelling were found in subfertile males [86]. Normal protamination of the spermatid nucleus provides both chemical and mechanical stability to the haploid genome [87] throughout their transit to fertilization [88,89]. Human sperm nuclei contain considerably fewer protamine (around 85%) than sperm nuclei of several other mammals [39] and therefore, they are less regularly compacted and frequently contains DNA strand breaks [90]. Infertile men possess a higher proportion of spermatozoa with an increased histone to protamine ratio than fertile controls [91].

Several studies have shown that the poor or aberrant protamination of sperm DNA during spermiogenesis is clearly a major factor in sperm chromatin damage induction [92,93].

Protamines are required for i) condensing the male genome to create a more compact and hydrodynamic nucleus.The spermatozoa with more hydrodynamic nucleus have the capacity to move faster and thus the more potential to fertilize the oocyte, ii) protecting the genetic informations from nucleases, mutagens or damage from reactive oxygen species or other toxic agents, iii) epigenetic remodeling during the process of spermiogenesis and iv) removing transcription factors and proteins to help reorganize the imprinting code in the oocyte [94].

Besides, most mammals express only protamine 1, whereas mice and humans express two types of protamine families. Human spermatozoa contain two types of protamines, P1 and P2, with a second type deficient in cysteine residues [95]. Also, the P1 family has been reported in all species of vertebrates studied to date [96,97].

Their structures differ and disruption of either gene in the mouse results in male infertility [33]. The ratio of P1 to P2, in normospermic human samples is around 1 [98]. Variations in the levels of protamination by an unbalanced P1/P2 ratio may result in male infertility. In oligospermic subjects, often a shortage of P2 is found in combination with the presence of P2 precursor proteins [99].

Mengual et al. [96] noted that the sperm nuclear P1 to P2 ratio

was significantly higher in the subgroup of men with oligospermia compared to the fertile controls. In infertile humans, where sperm nuclear protein have been more deeply analysed, the unbalance between P1/P2 ratio differentially impacts on the integrity of DNA and in the reproductive outcome of these couples [99,100]. It has been reported that P1/P2 ratio in human sperm correlates with the levels of sperm DNA fragmentation and also with the rate of sperm DNA damage [96]. Fertile control and subfertile patients showed a difference between P1/P2 ratio with different types of pathologies [101].

Observations in infertile men and transgenic mice models demonstrated that low PRM content in sperm or altered PRM1-PRM2 ratio is associated with infertility [102-105].

It has been shown that a sperm with protamine deficiency and increased histone leads to premature chromatin condensation and that is one of causes of failure in fertilization and embryo developments [106,107].

Sperm chromatin unpacking and reorganization

After fertilization, the highly packaged nucleoprotamine sperm genome must be decondensed. One of the first steps must be reduction of the protamine disulphide bonds to allow protamine removal and subsequent organization of the DNA in a nucleosomal structure. The chromatin changes and unpacking after fertilization potentially relevant to the function of protamines are reviewed elsewhere [108,109]. It is possible that differential marking of different sperm genomic DNA regions with P1 or P2 protamines or with histones, histone variants or with other proteins could contribute, after fertilization, to establish the order of paternal gene reactivation or even could be involved in setting up the appropriate imprinting of different paternal genes. The repair capacities of the oocyte arequite stable throughout oogenesis and persist after fertilization and may repair DNA damage from both parentalgenomes [110].

The Origin of DNA Damage and Mechanism

Sperm DNA is recognized as an independent measure of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters especially with assisted reproduction technique [111].

The exact mechanisms by which chromatin abnormalities/DNA damage arise in human spermatozoa are not exactly understood, but four main theories have been proposed at molecular level, namely defective sperm chromatin packaging, apoptosis, oxidative stress and genetic lesions.

Oxidative stress is likely to be one of the major culprits [112], although in some cases, exposure to xenobiotics might also be involved, as in the case of male smokers or men employed in occupations (wood and metal processing industries) that are significantly correlated with pathology in their children.

Also, exposure to environmental or industrial toxins, oxidative stress, smoking, etc, are known to cause sperm DNA fragmentation and infertility [113-116].

It is shown that the sperm from smokers men are significantly more sensitive to acid-induced DNA denaturation than non-smokers [26,113].

