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Sperm DNA Bridges from Sperm to Egg to Inculcate Genetic Variability: A Review

S. Mishra and S. Nanda
Department of Biotechnology, College of Engineering and Technology, IFTM Campus, Delhi Road, Moradabad 244 001, U.P., India

Corresponding Author: Dr. Sanjay Mishra, Department of Biotechnology, College of Engineering and Technology, IFTM Campus, Delhi Road, Moradabad 244 001, U.P., India

ABSTRACT

Sperm DNA fragmentation is known to compromise male fertility. The data entail that sperm DNA damage can be efficiently treated with oral antioxidant administration during a relatively short time period. It is an understanding that the 2 types of DNA appear to be distinct and independently packaged molecules; however, research has demonstrated the symbiotic nature of these structures in contributing to male infertility. Data must continue to be gathered to establish strong correlations between traditional semen analysis parameters and sperm DNA integrity; this information remains controversial and useless in clinical practice until new techniques for the diagnosis and treatment of sperm DNA can be established. More modern technology must be employed to correlate such information into useful clinical knowledge. This study is the compilation of reports pertaining to research on sperm DNA biochemistry and molecular biology to establish an opinion that sperm DNA integrity is both extremely fragile and exceedingly important for male fertility.

Key words: Apoptosis, male infertility, sperm DNA

INTRODUCTION

Sperm DNA fragmentation is known to compromise male fertility. The data entail that sperm DNA damage can be efficiently treated with oral antioxidant administration during a relatively short time period (Greco *et al*., 2005). An improvement of basic sperm parameters by oral treatment with antioxidants has been reported in a number of studies (Agarwal *et al*., 2004), but DNA damage has been addressed in only a few of them (Geva *et al*., 1998; Suleiman *et al*., 1996; Kodama *et al*., 1997; Comhaire *et al*., 2000; Keskes-Ammar *et al*., 2003). Only two of these studies were controlled and randomized (Suleiman *et al*., 1996; Keskes-Ammar *et al*., 2003) and none of them used a direct assay for the detection of DNA strand breaks. Infertility is a growing 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. Research now reveals that male infertility may contribute in up to two thirds of all couples who seek treatment for infertility. For many years a conventional semen analysis (concentration, motility and morphology) was seen as sufficient to diagnose male infertility; however, scientific examination must now take into account 2 different kinds of DNA that have been proven to contribute to this diagnosis. Nuclear DNA (nDNA), contained in the head of the sperm, is responsible for packaging all of the paternal genetic information that will be needed

for the fertilized egg. nDNA can be damaged or compromised via 4 interrelated courses: defective chromatin packaging, apoptosis, oxidative stress and genetic lesions. Mitochondrial DNA (mtDNA) is located in the midpiece of the sperm; when coupled with the tail, it is responsible for mobilizing the sperm toward the egg for fertilization. Scientists are only beginning to comprehend the relationship and interaction between these distinct DNA molecules and how they both contribute to male infertility. As the worldwide community continues to expand, an emerging subpopulation of couples has begun experiencing a common problem in making their contribution to the populace. 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 (Seshagiri, 2001). 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 growing (Feng, 2003). In infertile couples, responsibility for the lack of conception is generally 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 partners.

