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Sperm DNA Links from Sperm to Ovum to Implant Genetic Changeability: an Overview

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Abstract

Sperm DNA disintegration is known to cooperate male fertility. The data bring about that sperm DNA cleavage can be competently treated with orally administered antioxidant during a relatively short moment period. It is an accepting thought that the two types of DNA become visible to be distinctive and autonomously packaged molecules; though, investigation has established the symbiotic nature of these structures in contributing to male infertility. Data should continue to be gathered to ascertain strong correlation between conventional semen examination parameters and sperm DNA integrity; this information remains contentious and inadequate in clinical practice until certain novel techniques for the diagnosis and treatment of sperm DNA can be established. Rather more modern technology should be employed to associate such information into practical clinical awareness. This overview compiles certain specific reports pertaining to studies on sperm DNA physiology, biochemistry and molecular biology to provide further new insights into establishing hypothesis that sperm DNA integrity is both enormously fragile and remarkably significant for male fertility.

Keywords: Apoptosis; Male infertility; Sperm DNA

Introduction

Sperm DNA disintegration is known to cooperate male fertility. The data bring about that sperm DNA cleavage can be competently treated with orally administered antioxidant during a relatively short moment period. [1,2]. According to these reports, the rise of DNA fragmentation index (DFI) level can not only reduce the sperm motility but also affect the clinical outcomes of in vitro fertilization (IVF) and intracyto plasmic sperm injection (ICSI). While the outcomes of IVF and ICSI are statistically similar when DFI level is less than 15%, the curative effect of ICSI is better when DFI value exceeds 15%. An upgrading of basic sperm parameters by oral administration with antioxidants has been documented in a number of investigations, but DNA break has been reported in only a few of them [3,4-6]. Infertility is a rising problem among couples trying to conceive; in the past the female partner was singled out as the primary reason for being unable to bear a child. Investigation now results that male infertility may add in up to two thirds of all couples who look for treatment to overcome infertility. For several years a conventional semen analysis (concentration, motility, and morphology) has been noticed as insufficient to diagnose male infertility; however, scientific examination must now take into account two different kinds of DNA that have been proven to be worthwhile to this significant diagnosis. Nuclear DNA (nDNA), enclosed in the head of the sperm, is accountable for packaging all of the paternal genetic information, which will be needed for the fertilized egg. nDNA can be damaged or compromised through 4 interrelated courses: apoptosis, defective chromatin packaging, oxidative stress, and genetic lesions. Mitochondrial DNA (mtDNA) is cited in the mid piece of the sperm; as coupled with the tail, it is liable for mobilizing the sperm toward the ovum for fertilization. Scientists in relevance are only opening to figure out the correlation between these distinct DNA molecules and how they both contribute to male infertility. As the worldwide community continues to spread out, an emerging subpopulation of couples has started experiencing a general problem in enabling their contribution to the population. These couples are experiencing a major health crisis, commonly referred to as infertility. Infertility is classically defined as a state in which a couple desiring a child is unable to conceive following 12 months of unprotected intercourse [7.8]. It affects approximately 15% of couples who seek clinical treatment to conceive a child, and recent studies show that the number of infertile couples in the general population is increasing [9,10]. In infertile couples, liability for the deficiency of conception is usually divided into thirds, with one third due to male factors, one third due to female factors, and the final third due to overlapping factors from both the partners.

Application of Nutraceuticals in Sperm Creation

Nutraceuticals have been recognized as a way of potentiating sperm production and quality in the sub-fertile male. Recently, the administration of folic acid and zinc sulfate to sub-fertile males was shown to result in a significant improvement in sperm