Also, cigarette smoking is significantly associated with the percentage of "round–headed" spermatozoa and decreased superoxide dismutase levels in semen [27].

Despite association between DNA damage and male infertility, there is a detectable level of DNA damage in spermatozoa of fertile men [117].

Deficiencies in recombination during spermatogenesis, leading to cell apoptosis

Sperm DNA fragmentation may also occur during spermiogenesis as a result from aberrant chromatin packaging [118-120].

Stage –specific occurrence of transient DNA strand breaks during spermiogenesis has been observed [121,122].

DNA single strand breaks (SSBs) and Double (DSBs) during spermatogenesis (round and elongating spermatid) are necessary for transient relief of torsional stress, and aiding their replacement with transitional proteins and protamines during maturation of elongating spermatids [70,99].

Torosional stress increase during spermiogenesis, as DNA condensed and packed into the differentiating sperm nuclear protein. However, endogenous endonucleases (Topoisomerases) may induce DNA fragmentation as way of relieving this stress [123]. Macron and Boissonneault [123] Showed that DNA breaks are present in the whole population of fertile mouse and human spermatids and are part of the normal differentiation program of these cells.

Horak et al. [124] showed that the levels of bulky DNA adducts was 1.2 fold higher in smokers than non-smokers where a significant differences of 1.7 fold incease existed between current smokers and never smokers.

Perrin et al. [125] demonstrated that tobacco consumption is associated with benzo (a) pyrene-diol-epoxide-DNA adducts in spermatozoa.

Abnormal sperm maturation (Protamination disturbance)

Sperm chromatin integrity is essential for successful fertilization, embryo development, and normal pregnancy and protamine deficiency appeared to effect fertilization rate and embryo quality.

Defective maturation processes during spermiogenesis could resulting in a diminishing sperm chromatin packaging and make sperm cells more vulnerable for ROS–induced DNA fragmentation.

A less compacted sperm nucleus would be more vulnerable to any chemical or physical insults, such as those resulting fromreactive oxygen species [126].

De Yebra et al. [122] observed that infertile men have a high degree of variability in the relative sperm histone to total nuclear protein ratio.

Sperm protamine deficiency (partial or complete) is observed in a subset of infertile men and suggests that the relative histone to protamine ratio may be altered in the spermatozoa of these men [127,128].

Theoritically, any failure to fully protection of the DNA during epididymal passage like the presence of protamine 2 precursors,

slightly higher levels of residual histones, less disulphide bond formation, and decreased compaction of the spermnuclei may cause injury to the DNA [78].

It has been shown by [129] that defective sperm nuclear protein replacement, resulting in protamine deficiency, is positively associated with sperm DNA damage.

Besides, according to Zini et al. [130], the observed relationship between sperm head defects and percentage of High DNA Stainability (HDS) suggests that sperm head abnormalities may partially be due to imperfect sperm chromatin condensation.

Talebi et al. [131] suggested that the production of spermatozoa with less condensed chromatin may be one of the explanations of infertility due to varicocele. They used cytochemical tests for sperm chromatin evaluation and showed that the rates of aniline blue-reacted spermatozoa (with increase in residual histone) were significantly higher in infertile and varicocele patients than in the normal fertile donors.

Direct testicular hyperthermia and febrile illness has been shown to cause an increase in the histone protamine ratio and DNA damage in ejaculated spermatozoa [132].

Finally, the data show that certain behaviours are associated with increased scrotal heat (e.g., use of hot baths, saunas, down-filled blankets, laptop computers and driving for long times) [133].

Abortive apoptosis

Spermatozoa cannot undergo conventional programmed cell death called "Apoptosis" but are capable of exhibiting some of the hallmarks of apoptosis including caspase activity and phosphatidylserine exposure on the surface of sperm cell membrane which is termed as "abortive apoptosis" [37,134].

Apoptosis of testicular germ cells occurs normally throughout life, preventing their overproliferation [76,135]. It has been shown that an early apoptosis pathway, is intiated by Fas Protein and that Setroli cells express Fas ligand , which by binding to Fas leads to cell death via apoptosis [136,137], reducing the number of germ cells population to numbers Sertoli cells can support [76].