Application of nutraceuticals in sperm production: Nutraceuticals have been advocated as a way of potentiating sperm production and quality in the subfertile male. In a recent study, the administration of folic acid and zinc sulfate to subfertile 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 and 66 mg, respectively. Although the beneficial effect on fertility remains to be established, this finding opens new avenues of future fertility research and treatment (Wong *et al*., 2002). Arginine (Pryor *et al*., 1978), vitamin B12 (Sinclair, 2000), methylcobalamin (Moriyama *et al*., 1987) and ginseng (Salvati *et al*., 1996) have been used in the treatment of male infertility. However, most of these compounds have marginal effects on sperm production and quality and have not been tested for safety and efficacy in randomized placebo-controlled studies. In fact, ginseng has been shown to have estrogenic activity (Duda *et al*., 1996) and produce adverse reactions (Hammond and Whitworth, 1981; Dega *et al*., 1996). Because ROS overproduction has been associated with defective sperm function (Aitken *et al*., 1989), infertile patients have been treated with antioxidant compounds including, ascorbic acid (Fraga *et al*., 1991), vitamin E (Kessopoulou *et al*., 1995; Mishra *et al*., 2009), selenium (Scott *et al*., 1998), gluthatione (Lenzi *et al*., 1998), vitamins, selenium, gluthatione, ubiquinol and carnitine (Moncada *et al*., 1992; Lenzi *et al*., 1993; Costa *et al*., 1994; Sikka *et al*., 1995; Vitali *et al*., 1995; Hawkes and Turek, 2001; Vicari and Calogero, 2001; Lenzi *et al*., 2003; Sharma *et al*., 2009; Mishra *et al*., 2009). However, the effect of this treatment on sperm quality is still controversial (Ford and Whittington, 1998; Geva *et al*., 1998; Lenzi *et al*., 1998; Tarin *et al*., 1998; Comhaire *et al*., 1999). In a randomized, placebo-controlled, double-blind study, oral high doses of vitamins C and E to infertile males did not show any statistically significant improvement in semen parameters (Rolf *et al*., 1999). However, in this study, patient recruitment was solely based on having a sperm concentration below 50 million mL^{-1}. One of the main reasons studies looking at the efficacy of antioxidant therapy in the treatment of male infertility have not yet been conclusive may be due to inadequate patient selection. Not all infertile males have an increase in oxidative stress in their testis and semen. Therefore, in principle, only those men who have a quantifiable increase in oxidative stress should benefit from antioxidant therapy. Perhaps the best marker to identify these males would be Reactive Oxygen Species (ROS) levels in semen (Agarwal and Saleh, 2002). 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 (Donnelly *et al*., 1999).

Types of sperm DNA and fertilization: The cornerstone of the evaluation of the man remains semen analysis. Although, it gives some quantitative and qualitative information about the sperm sample, recent insight into the molecular biology and genetics of the sperm cell have demonstrated that morphology and motility alone are not the only grounds upon which sperm should be evaluated. Commonly overlooked 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. The second DNA type is called the mtDNA and is responsible for delivering the sperm to the egg by providing ATP for cellular acceleration. 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. This article hopes to elucidate male factor infertility as contributed by both kinds of DNA. Origins of nDNA 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 2 loops of wrapped DNA. The nucleosomes are then further coiled into regular helixes called solenoids, which increase the volume of the chromatin (Agarwal and Said, 2003). 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 (Sakkas *et al*., 1999; Agarwal and Said, 2003). 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. The arrangement of these loop domains ensures that the DNA can be delivered to the egg 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, supercoiled dough-nuts called toroids by replacing the nuclear histones with structures called protamines completes the levels of chromosomal organization. Human sperm contain 2 types of protamines that are about half the size of typical histones; throughout evolution, they have increased the number of positively charged residues, allowing formation of a highly condensed complex with the negatively charged paternal genomic DNA. Also, the addition of cysteine residues allows the formation of disulfide bonds between adjacent protamine molecules, thereby strongly stabilizing the nucleo-protamine complex (Oliva, 2006). Prior to this re arrangement, recombination is essential for spermatogenesis to occur (Carrel *et al*., 2007); as seen in studies using animal knockout models, decreased recombination is associated with diminished spermatogenesis. Many factors (both endogenous and exogenous) can influence this, contributing to male infertility (Mishra *et al*., 2003; Nanda *et al*., 2010a). Scientists agree on 4 distinct methods, although there may be others, by which nDNA can be compromised or damaged: defective sperm chromatin packaging, apoptosis, oxidative stress and genetic lesions (Agarwal and Said, 2003; Sharma *et al*., 2004; Lewis and Aitken, 2005; Shafik *et al*., 2006). 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 egg. Although, defects can arise at any of the 4 levels of packaging, the most common problems arise during DNA loop domain formation and histone-protamine replacement. nDNA loop domains can be difficult to arrange without inducing endogenous nicks to the nDNA (Sakkas *et al*., 1999). It is thought that these nicks exist naturally and serve to relieve torsional stress. The presence of these nicks is greatest

