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Potential Risk of Senescence on Male fertility and Sperm DNA damage on Progeny

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ABSTRACT

There is a growing concern of the potential risk of producing genetically defective sperm and transmitting germ-line mutations to progeny by fathers who prefer their children at old age. Men with male factor infertility do not readily make themselves available for evaluation until very late when they seek assisted reproduction technique. The objective of this review is to highlight the impact of senescence on oxidative DNA damage on spermatozoa and possible effects on the progeny. Relevant literatures on oxidative sperm damage were reviewed in addition to the experience and publications we have made over the years on male factor infertility. Older men produce more spermatozoa with oxidative DNA damage probably due to enhance generation of reactive oxygen species, aberrant DNA repair mechanism leading to production of spermatozoa with abnormal genetic materials that could have adverse consequences on the progeny. It is suggested that men should have their children early and those with male factor infertility should seek medical attention early before old age. Adequate precaution should be taken when selecting spermatozoa for use during assisted reproduction technique.

Introduction

It is generally believed that men can reproduce their kind even at old age, unlike the women whose fecundity decline sharply by the fourth decade of life. The changing pattern of men to have children at old age is worrisome because of the potential risk of producing genetically defective sperm and transmitting germ-line mutations. Male fertility may be affected by senescence even though spermatogenesis continues into old age [1-3]. But the potential risk of abnormal pregnancies, production of genetically defective spermatozoa and transmitting germ-line mutations have not been sufficiently reported. Moreover, the understanding of the risk of male senescence on fertility and deoxyribonucleic acid (DNA) damage is particularly important because of older men seeking reproductive assistance. The reliance on modern technologies such as Intracytoplasmic sperm injection (ICSI) and In-vitro fertilization (IVF) techniques which by-pass the natural barriers against fertilization by damaged spermatozoa [4] and increases the chances of fatherhood are relevant contributing factors that necessitated this review. Faulty sperm function is about the most single cause of male factor infertility. The objective of this review is to highlight the origin and contributions of sperm DNA damage to male factor infertility and the need to take adequate precaution in the selection of spermatozoa for use in assisted reproduction procedures.

The Human spermatozoon

The sperm cell is composed of a sperm head, a sperm neck and a sperm tail. Whole sperm is covered by the sperm plasma membrane called plasmalemma. The sperm head is composed of a nucleus and an acrosome. The nucleus contains sperm DNA (half number of chromosomes) while the acrosome has important enzymes that are vital for capacitation during fertilization. The sperm neck or midpiece has about 100 sperm mitochondria which generate energy for the sperm tail. The sperm tail has microtubule doublets which are connected by dynein arms.

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Germ cells mediate the transfer of genetic information from generation to generation and are thus pivotal for the maintenance of life. Spermatogenesis is a continuous and precisely controlled process that involves extremely marked cellular, genetic and chromatin changes resulting in a generation of highly specialized sperm cells. Spermatogonia stem cells replicate and differentiate into primary spermatocytes that undergo genetic recombination to give rise to round haploid spermatids [5].

Contributions of sperm DNA damage to fertility

The contribution of DNA damage to male factor infertility has attracted more attention in recent years. This is important because half of the progeny's DNA is inherited from the paternal unit. Some epidemiological studies have reported abnormal reproductive outcomes and transmission of genetic defects in men with advancing age [4,5], developmental and morphological birth defects[6], gene mutations[5,7], chromosomal abnormality[3], pregnancy loss [8] and other diseases such as prostate cancer [9]. Accumulating evidence has associated spermatozoa DNA damage with the risk of fetal development and gene mutation in progeny which include childhood cancer and infertility [10]. Male senescence has been linked with increased incidence in the production of sperm of uncommon genetic and chromosomal defects [3,7,11-13]. It was reported that older men make more spermatozoa with mutations associated with Apert syndrome and achondroplasia [12,14] as well as sperm DNA damage which was measured by high biomarker levels of DNA damage [15-18]. Similarly, significant association between male senescence and sperm DNA strand damage in non-clinical specimens of apparently healthy non-smokers has been reported [1]. It was observed that spermatozoa produced by older men had significantly higher incidence of DNA damage which was assayed in alkaline milieu and this represents alkali-labile DNA sites and single strand DNA breaks [1]. In the same report, it was observed that age did not correlate with sperm damage under neutral environment which was hypothesized to indicate double-strand DNA breaks. Similar observation was made by Wyrobek et al [15]. They reported age-associated effects on DNA fragmentation and achondroplasia mutations and not aneuploidy, Apert syndrome mutation or sex ratio [15,16].