The percentage of germ cells undergoing apoptosis in normal subjects is significantly lower than that seen in men with oligoasthenoteratozoospermia, Hodgkin's disease, and testicular cancer [138]. The incidence of caspase activation and DNA fragmentation is some what lower in samples from patients with hypospermatogenesis, in which some germ cells achieve the late elongated spermatid stage [139].

Oxidative stress

When levels of ROS rise above the body's antioxidant defense system, oxidative stress (OS) occurs. In this circumstance, the elevated levels of ROS damage cells, tissues, and organs [26,140].

ROS in seminal plasma are primarily produced by leukocytes and defective sperm [141]. The generation of reactive oxygen species (ROS) in male reproductive tract has been shown to be a concern because of their effects on sperm quality and function [28]. In sperm, ROS have been shown to have an important participation in the regulation of all the functional parameters, including motility, capacity, sperm–zona pellucida interaction, acrosome reaction and sperm –oocyte fusion [22,142].

They are highly reactive and cause beneficial or detrimental effects to sperm structure and function, depending on their nature and concentration [143].

It has been suggested that oxidative stress can probably damage male germ cells in epididymis due to long exposure of ROS [144].

The pathogenic effects of ROS occur when they are produced in excess of the antioxidant capabilities of the male reproductive tract or seminal plasma [145]. Overproduction of ROS depletes enzymatic and nonenzymatic antioxidants leading to additional ROS accumulation and cellular damage [146]. Oxidative stress occurs when the level of ROS exceeds the antioxidants protection resulting in sperm DNA damage [147].

Moustafa et al. [148] determined that infertile patients had high ROS levels in their seminal plasma and higher percentage of apoptosis than normal healthy donors.

Approximately, half of infertile men exhibit oxidative stress [149].

Factors such as increased oxidative stress or low levels of antioxidants may have implications on male reproductive health [150]. A number of factors can lead to oxidative stress, including tobacco and alcohol consumption, infection (viral or bacterial), exposure to xenobiotic. Chlamydia has been shown to cause fragmentation in human sperm DNA [151]. It has been demonstrated that this infection causes premature decondensation of sperm chromatin and DNA damage to human sperm [152]. Sperm are vulnerable to the oxidative –stress –mediated damage, due to their structure with a high proportion of poly unsaturated fatty acids in their plasma membrane [153].

Leukocytes found in male genital tract infections have the potential to produce reactive oxygen species (ROS) [154], and in significant numbers can overwhelm the anti-oxidant defence system and could cause DNA damage as a result of oxidative stress [29].

The presence of elevated levels (>1x106) of leukocytes in the semen is defined as leukocytospermia [155] and is associated with increased level of ROS, leading to sperm DNA damage [156]. Besides, ROS cause gene mutation such as point mutations and polymorphism [157,158].

Moreover, a possible consequence of sperm DNA damage is infertility in the offspring [112,159]. A concern emerging from studies conducted in smokers in an increased risk of childhood cancer observed in the offspring of men with a high proportion of sperm with fragmented DNA in their semen. This study revealed that the children of these men, whose ejaculates are under oxidative stress [119] and characterized by a high level of chromatin fragmentation, are 4-5 times more likely to develop cancer in childhood than the children of non-smoking fathers [160].

Cigarette smoking causes oxidative stress either by producing high levels of free radicals or by decreasing the antioxidant capacity of seminal plasma [161].

In addition to direct oxidative damage to tissue, oxidative free radicals modulate the immune-inflammatory system in part, through enhanced expression of pro-inflammatory genes [162]. Inflammation in turn, enhances oxidative stress. For example, in emphysema, increased TNF-α and TGF-β induces decreased glutathione synthesis and cellular glutathione, raising the cell's susceptibility to oxidative damage [163-165].

How Cigarette Smoking Affects DNA Integrity and Other Sperm Parameters

Life style choice plays an important role in male infertility, such as smoking, alcohol, and caffeine consumption have been associated with chromosomal aberration and genomic alterations in somatic cells [166-168]. In addition, cigarette smoking, alcohol, and drugs are the main stimulants exerting a negative effect on the male and female reproductive system [169].