during transition from round to elongated spermatids in the testis and occurs before complete protamination within the sperm. Topoisomerase II is the enzyme that creates an ligates the nicks within nDNA during this process (Sakkas *et al*., 1999; Henkel *et al*., 2004). 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 increase the levels of internal nDNA breaks by preventing their repair and increasing their susceptibility to damage (Lewis and Aitken, 2005). Also involved in sperm chromatin packaging is the replacement of histones with protamines. Protamines are major DNA-binding proteins essential for chromatin condensation (Vogt, 2004). During epididymal transport, histones are replaced by transition proteins, only to be replaced by protamines (Oliva, 2006); both intermolecular and intramolecular disulfide cross-linking among the cysteine-rich protamines compresses the DNA into one sixth the volume occupied by somatic cell nDNA (Lewis and Aitken, 2005; Zini and Libman, 2006). This high rate of cross-linking affords the sperm nDNA a measure of protection against exogenous assault and compensates for an impaired DNA-repair capacity. Human spermatozoa retain approximately 15% histones in their structure, leading to a less compact chromatin arrangement than in other mammals (Erenpreiss *et al*., 2006), perhaps to allow access for oocyte repair mechanisms. Human sperm contain 2 different types of protamines, which are believed to be present in equal amounts in fertile men: P1 and P2 (Carrell *et al*., 2007). Experiments have shown that the ratio of P1 to P2 is critical to male fertility (Oliva, 2006; Vogt, 2004; Erenpreiss *et al*., 2006; Carrell *et al*., 2007), more specifically to the sperm's fertilization ability (Aoki *et al*., 2006). In addition, recent testing has demonstrated 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, reduced sperm motility and male infertility (Tseden *et al*., 2007). Also, a low pre- P2: P2 ratio suggests a link between deficient protamine processing and decreased nDNA integrity (Torregrosa *et al*., 2006). Apoptosis is the controlled disassembly of a cell from within; it is believed to have 2 roles during normal spermatogenesis (Seli *et al*., 2004; Shrama *et al*., 2004; Spano *et al*., 2005). 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 phosphatidylserine (PS) relocation, Fas expression, nDNA strand breaks and caspase 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 (Henkel *et al*., 2004). 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 (Spano *et al*., 2005). Bcl-2 and Bcl-xL are both prosurvival 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 (Henkel *et al*., 2004); 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 (Erenpreiss *et al*., 2006). Ligation of Fas ligand to the Fas receptor triggers activation of cytosolic aspartate-specific proteases, or simply caspases. Once caspase 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 (Agarwal and Said, 2003). 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 (Sakkas *et al*., 2002; Lewis and Aitken, 2005; Erenpreiss *et al*., 2006). 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 (Agarwal and Said, 2003). It is believed that if the apoptotic cascade is initiated at the round spermatid 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 (Martinez *et al*., 2002). 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 (Shafik *et al*., 2006). 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 spermatid (Shafik *et al*., 2006). 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 (Nanda *et al*., 2010b). 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 (Baker and Aitken, 2005). 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 (Saleh *et al*., 2003). The ROS are created by metabolizing ground-state oxygen into the superoxide anion and H_2O_2 (Vernet *et al*., 2001). They play an important physiologic role, modulating gene and protein activities vital to sperm proliferation, differentiation and function (Erenpreiss *et al*., 2006). The 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 (Vernet *et al*., 2001; Balercia *et al*., 2004; Sharma *et al*., 2009). 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 (Erenpreiss *et al*., 2006). The second proposed source is through NADPH oxidase in sperm. This theoretic oxidase would serve to transfer electrons from NAD (P) Hto 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 (Vernet *et al*., 2001; Saleh *et al*., 2003; Baker and Aitken, 2005; Balercia *et al*., 2004; Nanda *et al*., 2010b). The third proposed intracellular source of ROS production is through the generation of Nitric Oxide (NO) (Sanchez-Pena, *et al*., 2004; Sharma *et al*., 2009). The NO is a free radical created from the oxidation of L-arginine by 3 isoforms of Nitric Oxide Synthase (NOS). The 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 organophosphorous pesticides that disrupt the endocrine system. These agents possess estrogenic properties that are capable of inducing ROS production by male germ cells (Agarwal and Said, 2005; Baker and Aitken, 2005). Cigarette smoking is also known to increase ROS levels through increased leukocyte generation. Infertile smokers are known to harbor increased levels of spermatid oxidative stress compared with infertile nonsmokers. This increase is associated with increased seminal leukocytes (Love and Kenney, 1999). Finally, scrotal heat stress has been demonstrated in stallions to damage sperm chromatin structure, possibly by oxidative stressors (Sheynkin *et al*., 2005). Recently similar analyses were performed on humans regarding the use of laptop computers in respect to elevated scrotal temperature (Tesarik *et al*., 2002). 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 (Vogt, 2004). 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 (microdeletions), 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 oocyteactivating factors within the spermatozoa, implicating faulty sperm chromatin packaging and nDNA damage (Tesarik *et al*., 2004). 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 (Kao *et al*., 1998). 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. mtDNA The mtDNA of a sperm is completely located in the sperm midpiece; it exists as a circular, double-stranded DNA molecule composed of 16 569 base pairs (St. John *et al*., 2000a). 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 important 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, which drives the ETC (O'Connell *et al*., 2002a). It is important to remember the differences between mtDNA and nDNA (Ruiz-Pesini *et al*., 1998). The mtDNA is not afforded the same protection or basic upkeep that nDNA is given. First, there is no protection from histones or DNA-binding proteins within mtDNA; also it lacks introns. Because of this, every mutation in mtDNA has the potential to damage the function of the cell. mtDNA also lacks a significant proofreading system and replicates much more rapidly than nDNA; this causes the mutation rate found in mtDNA to be 10 to 100 times more than that of nDNA. mtDNA Deletions Because of the increased rate of occurrence, mitochondrial deletions have been exclusively investigated; of particular interest has been the relationship to sperm motility and forward progression. Deletions in the mitochondrial genome would directly affect the sperm's ability to synthesize ATP through the ETC. Direct correlations have been found involving mtDNA deletions and decreased sperm motility (O'Connell *et al*., 2003). There are 6 distinct 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 (St. John *et al*., 2001). 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 (St. John *et al*., 2000a; O'Connell *et al*., 2002a, 2003; Thangaraj *et al*., 2003). Small-scale deletions, however, can be equally devastating. Deletions as small as 2 base pairs have been proven to insert a stop codon into the mtDNA sequence and truncate vital proteins to ETC function (Rodrigo *et al*., 2004). It is important to note that no single deletion has been found to be indicative of poor sperm quality (Thangaraj *et al*, 2003). mtDNA deletions have also been compared to the ages of individuals seeking infertility treatment. Some authors argue that mtDNA deletions accumulate