In a study that evaluated male participants in In-vitro fertilization (IVF) program, it was observed that sperm DNA damage correlated positively with donor age and with malfunctioning of post fertilization embryo cleavage. It was an indication of high level of decline in the integrity of

sperm DNA in older male participants [17]. The three types of DNA damage that occur in human genome are mainly single strand breaks, double strand breaks and alternation of bases.

Causes of Sperm DNA Damage

The origin of Sperm DNA damage can generally be divided into two- Intrinsic and Extrinsic factors.

1. **Intrinsic factors**

(a) *Defective maturation process of spermatozoa*

Naturally, sperm cells have small amount of cytoplasm hence limited contents of cytoplasmic antioxidants. This inherent nature makes the spermatozoa to be susceptible to oxidative stress. The plasma membrane of sperm cells is made up of high levels of polyunsaturated fatty acids which help to maintain the fluidity of the membrane. Again, these polyunsaturated free fatty acid contents readily attract free radical injury. These mechanisms make worse oxidative damage of spermatozoa [18-22]. Sperm DNA packaging process is a highly complicated system and any mistake may expose the DNA to damage by means of inappropriate execution of any of the steps [23-28].

(b) *Oxidative stress*

When there is excess generation of free radicals that exceeds the neutralizing potentials of naturally available cellular antioxidants, oxidative stress is said to occur. Studies have shown that upto 40% of infertile males have higher levels of reactive oxygen species (ROS) than fertile males, which often lead to a cascade of events of lipid peroxidation and damage to cellular macromolecules. The structural and natural composition of spermatozoa in addition to limited antioxidant availability makes sperm cells susceptible to oxidative stress. The presence of leukospermia and varicocele has been associated with elevated free radicals in semen. Varicocele may exacerbate seminal DNA damage directly through increased scrotal temperature or indirectly via increased generation of ROS. Conversely, leukocytoplasmia contributes to an increased generation and secretion of pro-inflammatory cytokines that could change the regulatory mechanisms of spermiogenesis and DNA damage [29].

(c) *Abortive Apoptosis*

By this mechanism, spermatozoa with damage DNA may escape apoptosis and are incorporated into the gene pool. Apoptosis is a programmed cell death, which is a natural process aimed at removing old and senescent sperm cells. During spermatogenesis, the body uses apoptosis to regulate the number of proliferative germ cells [30]. The Fas cell surface proteins(transmembrane protein that belongs to tumour necrosis factor family) help to control apoptosis in sperm cells. These proteins and the associated ligands are used to assess genetic damage in spermatozoa. Studies have shown high levels of these biomarkers in men with abnormal sperm indices [31,32]. The presence of high levels of Fas cell surface proteins in infertile men may be due to the failure of the Sertoli cells to activate Fas ligand generation and carry-out apoptosis, such that immature sperm cells with high levels of Fas cell surface proteins that avoided apoptosis could get matured and their damaged DNA enter into the gene pool [18].

Any fault in the natural apoptosis pathway itself involving inadequate caspase activation can also occur. Caspases (cysteine-aspartate proteases) are a group of cysteine protease family which takes part in the initial steps of apoptosis cascade. It was observed that if the apoptotic pathway from caspase 8/9 to caspase 3 (final executioner of apoptosis) to caspase-activated deoxyribonuclease (CAD) is ineffectively or accurately carried out, apoptosis of the spermatozoa is inhibited [33]. When this occurs those spermatozoa with damaged DNA which were previously destined for death escape and proceed to maturation.

2. **Extrinsic factors**

Life style behaviours (smoking, obesity, excessive alcohol and caffeine consumption),inadequately treated sexually transmitted infections, radiation, medication and substance abuse are some of the predisposing external factors for sperm DNA damage. Cigarettes, medications, recreational drugs adversely impact sperm DNA damage, since they contain chemicals that are involved in DNA strand breaks or act indirectly through secondary oxidative methods. Some authors have reported that cigarette smoke can cause DNA damage in sperm cells via oxidative stress. Many of these chemicals and their metabolites could trigger the release of pro-inflammatory cytokines and the generation of ROS in seminal plasma. They can also cause the release of other DNA adducts [34] which are responsible for mis-matched pairs, improper DNA replication and incorrect protein synthesis [23,24] Drugs such as cocaine and caffeine can impact sperm DNA strand breaks leading to apoptosis [35]. Their excessive consumption has been reported to cause double strand breaks in sperm DNA [36].