concentration compared to placebo. Treatment lasted 25 weeks and the daily dose of folic acid and zinc were 5 mg and 66 mg, respectively. Although the beneficial effect on fertility remnants to be recognized, this finding opens new avenues of future fertility research and treatment [11]. Arginine , vitamin B12, methylcobalamin and ginseng have been used in the treatment of male infertility[11,12]. Though, most of these compounds have minor effects on sperm production and quality and have not been tested for safety and effectiveness in randomized placebo-controlled studies [13]. In fact, ginseng has been shown to have estrogenic activity and produce adverse reactions [13]. Because ROS overproduction has been associated with defective sperm function , infertile patients have been treated with antioxidant compounds including, ascorbic acid, vitamin E, selenium, glutathione [14,15-19]. However, the effect of this treatment on sperm quality is still controversial [4]. In a randomized, placebo-controlled, double-blind investigation, oral high doses of vitamins C and E to infertile males could not show any statistically significant improvement in semen parameters [4]. Although, in this study, patient recruitment was solely based on having a sperm concentration less than 50 million/ml, and, thus studies looking at the effectiveness of antioxidant therapy in the treatment of male infertility have not yet been decisive may be due to insufficient patient selection. Not all infertile males have an augment in oxidative stress in their testis and semen. Therefore, principally, only those men who have an experimental increase in oxidative stress should gain from antioxidant therapy. Possibly the best marker to identify these males would be reactive oxygen species (ROS) levels in semen [20-22]. Another important aspect of antioxidant therapy is whether the antioxidant(s) and dose used in vivo are appropriate. Previous studies have indicated that the combination of vitamins E and C at high doses in vitro results in DNA fragmentation [6,22].

Major Types of Sperm DNA and Fertilization

The foundation of the evaluation of the human remnants semen analysis. Though it gives some quantitative and qualitative information about the sperm sample, latest insight into the physiology, biochemistry and molecular biology of the sperm cell have demonstrated that morphology and motility alone are not the only basis upon which sperm should be analyzed [23]. Over the last years, major improvements in the field of male infertility diagnosis have been achieved. The diagnostic usefulness of sperm DNA integrity and sperm vacuolization for predicting outcome in infertile couples undergoing in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatments [23]. A cohort study from 152 infertile couples undergoing sperm DNA fragmentation and high-magnification tests prior to an assisted reproduction treatment was designed. The most predictive cutoff for pregnancy has been observed to be 25.5% of DNA fragmentation with a negative predictive value of 72.7% (P=0.02). For the degree of vacuolization, the best predictor of pregnancy was 73.5% of vacuolated sperm grades III+IV with a negative predictive value of 39.4% (P=0.09), which was not statistically significant [23]. Consequently, sperm DNA fragmentation greater than 25.5% could be associated with higher probability of failure IVF treatment. Regarding the results of the sperm analysis at high magnification, they do not allow us to predict whether or not patients will become pregnant [23]. Generally unnoticed is the fact that sperm carry 2 different kinds of DNA. The nDNA, commonly called the genome, is located in the head of the sperm [8]. The second DNA type is called the mtDNA and is responsible for delivering the sperm to the egg by providing ATP for cellular acceleration [22]. Both types of DNA work toward the common goal of fertilization, but each is susceptible to a myriad of factors that could derail the fertilization process. Imperfections in both types of DNA contribute equally to the problem at hand [22]. Damage nDNA in somatic cell nuclei is packaged into structures called nucleosomes. These structures consist of a protein core formed by an octamer of histones with two loops of wrapped DNA. The nucleosomes are then further coiled into regular helixes called solenoids, which increase the volume of the chromatin [22,24]. Sperm nuclei, however, need to be packaged much differently and more compactly to assure proper delivery of the nDNA. There are believed to be 4 levels of organization for packaging spermatozoon nDNA [24,25]. One level consists of anchoring the chromosomes to the nuclear annulus. In another, DNA loop domains are created as the DNA attaches itself to the newly added nuclear matrix [22]. The arrangement of these loop domains ascertains that the DNA can be delivered to the ovum in a form that is both physically and chemically accessible to the growing embryo. Chromosome repositioning and organization within the matrix of the sperm head is another level. Condensation of nDNA into tiny, super coiled dough- nuts called toroids by replacing the nuclear histones with structures called protamines completes the levels of chromosomal organization. Human sperm contain two major types of protamines, which are about half the size of typical histones; throughout evolution, they have augmented the number of positively charged residues, allowing formation of a highly condensed complex with the negatively charged paternal genomic DNA. Besides, the addition of cysteine residues allows the formation of disulfide bonds between adjacent protamines molecules, thereby strongly stabilizing the nucleo-protamine complex [22, 26-28]. Prior to this re arrangement, recombination is essential for spermatogenesis to occur [22, 26-28]; as seen in studies with animal knockout models, lowering recombination is associated with diminished spermatogenesis. Several factors (both endogenous and exogenous) can influence this, contributing to male infertility. Scientists agree on 4 principal techniques, although there may be others, by which nDNA can be compromised or damaged: defective sperm chromatin packaging, apoptosis, oxidative stress, and genetic lesions [21,22,24,29,30] . The effects of these damaging methods are often found to be interrelated. Defective Chromatin packaging refers to the highly complex and specific structure into which nDNA is folded to properly deliver the genetic information to the ovum. Though defects can come up at any of the 4 levels of packaging, the most general problems occur during DNA loop domain formation and histone-protamine replacement. nDNA loop domains can be difficult to organize without inducing endogenous nicks to the nDNA [22,31]. It is contemplation that these nicks survive naturally and provide to