Lewis et al. [170] associated cigarette smoking with decreased sperm count, alterations insperm motility and overall increased number of abnormal spermatozoa. Besides, smoking and consumption of alcohol and caffeine have been associated to the increase in nuclear DNA damage of the white blood cells [171,172]. On the contrary, very little is known about their effect on sperm DNA [172]. [173] Smith et al. added that impaired spermatogenesis is generally associated with increased sperm DNA damage.

Several studies showed a deleterious effect of lifestyle factors on the male fertility, only a few studies focused on the effect of tobacco smoking and alcohol consumption on male germ cells' genetic integrity and showed contradictory results concerning sperm aneuploidy and DNA fragmentation [174,175]. The impact of cigarette smoking on sperm DNA integrity is somewhat conflicting.

Shen et al. [176] reported on a positive correlation between 8-OHdG amount and blood cotinine, the metabolite of nicotine, levels.

Cigarette smoke has been associated with an overall decrease in semen quality, a reduction in sperm count and motility and increase in number of morphologically abnormal sperm cells [113].

It has been demonstrated that the DNA fragmentation index (%DFI) and high DNA stainability (%HDS) are significantly higher in fertile men who smoked than non smokers [28,113].

Similarly [175] Sepaniak et al. demonstrated an association between cigarette smoking and DNA fragmentation.

It is shown that the sperm from smoker men are significantly more sensitive to acid-induced DNA denaturation than non-smokers [26,113].

It is possible that high levels of sperm DNA fragmentation of infertile smokers could be associated with other undetermined factors as tobacco smoking, a source of reactive oxygen species that can increase the oxidative sperm DNA damage [6,177].

Linschooten et al. [178] indicated that spermatozoa of smokers encounter higher levels of oxidative stress that even expression of antioxidant enzymes and seminal vitamin C were insufficient to provide full protection of spermatozoa against such sperm DNA damage.

Elshal et al. [27] showed that sperm DNA fragmentation index, high sperm DNA stainability and round-head spermatozoa are increased in idiopathic infertile men associated with cigarette smoking attributed to increased oxidative stress and insufficient scavenging antioxidant enzymes in the seminal fluid.

Belcheva et al. [179] pointed to that although sperm DNA integrity of healthy smokers remains in the normal range; a clear negative trend is observed in respect of disturbed plasma membrane phospholipid asymmetry.

Calogero et al. [180] demonstrated that cigarette smoke extract could suppress sperm motility, has a detrimental effect on sperm chromatin condensation and apoptosis, increases spermatozoa with phosphatidylserine externalisation, and early apoptotic sign and fragmented sperm DNA, a late apoptotic sign, in a concentration- and time dependent manner.

Smoking increases oxidative stress, which results in deplation of antioxidants in the seminal plasma, thereby inducing oxidative DNA damage of the sperm [161] and mutagenic adducts [181].

Selit et al. [182] demonstrated that smoking has a negative impact on sperm DNA and RNA abnormalities and that is accentuated in heavy smokers compared to light smokers.

Hammadeh et al. [105] suggested that induced oxidative stress by cigarette smoking have significant inverse effects on the protamination process by disrupting protamine-2.

In a second study, [183] Hamad et al. proposed that smokers retain a higher proportion of spermatozoa with a higher histone H2B to protamine ratio than non-smokers, which will cause alterations in the sperm chromatin structure resulting in abnormal spermatogenesis ending with infertility.

This study was confirmed by Yu et al. [184]. In their work, they concluded that both smoking and defective semen quality are strongly associated with the histone-to-protamine transition in mature human sperm and smoking may interfere with the transcription of protamine mRNA. All together, sperm histone transition could be affected by cigarette smoking at the leval of protamine mRNA trancription.

Besides, there is a very strong and significant correlation between smoking and genetic defects in the sperm [185,186]. Others, using various techniques like COMET, TUNEL, CASA and 8-oxodG analysis could not confirm the association between tobacco smoking and sperm DNA injury [187,188,189].

Sergerie et al. [190] failed to demonstrate significant association between smoking and the usual semen parameters.

Cigarette smoking or product of cigarette smoke increases superoxide generation by both endothelial and smooth muscle cells from NADPH oxidase and uncoupled eNOS, and upregulates proinflammatory cytokines and the RhoA/ROCK contractile pathway. This results in reduced NO bioavailability, increased vasoconstriction, and endothelial dysfunction [191,192].