with increased age (O'Connell *et al*., 2002a), while others have found that there is no significant correlation between the two (St. John *et al*., 2000b). Epididymal and testicular mtDNA deletions have also been compared, suggesting that testicular sperm may be superior to epididymal sperm for use in ART (O'Cornell *et al*., 2003), however, recent publications suggest the opposite (O'Cornell *et al*., 2003; Buffat *et al*., 2006). Lastly, comparisons have been drawn between the incidences of nDNA deletions in combination with mtDNA deletions. Although results have only come out of a single laboratory, strong correlations between the 2 types of deletions have been found (O'Connell *et al*., 2002b; Buffat *et al*., 2006). mtDNA Copy Number 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 (May-Panloup *et al*., 2003). Laboratories have been trying to pinpoint the specific mtDNA copy number that is considered normal for fertile men, but have had little success; reported copy numbers for normal, fertile men range from 3.8 in 100% density layers (Kao *et al*., 2004) to 74.1 6 2.0 in healthy men (Diez-Sanchez *et al*., 2003) to 717 6 394 in motile spermatozoa (Marchetti *et al*., 2002). These discrepancies are usually attributed to the method of analysis used or the crosshybridization of mitochondrial pseudogenes found in the nDNA. All reports, however, appear to correlate on 1 important fact: progressive cells possess fewer mtDNA copy numbers than do nonprogressive spermatozoa. Mitochondrial DNA and Apoptosis While nDNA rearrangements can be associated with any number of possible abnormalities causing male infertility; mtDNA injury is most often attributed to the apoptotic pathway. Mitochondrial membrane potential is a measurable factor that has been used to predict the spermatozoa's risk for apoptosis (Donnelly *et al*., 2000). There is an ongoing debate over the cause and effect of apoptotic signaling in mitochondria. In other words, does the sperm mitochondria respiratory system contribute to the ROS environment, causing apoptosis, or does the increased ROS environment cause mitochondrial respiratory failure? The first theory 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 (Sharma *et al*., 2009; Nanda *et al*., 2010b). Proposed free radical generation by mitochondrial involvement has been explained by Ozawa's hypothesis (Nanda *et al*., 2010b). This hypothesis outlines a viscous cycle whereby mtDNA deletions cause the mitochondria to deprive the sperm of ATP, inducing an energy crisis within the spermatozoa; as the cellular demand for ATP continues, the acceleration of electron leakage associated with ROS generation is increased. This cycle will continue, having catastrophic results and eventually concluding with the spermatozoa entering the apoptotic pathway (Sharma *et al*., 2009). The latter theory, indicating that the ROS environment causes mitochondrial respiratory failure, is supported by the idea that the appearance of mtDNA damage can be seen before any other indications of the apoptosis pathway (Sutovsky *et al*., 2004). The first signs of stress induced by increased ROS levels are seen in the disruption of the mitochondrial membrane potential (Donnelly *et al*., 2000). In addition, 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 (May-Panloup *et al*., 2003). 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 (St. John *et al*., 2001); however, the pathway by which paternal mitochondria are eliminated is still debated. A few existing theories involve paternal mtDNA dilution within the