relieve torsional stress. The presence of these nicks is maximum during transition from round to elongated spermatids in testis and occurs before complete protamination within the sperm. Topoisomerase II is a specific enzyme, which creates instantly and ligates the nicks within nDNA during this process [22,32]. Any defect in the enzyme itself will negatively affect the packaging of the genetic information and will contribute to male infertility. The enzyme may leave the nDNA fragmented with single- or double-stranded breaks; this may indicate an early apoptotic process in somatic cells and incomplete sperm maturation in the case of spermatozoa. Topoisomerase inhibitors have been proven to augment the levels of internal nDNA breaks by preventing their repair and increasing their susceptibility to damage [29]. Also involved in sperm chromatin packaging is the replacement of histones with protamines. Protamines are major DNA-binding proteins essential for chromatin condensation [31,33]. During epididymal transport, histones are replaced by transition proteins, only to be replaced by protamines [26]; both intermolecular and intra molecular disulfide cross-linking among the cysteine-rich protamines compresses the DNA into one sixth the volume occupied by somatic cell nDNA [22,34]. This high rate of cross-linking affords the sperm nDNA a measure of defense against exogenous assault and compensates for an impaired DNA-repair capability. Human spermatozoa preserve approximately 15% histones in their native structure, leading to a less compact chromatin arrangement compared to other mammals [35,36], perhaps to allow access for oocyte repair mechanisms. Human spermatozoa contain 2 different types of protamines that are thought to be present in equal amounts in fertile men: P1 and P2 [27,36]. The ratio of P1 to P2 is critical to male fertility [22,26,27,33,35], more particularly to the sperm's fertilization ability [36]. Besides, recent analysis has confirmed that P2 precursors (pre-P2) are vital in maintaining the delicate P1:P2 ratio. Translation of pre-P2 mRNA appears to cause abnormal head morphogenesis, decreased sperm motility, and male infertility [37]. Infertility and problems of impaired fruitfulness have been a concern through ages and is also a noteworthy clinical problem in modern era, ultimately affecting about 10% of couples globally. Of all infertility cases, around 40–50% are due to "male factor" infertility and as many as 2% of all men will show suboptimal sperm parameters. It may be one or a combination of low sperm concentration, poor sperm motility, or abnormal morphology. The rates of infertility in less industrialized nations are noticeably higher and infectious diseases are accountable for a greater proportion of infertility [37]. Moreover, a low pre- P2: P2 ratio suggests a link between deficient protamines processing and decreased nDNA integrity [37,38]. These reports are thought to help in knowing the trends of male factor infertility in developing nations like India and to find out in future, various factors that may be responsible for male infertility. Apoptosis is the controlled disassembly of a cell from within [19]; it is believed to have 2 roles during normal spermatogenesis [21,22,39,40]. The first role is to limit the germ cell population to numbers that can be supported by the surrounding Sertoli cells. The second is for the depletion of abnormal spermatozoa. As seen in the prior section, abnormal spermatozoa can be produced via defective