It has been shown that sperm DNA has an elevated level of 8-OHdG in smokers [105], and this level inversely correlates with the intake and seminal plasma concentration of Vitamine C which is the most important antioxidant in sperm cell [188].

Zhang et al. [193] demonstrated that seminal Zn was lower in the medium, heavy and long term smokers than in the non-smokers being negatively correlated with the amount and duration of cigarette smoking .

Dissanayake et al. [194] correlated the deterioration of semen parameters in addition to semen pH and viscosity with the decreased seminal Zn.

The Relationships of DNA Damage as Consequences of Smoking and Others on IVF/ICSI Outcome

Sperm chromatin is a highly organized, condensed, and compact structure, which is considered to be an important factor for normal fertilization and pregnancy outcome [195].

Environmental stress, gene defects, and chromosomal abnormalities can disturb critical biochemical compaction processes that occur during spermatogenesis, and may also cause an abnormal chromatin structure that would finally interfere with fertility [42,128,196,197].

It has been demonstrated that abnormalities in the male genome, characterized by disturbed chromatin packaging and damaged sperm deoxyribonucleic acid (DNA) may be a cause for male infertility regardless of routine semen parameters [44,199]. Also, it has been shown that the sperm cells from infertile men may contain a range of nuclear anomalies, including abnormal chromatin structure, microdeletions, chromosomal rearrangements, aneuploidy and DNA strand breaks [200]. There is growing evidence that sperm carry information about the ancestral environmental that can influence the development and health of next generation (s), presumably through enduring alterations in gene expression [201-206].

Several authors suggested a negative relationship between disorganization of the chromatin material in sperm nuclei and the fertility potential of spermatozoa both in vivo and *in vitro* [105,195,207,208]. It has been shown how a higher percentage of spermatozoa with alterations in chromatin structure have a negative effect on ART procedure outcome [45,209-212].

Sperm DNA damage measured by various techniques has been closely associated with all the stages of ART outcome such as fertilization, embryo quality, implantation, pregnancy and spontaneous abortion [213,214]. An increase in sperm DNA damage is associated with decreased implantation, thereby a decrease in pregnancy rates [144]. Furthermore, sperm with abnormal chromation packaging and DNA damage is showed to result in decondensation failure, which results in fertilization failure [42,45]. In addition, a possible consequences of sperm DNA damage might be microdeletions in the Y chromosome, which will lead to infertility in the male offspring [215]. Sperm with damaged DNA are still capable of fertilization [147] but its effect is prominent in the later stages [216]. When Sperm DNA is damaged, infertility, miscarriage, and birth defects in offspring can occur [217].

A study conducted by Surahan et al. [218] has demonstrated that 15% of all childhood cancers are directly attributed to paternal smoking. This study suggests that there may be a link between sperm DNA damage and the subsequent development of childhood diseases, but other researcher could not find the association [219]. Moreover, men suffering from male infertility have higher levels of sperm with DNA damage, which result in a negative impact on their ART outcome [143,210,220].

Therapeutic Strategies to Reduce ROS Production and Improve IVF/ICSI Outcome

Several *in-vivo* and *in-vitro* studies demonstrated that antioxidants have positive effects on oxidative-induced sperm DNA damage and so, these agents can manage male infertility and subfertility [221]. Vilorial et al. [222] found lower level of sperm antioxidant enzymes in smokers as compared to non-smokers, however, without any difference in the degree of DNA damage between the two groups.

The concentration of antioxidants in seminal plasma is 10 times greater than in blood plasma [126], and the presence of antioxidants in the seminal plasma protects the functional integrity of the sperm against the oxidative stress [223]. Several studies have reported that sperm DNA damage is associated with oxidative stress, and this represents the basis for the use of antioxidants in the treatment of sperm DNA damage [25,224,225]. A series of antioxidants enzymes and numerous endogenous and dietary antioxidants compound maintain defenses against stress by scavenging ROS.

Glutathione peroxidase (GPx) is selenium–containing enzymes which reduce hydrogen peroxide to water and lipid peroxides to their corresponding alcohols. Thioredoxin peroxidase (TrPx) is a member of the peroxiredoxin family, which reduces hydrogen peroxide [226].