fertilized egg or oxidative damage to the entering paternal mtDNA as possible explanations for maternal mtDNA dominance. The most plausible theory involves the ubiquitination of the paternal mtDNA (Sutovsky *et al*., 2000). 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 believed to be ubiquitinated is prohibin (Sutovsky *et al*., 2000; Hayashida *et al*., 2005), which is an evolutionarily conserved, 30-kd integral protein of the inner mitochondrial membrane that is 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 eliminate the mtDNA. This theory is further supported by the fact that when gel electrophoresis is performed upon mature spermatozoa mtDNA, there exists 3 distinct bands for the protein prohibin: 1 at the predicted 30 kd location and 2 others in the range of 47 to 50 kd, possibly signifying the phosphorylation of the protein in preparation for the attachment of the ubiquitin tag (Hayashida *et al*., 2005). Recent evidence, however, disputes the ubiquitin tag hypothesis in the elimination of paternal mtDNA (St. John *et al*., 2000a). A protein known as t-tpis, located in the testis and involved in spermatogenesis (full function unknown), has been given special attention due to its involvement in a vital Tom complex within the mitochondria of spermatozoa. Tom complexes are translocators of the mitochondrial outer membrane. T-tpis is found to be expressed solely in the midpiece of spermatozoa, linking it to possible mitochondrial function. Further investigation has revealed that t-tpis is a protein member of the Tom complex assembled using Tom 22 and 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 possible way of paternal mitochondrial recognition and elimination. Contrary evidence of exclusive maternal mitochondrial inheritance comes from abnormal embryos that failed to eliminate paternal mtDNA; however, these embryos frequently fail to develop past the blastocyst stage (Quintana-Murci *et al*., 2001). In the rare event that paternal mtDNA is observed in adults (Emiliani *et al*., 2001; Schwartz and Vissing, 2002; Bandelt *et al*., 2005; Tesarik *et al*., 2006), recombination events are often attributed to this phenomenon. Nonetheless, it is generally more accurate to consider artificial recombination (i.e., errors in testing) before considering actual recombination events to have occurred. Treatment of sperm DNA for better ART outcomes unfortunately, 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 method of treatment involves cessation of all activities that are known to be harmful to the production of healthy sperm; this includes smoking and exposure to possible environmental estrogens, such as pesticides (Agarwal and said, 2003; Sharma *et al*., 2009; Mishra *et al*., 2009; Nanda *et al*., 2010 b). This is generally accompanied by oral antioxidant treatment at least 2 months prior to ART treatment in an attempt to minimize oxidative stress (Martinez *et al*., 2002). Another suggested line of treatment is the use of surgically retrieved testicular spermatozoa instead of epididymal sperm. The reason to use testicular sperm is to minimize sperm with fragmented nDNA and obtain specimens with better mtDNA for use with *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedures (Martinez *et al*., 2002). However, recent evidence suggests the exact opposite, indicating epididymal sperm to be superior to testicular sperm for ICSI outcome (Rodrigo *et al*., 2004; Buffat *et al*., 2006). Also, a high-magnification optical system can be used to select better