Senescence, Infertility, oxidative stress and DNA damage

Older men may produce more spermatozoa with DNA damage as a result of age-related increased generation of oxidative stress in their reproductive tract [37-39]. We previously reported on the major causes, burden of male infertility and the relevance of proper diagnosis and treatment at subsidized cost among Nigerians [40-44]. Oxidative stress has harmful effect on sperm DNA and can damage sperm DNA, mitochondrial and nuclear membranes [10, 43,45]. An association between oxidative stress and nuclear membranes has been reported [46]. Similarly, the importance of high antioxidant intake in the management of male factor infertility has been suggested. High intake of antioxidants was associated with better semen indices in study participants [47]. Oxidative stress can cause lipid peroxidation, protein dysfunction, nucleic acid oxidation and impaired DNA repair. These could result in gene mutation and carcinogenesis [45,48]. Our group previously reported high levels of seminal plasma caspase 3, cytochrome c and low total antioxidant capacity in infertile men in Nigeria [49]. Defective mitochrondrial dependent apoptotic signaling pathway may be an important contributing factor to infertility [49-54]. The control spermatogenesis is nurtured and aided by Sertoli cell and in the presence of large number of spermatozoa with damaged DNA, the Sertoli cells express FasL which induces sperm cell apoptosis by Fas/FasL pathway in order to maintain equilibrium necessary for normal spermatogenesis [55,56]. In older men with increased oxidative stress, the equilibrium mentioned above is not achieved. This may lead to increased rate of apoptosis, DNA damage and infertility [44]. High levels of DNA fragmentation and active caspase 3 were reported in testes of men with Sertoli cell only syndrome and maturation arrest [57].

We earlier reported lower levels of total antioxidant capacity in infertile than control male subjects [49]. The increased generation of ROS often observed in older men [37-39], may be due to several factors and include routine medical prescription and environmental pollutants [58]. The generation of ROS could be made worse by infection, prolonged stasis and abnormal spermatozoa, environmental and life style changes [44,45,59-61].

Oxidative stress and sperm function

Sperm motility is about the first function to be affected by oxidative stress and lipid peroxidation. Studies in human and experimental animals have associated lipid peroxidation with abnormal sperm motility [61-63]. The prolongedexposure of human spermatozoa to ROS using xanthine oxidase as free radical generating system has shown that sperm motility is readily affected by oxidative attack and that hydrogen peroxide is the most cytotoxic oxygen specie [64-68]. The mechanism by which sperm motility is lost in the presence of oxidative stress is not very clear, but oxidative injury to axonema and decreased intracellular adenosine triphosphate (ATP) have been suggested [64,69-71]. Several authorshave shown that oxidative stress may compromise the fertilizing capacity of sperm cells even when motility is normal [71,72]. In this situation, it is the capacity of the spermatozoa topenetrate the vitelline membrane of the oocyte that is affected. A study of the impact of oxidative stress on sperm-oocyte fusion has shown a biphasic response depending on the levels of oxidants [73]. Some authors demonstrated that at low level of oxidative stress sperm-oocyte fusion rates were increased supporting the hypothesis which suggests the role ROS play in activation of the tyrosine phosphorylation events that occur during sperm capacitation [74] and the importance of sterol oxidation in driving the efflux of cholesterol from sperm plasma membrane [75]. Conversely, at higher levels of oxidative stress, lipid peroxidation was induced in the plasma membrane and sperm-oocyte fusion was impaired, probably as a result of damage to acrosome which is involved in the fusion process between spermatozoa and oocytes [76]. Again, our group previously demonstrated low acrosin activity in seminal plasma of infertile Nigerians compared to fertile subjects [77]. Acrosin is a sperm acrosomal enzyme that is involved in acrosomal reaction during sperm-oocyte fusion, that is, the union of spermatozoa to the zona pellucida and penetration of spermatozoa through the zona pellucida. The acrosomal membrane of the sperm head has been suggested to possess specific molecules for joining to the zona pellucida before penetration of the oocyte. This enzyme hydrolyzes the oocyte membrane to provide access for spermatozoa to penetrate the interstices of the corona radiata at adequate calcium environment [78-80]. This process may be affected by senescence. From our previous report, it was observed that seminal plasma

calcium levels decreased with decreasing concentrations of sperm density [81].

Impact of senescence on Progeny due to oxidative DNA Damage

Association between paternal age and abnormalities ofprogeny via oxidative DNA damage in spermatozoa was aptly described in a study using senescence-accelerated mouse prone 8 (SAMP8) [82]. The experimental mouse is a strain that possesses a suite of naturally occurring mutations resulting in accelerated senescence characteristics (phenotype) occasioned by oxidative stress. This oxidative stress was further enhanced by a mutation in the Ogg1 gene, significantly decreasing the ability of the enzymes to completely remove 8OHdG adducts. A study of the reproductive charateristics of the male mice revealed a significantly higher level of DNA damage in epididymal spermatozoa examined using alkaline comet assay technique. Further examination of the lesions showed that they were oxidative damage as occur in nature as demonstrated by the presence of higher levels of 8OHdG adducts in the testicular tissue and mature sperm cells than control strains [62,82]. Since senescence correlated with oxidative DNA damage of spermatozoa, it is logical to expect these pathologies to reflect in the incidence of morbidity in the progeny of ageing fathers [64]. In fact, three main types of paternal age-associated pathologies have been described which are miscarriage, dominant genetic mutations and complex neurological conditions [64]. Other paternal age-related abnormalities include multiple endocrine neoplasias, Aper syndrome and achondroplasia [82,83]. These conditions occur as a result of replication error in the germ-line.