Besides, antioxidant enzymes such as the different isozymes of glutathione peroxidase (GPX) plays a central role in the protection of both the epididymal epithelium and spermatozoa during their passage through the epididymis [227]. It has been shown that this damage progressively increases from the caput to the cauda epididymis and is related to a decrease in the levels of the isozyme GPX-5 [227].

A specific sperm nuclear glutathione peroxidase (snGPx) with properties similar to that of phospholipid hydroperoxide glutathione peroxidase (PHGPx) and identified as a 34-kDa selenoenzyme, acts as a protamine thiol peroxidase and is directly involved in the stabilization of the condensed chromatin by specific cross-linked protamine disulphide bridge [228].

The nonenzymatic antioxidants include, among others, vitamin C (ascorbic acid), vitamin E (alpha-tocopherol) ß—carotene, and reduced glutathione [226].

Numerous nonspecific antioxidants can scavenger ROS. Vitamine E for example is a lipid –soluble antioxidant, which protect Low density Lipoprotein angainst oxidation.

Vitamine C is water-soluble antioxidants, which very effectively scavenges a wide array of ROS, and also prevent oxidation of BH4, an essential NOS cofactor [229].

Antioxidants such as vitamin E, vitamin C, and carotenoids can restore a proper pro-oxidant–antioxidant balance and maintain the integrity of sperm cells. Selenium (Se) and vitamin E supplementation seem to improve sperm quality and fertility [196,230,231].

Oral administration of the antioxidants has been shown to significantly increase antioxidants levels in the seminal plasma and

an improvement in the semen quality [232,233] and to significantly decrease sperm DNA damage [234] and the incidence of aneuploidy in sperm [235], thereby increasing the assisted reproductive success [236].

Besides, ascorbate and catalase, which are both found naturally in seminal plasma, reduce the level of ROS and improving the quality of sperm following cryopreservation prior to ART [237].

Metal chelators can also be useful in reducing ROS generation and preventing lipid peroxidation of sperm membranes, thereby protecting sperm nuclear DNA [238]. In the cases of male infertility which are suggested to be a result of existing sperm DNA damage, the patients can be given oral antioxidant during two months (at least one spermatogenesis cycle) before an ICSI attempt. The subsequent ICSI cycle led to a significant increase in implantation rates and clinical pregnancy in comparison to the pretreatment ICSI outcomes despite of the absence of differences in fertilization and cleavage rates or in embryo quality [234].

Omu et al. [239] showed that zinc therapy improves the spermatozoal quality through possible mechanisms such as increased expression of Zn-Cu superoxide dismutase and anti-apoptotic Bcl-2 and reduced Bax, decreased seminal antisperm antibody titers and also reduction in sperm DNA fragmentation rates.

Hence, men with marginal semen quality were advised to get benefit from quitting smoking where sperm motility and morphology improved after 6 months of follow-up [7,240].

Hence, the intake of antioxidants from diet or supplements may have a major influence on the *in vitro* susceptibility of lipids to peroxidation and may account for the reported differences in lipid peroxidation between smokers and nonsmokers independent of the effects of cigarette smoking [241]. In a study exclusively of smokers, a combined antioxidant supplement resulted in increased oxidative resistance to lipid peroxidation [242] .

Santos et al. [243] evaluated sperm quality after a 3-month smoking cessation programme by sperm analysis, objective sperm motility analysis, protein tyrosine phosphorylation in capacitating conditions and DNA fragmentation (TUNEL). They found that sperm analysis after smoking cessation revealed a distinctive improvement in sperm concentration, fast spermatozoa (≥35 μm/s), sperm vitality, percentage of spermatozoa recuperated after an enrichment technique and protein tyrosine phosphorylation.

Conclusion

These results reveals that spermatozoa from smokers have a higher levels of histone H2B, less condensed chromatin and higher DNA fragmentation than do non-smokers, suggesting that the spermatozoa of smokers retain higher level of H2B, which make these spermatozoa more susceptible for DNA damage by oxidative stress and other factors like smoking. Therefore, physicians should made aware their patients of simple lifestyle changes that could impact their fertility. All patients should be encouraged to strive for a healthy, sustainable lifestyle. In areas where there is clear evidence that a lifestyle behavior may impact fertility, such as smoking, the patient should be encouraged to modify behavior.

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