sperm chromatin packaging, among other ways. In somatic cells, cells that enter into an apoptotic pathway usually have several classical indicators, such as phosphatidyl serine (PS) relocation, Fas expression, nDNA strand breaks, and capsize activity. PS relocation is perhaps the earliest indicator of apoptosis; normally located on the inner leaflet of the plasma membrane, PS migrates to the outer membrane once the apoptotic signal has been given [32,41]. To help control this signal, both pro- and anti- apoptotic proteins are present in the testis; they are members of the Bcl-2 family of proteins and provide a signaling pathway that is imperative to maintaining male germ cell homeostasis [40,42]. Bcl-2 and Bcl-xL are both pro survival proteins, while Bax is a pro-apoptotic protein. Disturbing the balance of these proteins from the Bcl-2 family has been demonstrated in mice to contribute to male infertility by disrupting normal apoptosis levels. Fas expression is another indicator that the apoptosis signal has been given. Fas is the type I cell surface protein, belonging to the tumor necrosis/nerve growth factor receptor family [32,43]; it is induced by the binding of Fas ligand to the Fas receptor on the plasma outer membrane. Sertoli cells are known to express Fas ligand, demonstrating the fact that apoptosis is a commonly used mechanism to control the germ cell population at a level that can be supported by the Sertoli cells [35,44]. Ligation of Fas ligand to the Fas receptor triggers activation of cytosolic aspartate-specific proteases, or simply capsizes. Once capsize activation has taken place, a signal is transduced to synthesize caspase-activated deoxyribonuclease, which leads to DNA degradation by forming single and double-stranded breaks within the nDNA [24]. In infertile men, ejaculated spermatozoa often possess partially degraded nDNA, usually considered to be indicative of the apoptosis pathway; this ''escaping'' of the apoptosis signal is referred to as ''abortive apoptosis'' [29,35,45-47]. The apoptotic pathway is an all or nothing response, meaning that once the signal has been given there is no reversing the process. Abnormalities in this pathway are often attributed to 1 of 2 possibilities: infertile men may not produce enough sperm to trigger Sertoli cell activation to produce Fas, or there may be a problem in activating the Fas mediated apoptosis signal [24,48]. It is believed that if the apoptotic cascade is initiated at the round spermatids phase when transcription is still active, this may be the origin of the nDNA breaks commonly seen in abortive apoptosis in ejaculated spermatozoa. However, nDNA breaks are known to be common during condensation of the genome. It is currently unclear whether these breaks are caused by an aborted apoptotic pathway or simply by incomplete chromatin packaging. Also, not all caspase activity has been shown to be indicative of the apoptotic signal. Recent work has demonstrated that there appears to be some caspase activity in human germ cells that is not associated with apoptosis and may indeed serve a viable function [48,49]. Another well-known inducer of the apoptotic pathway is telomere shortening. Telomeres are capping structures at chromosome ends that protect against rearrangements, preventing ends from being recognized as nDNA breaks [30,50]. They are usually composed of tandem TTAGGG sequence repeats that are bound to a complex array of proteins. Telomerase is a specialized reverse transcriptase