As men age, the risk of mutation increases due to increased incidenceof replication error because the germ cells of older men experience multiple rounds of pre-meiotic replication and cellular iteration or repetition. However, the exception to this hypothesis is the fibroblast growth factor receptor 2 (FGFR2) mutation associated with Apert syndrome. In this case there is correspondence between the incidence of mutation in spermatozoa and the occurrence of the condition in children [84]. The underlying cause is not just replication error but over-expression of the mutation that caused the condition in the spermatozoa as a result of age-dependent clonal expansion, which are mutant spermatogonial stem cells that have a proliferative advantage over normal cells. Studies have also suggested that such mutations take place in clusters within the seminiferous tubules probably due to failures of unequal division within the germ-line [84].

Abnormal Repair of DNA damage

Abnormal (incomplete) repair of oxidative DNA damage in the mature spermatozoa that escaped apoptosis and used to fertilize oocytes was explained as one of the causes of paternal age effect on offspring. This may explain the increased rate of miscarriage observed as a function of male senescence [85] and other various complex polygenic conditions that are associated with paternal age at the time of conception. Paternal age has also been associated with increased incidence of complex polygenic neurological conditions such as epilepsy, schizophrenia and autism in the progeny [86]. In a study conducted among Icelandic population it was observed that the mutation load inherited by progeny was overwhelmingly associated with the age of their fathers at the time of conception and the moment this load exceeds a certain critical level, overt abnormalities occur in the progeny [87]. The relationship between age-dependent increase in mutational load in progeny and the unusual repair of oxidative sperm DNA damage in the zygote is not completely understood. The potential contributions of a wide range of environmental and lifestyle factors interacting with the human genome to enhance oxidative DNA damage cannot be ruled out. It was reported that about 4% of new born children in Australia are products of artificial reproductive technique (ART) [88]. Most couples with infertility in Nigeria (those that can afford) are increasingly embracing the use of ART to resolve their problem. Since about 40-50% of this condition is occasioned by male factor infertility, careful selection of spermatozoa is needed to avoid mutations which would not have taken place if natural method was adopted for conception. Recent studies have reported that the incidence of birth defects following ART has doubled and imprinting disorder are frequently seen in children conceived in-vitro [89,90]. Some authors observed that children born by ART were more likely to be admitted to neonatal intensive care unit, to stay in hospital longer than those naturally conceived [86]. Abnormal patterns of retinal vascularization and high incidence of undescended testicles in boys conceived by Intra cytoplasmic sperm injection (ICSI) have been reported [91-93].

Impact of Senescence on DNA Repair processes in the Germ line

Age exerts profound influence on DNA repair in the germ-line. The occurrence of oxidative injury in early stages of spermatogenesis results in several oxidative damages in germ cells entering meiosis and these may precipitate an increase in apoptosis. But milder levels of oxidative stress may induce compensatory mechanisms on the spermatocytes that confer longevity and survival of the progeny. A good example of the impact of paternal age is on DNA repair in the germ line is on telomere length. One of the ways that the germ line responds to senescence-associated oxidative stress is by upregulation of telomerase activity and increase the length of telomeres in the spermatozoa [94]. Telomere length is a paternally inherited trait such that children of ageing fathers confer longevity on the progeny since telomere length is associated with longevity [95]. This perhaps may be one of the few benefits of having an older father; he may confer upon the progeny the molecular basis for a long life [1]. Conversely, if the paternal germ line experienced adverse oxidative damage after meiosis when the telomerase can no longer increase then telomere length in the spermatozoa will be abnormally short and this may adversely affect the health of progeny conceived by ART [96].

Conclusion

In order to minimize sperm DNA damage, it is important to avoid those extraneous factors that predispose an individual to oxidative DNA damage. Lifestyle modifications such as avoiding smoking, excessive alcohol and caffeine consumption as well as observing adequate exercise should be taken into consideration. It is suggested that men should endeavour to have their children early before old age to avoid the transmission of aberrant genome to their progeny. Adequate precaution should be taken when selecting spermatozoa to be used for fertilization during the process of assisted reproduction technique.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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