that contains a catalytic subunit that synthesizes new telomeric repeats. In the absence of telomerase, telomeric sequences are lost after each round of replication, eventually creating a shifted sequence that could be recognized as an nDNA double-stranded break; this would then be recognized by a genomic surveillance mechanism that appears in the elongating spermatids [30]. This recognition is another way to induce an apoptotic response, possibly contributing to the ''abortive apoptosis'' theory. Abortive apoptosis is a theory that still requires much scientific evidence to be considered valid. Because of naturally occurring processes within the spermatozoa that mimic somatic cell apoptosis, many believe that this theory requires additional evidence. Oxidative stress upon spermatozoa is induced by an increase in the amount of reactive oxygen species (ROS) that are present in the fluids filling the male genital tract [51]. Sperm are particularly susceptible to oxidative stress due to the high content of unsaturated fatty acids in their membranes, as well as their limited stores of antioxidant enzymes [52,53]. Their increased susceptibility is enhanced by defective chromatin packaging, causing further damage to the genome; individuals with varicoceles are particularly susceptible to this type of damage [53,54]. ROS are created by metabolizing ground-state oxygen into the superoxide anion and H_2O_2 [55,56]. ROS also promote tyrosine phosphorylation to support sperm capacitation. Fertile men control ROS generation through seminal antioxidants; the pathogenic effects of ROS are apparent only when they are produced in excess of the antioxidant capabilities. It is known that the main source of excess ROS generation in semen is leukocytes; genital tract infections are considered to be the most common cause. However, secondary contributors are known to play an important role as well when an infection is not present. The origin of these secondary contributors has yet to be pinpointed in human sperm, but there are many sources under investigation. Three possible sources of excess ROS generation are from within the human sperm itself. The first is through leakage of electrons from the mitochondrial transport chain [56-59]. This was proposed because of tests performed on rat spermatozoa indicating increased translocation of mitochondrial free radicals into the sperm genome. However, further investigation has demonstrated that mitochondrial blockers do not have the same effects on human spermatozoa [35]. The second proposed source is through NADPH oxidase in sperm. This theoretic oxidase would serve to transfer electrons from NAD(P)H to ground-state oxygen to create the superoxide anion. It is known that NAD (P) H in leukocytes helps to contribute to ROS production in rat spermatozoa, but it has yet to be demonstrated in humans [51,52,54,57,60]. The third proposed intracellular source of ROS production is through the generation of nitric oxide (NO) [19,61,62]. NO is a free radical created from the oxidation of L-arginine by 3 isoforms of nitric oxide synthase (NOS). NOS activity has been shown to be associated with the acrosome reaction and capacitation of mouse sperm, thus influencing their fertilizing potential. In humans, decreased NO concentrations are known to increase sperm capacitation and zona pellucida binding. The exact mechanism of its influence has yet to be elucidated. Other proposed sources of ROS come from outside the sperm's immediate environment, usually from outside of the host's body. They include xenobiotic agents such as organ ophosphorous pesticides that disrupt the endocrine system. These agents possess estrogenic properties that are capable of inducing ROS production by male germ cells [52,63,64]. Cigarette smoking is also known to increase ROS levels through increased leukocyte generation. Infertile smokers are known to harbor increased levels of spermatids oxidative stress compared with infertile nonsmokers. This increase is associated with increased seminal leukocytes [65]. Finally, scrotal heat stress has been demonstrated in stallions to damage sperm chromatin structure, possibly by oxidative stressors [66]. Recently similar analyses were performed on humans regarding the use of laptop computers in respect to elevated scrotal temperature [67]. These findings also recognized the elevated temperature of the scrotal environment as having a negative effect upon spermatogenesis, warranting further research. Genetic Lesions Genetic lesions are another possible means of attack through which nDNA can influence male infertility; these lesions create insults or gaps within the genome and may yield effects ranging from minimal to catastrophic. They can be divided into 3 classes based on the type of impact they present[8,33]. The first class consists of chromosomal aneuploidies and rearrangements in which batteries of genes on specific chromosomes have changes in expression dosages or changes in their normal genomic environments. The second class embodies submicroscopic deletions (micro deletions), in which deletions or rearrangements of multiple genes mapped in a molecular environment have changes in their expression patterns. The third class is made up of single gene defects in which expression of a single gene (or key element) is changed or lost, causing male infertility. These lesions can affect all of the human chromosomes, including any of the 300 genes estimated to be involved in male fertility. They can occur within introns as well as exons, making their impact difficult to predict. Paternal nDNA effects Prior to analyzing the second type of DNA found in spermatozoa, it is important to establish that nDNA integrity, as it relates to embryo quality, is still an intense topic of discussion. Paternal effects upon the embryo have been classified as both ''early'' and ''late.'' Early paternal effects appear to be mediated by centrosome destruction or a deficiency in oocyte activating factors within the spermatozoa, implicating faulty sperm chromatin packaging and nDNA damage [68,69]. Early effects are observed before the major activation of embryonic genome expression, which begins at the 4-cell stage in humans. Late paternal effects may involve sperm aneuploidy, nDNA damage, or abnormal chromatin packaging, which can influence the orderly activation of paternal gene expression [70]. It has been found that there is no correlation between sperm nDNA fragmentation and the early paternal effect; however, many assisted reproductive technology (ART) clinics perform embryo transfers on the third day after embryo retrieval, prior to the time when late paternal effects can be fully observed. Because of this fact, blastocyst transfer may be preferable, at the risk of having fewer eggs to transfer. The mtDNA of a sperm is completely located in the sperm mid piece; it exists as a circular, double-stranded DNA molecule composed of 16569 base pairs [71-73].

Severe asthenozoospermia is one of the leading causes of male infertility as spermatozoa cannot reach the oocyte and/or penetrate normally. Identifying structural causes of sperm immotility was of great concern before the advent of intracytoplasmic sperm injection (ICSI), because immotility was the limiting factor in the treatment of these patients. In these cases, *in vitro* methods are used to identify live spermatozoa or stimulate sperm motility to avoid selection of non-viable cells. With these advances, fertilization and pregnancy results have improved dramatically. The identification of genetic phenotypes in asthenozoospermia is important to adequately inform patients of treatment outcomes and risks. The one sperm characteristic that seriously affects fertility prognosis is teratozoospermia, primarily sperm head and neck anomalies. Defects of chromatin condensation and acrosomal hypoplasia are the two most common abnormalities in severe teratozoospermia. The introduction of microscopic methods to select spermatozoa and the development of new ones to evaluate sperm quality before ICSI will assure that ultra structural identification of sperm pathologies will not only be of academic interest, but will also be an essential tool to inform treatment choice [73]. The genetic information encoded by the mtDNA consists of 2 ribosomal RNAs, 22 transfer RNAs, and 13 polypeptides essential for mitochondrial respiration and oxidative phosphorylation associated with the electron transport chain (ETC). The most significant function of the sperm mitochondria is to manufacture ATP. The mitochondria itself is composed of 2 distinct membranes, an inner membrane and an outer membrane. The outer membrane is relatively permissive and allows the transit of large molecules through nonspecific porin channels; the inner membrane is much more discriminatory. The inner membrane is heavily invaginated and forms cristae; enzymes for the ETC are located on the inner membrane, and the particular nature of inner membrane transport helps to maintain the mitochondrial membrane potential that drives the ETC [74]. It is important to remember the differences between mtDNA and nDNA [75]. mtDNA is not afforded the same defense or basic upkeep that nDNA is given. First, there is no protection from histones or DNA-binding proteins within mtDNA; moreover it lacks introns. Because of this, every mutation in mtDNA has the potential to damage the function of the cell. mtDNA also lacks an important proofreading system and replicates much more rapidly than nDNA; this results in the mutation rate created in mtDNA to be 10 to 100 times more compared to that of nDNA. Deletions in the mitochondrial genome would directly affect the sperm's capability to synthesize ATP through the ETC. Direct correlations have been noticed to involve mtDNA deletions and decreased sperm motility [76,77]. There are six different respiratory chain complexes that are required for the ETC to function properly. Of them, all but complex II are encoded by the mitochondrial genome; complex II is encoded by the nuclear genome and imported to the inner membrane of the mitochondria [76,78]. Dysfunctions in these complexes are considered direct indications of mtDNA deletions. Deletions have been found to fall into 2 categories: small and large scale. While some large-scale deletions appear to be found in fertile men and may be considered ''common,'' they are usually associated with spermatozoa with low motility [74,76,79,80] Small-scale deletions, on the other hand, can be equally disturbing. Deletions as small as two base pairs have been proven to insert a stop codon into the mtDNA sequence and shorten vital proteins to ETC function [81,82]. It is important to note that no single deletion has been found to be indicative of poor sperm quality [79]. mtDNA deletions have also been compared to the ages of individuals looking for infertility treatment. Some authors discuss that mtDNA deletions gather with increased age [74], while others have registered that there is no noteworthy correlation between the two [71,72]. Epididymal and testicular mtDNA deletions have also been compared, signifying that testicular sperm may be superior to epididymal sperm for use in ART [76], however, recent publications suggest the opposite [76,83]. Finally, comparisons have been drawn between the incidences of nDNA deletions in combination with mtDNA deletions. Though results have only come out of a single laboratory, strong correlations between the two types of deletions have been observed [83-85]. The number of mtDNA molecules in a single spermatozoon is known as its mtDNA copy number. mtDNA copy number is controlled by the down-regulation of nuclear-encoded mitochondrial transcription factor A [70,86-88]. These data are usually attributed to the technique of analysis used or the cross-hybridization of mitochondrial pseudo genes found in the nDNA. All reports, however, appear to correlate on an important fact: progressive cells acquire fewer mtDNA copy numbers compared to non-progressive spermatozoa [89]. There is an ongoing debate over the cause and effect of apoptotic signaling in mitochondria reflecting whether the sperm mitochondria respiratory system contributes to the ROS environment, causing apoptosis, or increased ROS environment results in mitochondrial respiratory failure. The first hypothesis is supported by the fact that the mitochondrial respiratory system is a substantial producer of intracellular free radicals that might be able to escape the mitochondria and influence the production of ROS [19,22,51]. Proposed free radical generation by mitochondrial involvement has been explained by Ozawa's hypothesis [22,51]. This hypothesis outlines a ''viscous cycle'' while mtDNA deletions cause the mitochondria to remove ATP from the sperm, inducing an energy crisis within the spermatozoa; as the cellular demand for ATP continues, the acceleration of electron leakage connected with ROS generation is increased. This cycle is likely to continue, having catastrophic results and eventually concluding with the spermatozoa entering the apoptotic pathway [19]. The latter hypothesis, signifying that the ROS environment leads to mitochondrial respiratory failure, is supported by the thought that the appearance of mtDNA damage can be seen before any other indications of the apoptosis pathway [19,90]. The first signs of stress induced by increased ROS levels are seen in the distraction of the mitochondrial membrane potential [89,91]. Besides, structural evidence implies that the location of the mitochondria within the sperm midpiece leaves these structures in closest proximity to the stressors of increased ROS levels [87]. Because of mtDNA's lack of DNA protection, this implies that these DNA molecules would be the first to be damaged. Although both theories have substantial support, further research is

necessary to distinguish the cause and effect pathway. Elimination of Paternal Mitochondria by the Egg It is well known that mitochondrial inheritance is of maternal origin [78,87,91]; on the other hand, the pathway by which paternal mitochondria are eliminated is still debated. A few existing hypotheses involve paternal mtDNA dilution within the fertilized egg or oxidative damage to the entering paternal mtDNA as probable explanations for maternal mtDNA dominance. The most reasonable theory involves the ubiquitination of the paternal mtDNA [87,92]. Ubiquitination is a process through which a ubiquitin tag is attached to a protein molecule, imprinting it for destruction. The protein molecule within paternal mitochondria most commonly thought to be ubiquitinated is prohibin [91-93], which is an evolutionarily conserved, 30-kd integral protein of the inner mitochondrial membrane, expressed during spermatogenesis as well as after fertilization. Studies demonstrate that prohibin is ubiquitinated by the spermatozoa itself and is already destined for destruction before it even fertilizes the egg. Upon fertilization, prohibin would encounter the egg's cytoplasmic destruction machinery, recognizing the ubiquitin tag, and would remove the mtDNA. This hypothesis is further supported by the fact when gel electrophoresis is performed upon mature spermatozoa mtDNA, there exists three different bands for the protein prohibin: 1 at the predicted 30-kd location and 2 others in the range of 47 to 50 kD, probably suggesting the phosphorylation of the protein in preparation for the attachment of the ubiquitin tag [93,94]. Latest evidence, though, disputes the ubiquitin tag hypothesis in the elimination of paternal mtDNA [71,72]. A protein known as t-tpis, located in the testis and involved in spermatogenesis (complete function still obscure), has been given special focus for the cause of its involvement in a vital Tom complex within the mitochondria of spermatozoa. Tom complexes are translocations of the mitochondrial outer membrane. t-tpis is found to be expressed solely in the mid-piece of spermatozoa, connecting it to probable mitochondrial function. Further investigation has revealed that t-tpis is a protein member of the Tom complex assembled using Tom 22 and Tom 40 complexes; they are known to be required for cell viability and are localized on the cytosolic side of the mitochondrial outer membrane. A potential ''knob and key-hole'' model involving t-tpis expression has been proposed as a probable way of paternal mitochondrial recognition and elimination. Contrary substantiation of exclusive maternal mitochondrial inheritance comes from abnormal embryos, which failed to eliminate paternal mtDNA; never-the-less, these embryos frequently fail to develop past the blastocyst stage [95]. In the rare event that paternal mtDNA is observed in adults [96-100], recombination events are often attributed to this phenomenon. Nonetheless, it is generally more accurate to consider artificial recombination (ie, errors in testing) before considering actual recombination events to have occurred. Treatment of Sperm DNA for better ART outcomes. Regrettably, there is no treatment for mtDNA deficiencies; instead, scientists have focused upon ways in which to isolate sperm with improved nDNA status, as well as selecting better sperm for ART use to generate better ART outcomes. The first technique of treatment involves cessation of all activities that are recognized to be harmful to the production of healthy sperm; this includes smoking and exposure to probable environmental estrogens, such as pesticides [15,19,22,24,51]. This is in general accompanied by oral antioxidant treatment at least two months prior to ART treatment in an effort to minimize oxidative stress [22,49]. One more suggested line of treatment is the use of surgically retrieved testicular spermatozoa as an alternative of epididymal sperm. The motive to use testicular sperm is to minimize sperm with fragmented nDNA and acquire specimens with better mtDNA for use with *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedures [22,49,101]. However, latest evidence suggests the exact opposite, indicating epididymal sperm to be superior to testicular sperm for ICSI outcome [81,83]. Never-the-less, a high-magnification optical system can be employed to sort better spermatozoa for ICSI. In this way, spermatozoa can be selected by visualizing morphology under conditions not possible with normal laboratory equipment. Subtle morphologic abnormalities become visible under this high magnification (66006) that cannot be seen under normal high power objectives (4006), permitting the embryologist to select better sperm for ICSI fertilization [22,96,100,101]. Other ways to upgrade sperm nDNA include enhanced preparation techniques. This involves reducing the centrifugal forces exerted on the sperm while concentrating it and eliminating leukocytes as rapidly as possible from the sample. Besides, the swim-up technique can be employed to avoid use of the centrifuge. It is hypothesized that the supplementation of sperm wash medium to raw semen prior to liquefaction may inhibit bacterial binding to the sperm surface as well as diminish nDNA damage caused by ROS. Oddly, *in vitro* culture of surgically retrieved testicular spermatozoa for 48 to 72 hours at 37 0C has been recommended to improve motility, along with decreasing the proportion of spermatozoa containing single-stranded nDNA breaks [22,102]. Very recently, a novel sperm selection assay has been proposed to select viable sperm free of chromosomal anomalies for use with ICSI. Sperm hyaluronic acid (HA) binding has demonstrated the ability to isolate mature, viable sperm with unreacted acrosomal status, without damaging the specimen [22,51,101,103]. One principle of this assay lies in the expression of the chaperone protein HspA2; in spite of its pivotal role in meiosis, HspA2 levels have become physiological and biochemical markers of sperm maturation [22,101,104]. Low levels of HspA2 expression are connected with diminished sperm maturity, increased frequency of chromosomal aneuploidies, presence of apoptotic processes, and fragmented nDNA. The second principle involved considers remodeling of the cytoplasmic and membrane-specific physiological and biochemical markers, facilitating the formation of sperm binding sites for the zona pellucida of oocytes and for the binding sites of HA. Immature sperm, which fail to undergo membrane remodeling are unable to bind to immobilized HA and thus not likely to be selected in this assay [22,101,104]. Chromosomal disomies are said to be reduced between fourfold and fivefold in HA-selected sperm compared with semen sperm [22,51,101,105] reflecting that HA preferentially selects for chromosomally normal sperm. Because of such fascinating consequences, a kit for this specific assay has become commercially available. The sperm-hyaluronic binding assay

(HBA) has been marketed for routine testing of sperm motility and fertility [101,106,107]. Regrettably, HBA data have been observed quite inadequate of expectations in predicting successful fertilization rates in IVF, expressing rather less significance compared to sperm morphology and limiting its clinical predictive worth. Besides, comprehensive research is required to upgrade the existing protocols so that to improve the quality of spermatozoa likely to be sorted out for fulfilling the purpose of majority of ART techniques to elevate outcomes.

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