spermatozoa for ICSI. In this manner, 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), allowing the embryologist to select better sperm for ICSI fertilization (Emiliani *et al*., 2001; Tesarik and Mendoza, 2007). Other ways to improve sperm nDNA include enhanced preparation techniques. This involves lowering the centrifugal forces exerted on the sperm when concentrating it and removing leukocytes as quickly as possible from the sample. Also the swim-up technique can be used to avoid use of the centrifuge. It is postulated that the addition 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 h at 37°C has been suggested to improve motility, along with decreasing the proportion of spermatozoa containing single-stranded nDNA breaks (Huszar *et al*., 2003). 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 (Jakab *et al*., 2005; Nanda *et al*., 2010a, b). One principle of this assay lies in the expression of the chaperone protein HspA2; in spite of its key role in meiosis, HspA2 levels have become indicative of sperm maturation (Huszar *et al*., 2006). Low levels of HspA2 expression are associated with diminished sperm maturity, increased frequency of chromosomal aneuploidies, presence of apoptotic processes and fragmented nDNA. The second principle involved takes into account remodeling of the cytoplasmic and membrane-specific biochemical markers, facilitating the formation of sperm binding sites for the zona pellucida of oocytes and for the binding sites of HA. Immature sperm that fail to undergo membrane remodeling are unable to bind to immobilized HA and will not be selected in this assay (Huszar *et al*., 2006). Chromosomal disomies are said to be reduced between fourfold and fivefold in HA-selected sperm compared with semen sperm (Ye *et al*., 2006) reflecting that HA preferentially selects for chromosomally normal sperm. Because of such promising results, a kit for this assay has become commercially available. The sperm Hyaluronan Binding Assay (HBA) has been marketed for routine testing of sperm motility and fertility (Nasr-Esfahani *et al*., 2008). Unfortunately, HBA results have fallen well short of expectations in predicting successful fertilization rates in IVF, demonstrating less significance than sperm morphology and limiting its clinical predictive value. Further research is needed to perfect techniques that will be employed to improve the quality of spermatozoa that are selected for use in all ART techniques to improve outcomes.

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REFERENCES

Agarwal, A. and A. Saleh, 2002. Role of oxidants in male infertility: Rationale, significance and treatment. Urol. Clin., 29: 616-627.

Agarwal, A. and T.M. Said, 2003. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum. Reprod. Update, 9: 331-345.

Agarwal, A., K.P. Nallella, S.S. Allamaneni and T.M. Said, 2004. Role of antioxidants in treatment of male infertility: An overview of the literature. Reprod. Biomed. Online, 8: 616-627.

Agarwal, A. and T.M. Said, 2005. Oxidative stress, DNA damage and apoptosis in male infertility: A clinical approach. Br. J. Urol. Int., 95: 503-507.

Aitken, R.J., J.S. Clarkson and S. Fishel, 1989. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol. Reprod., 41: 183-187.

Aoki, V.W., L. Liu, K.P. Jones, H.H. Hatasaka, M. Gibson, C.M. Peterson and D.T. Carrell, 2006. Sperm protamine1/protamine 2 ratios are related to *In vitro* fertilization pregnancy rates and predictive of fertilization ability. Fertility Sterility, 86: 1408-1415.

Baker, M.A. and R.J. Aitken, 2005. Reactive oxygen species in spermatozoa: Methods for monitoring and significance for the origins of genetic disease and infertility. Reprod. Biol. Endocrinol., 3: 67-75.

Balercia, G., S. Moretti, A. Vignini, M. Magagnini and F. Mantero *et al*., 2004. Role of nitric oxide concentrations on human sperm motility. J. Androl., 25: 245-249.

Bandelt, H.J., Q.P. Kong, W. Parson and A. Salas, 2005. More evidence for nonmaternal inheritance of mitochondrial DNA? J. Med. Genet., 42: 957-960.

Buffat, C., C. Patrat, F. Merlet, J. Guibert and S. Epelboin *et al*., 2006. ICSI outcomes in obstructive azoospermia: Influence of the origin of surgically retrieved spermatozoa and the cause of obstruction. Hum. Reprod., 21: 1018-1024.

Carrell, D.T., B.R. Emery and S. Hammoud, 2007. Altered protamine expression and diminished spermatogenesis: what is the link? Hum Reprod Update, 13: 313-327.

Comhaire, F.H., A.M. Mahmoud, C.E. Depuydt, A.A. Zalata and A.B. Christophe, 1999. Mechanisms and effects of male genital tract infection on semen quality and fertilizing potential: the andrologists view point. Hum. Reprod. Update., 5: 393-398.

Comhaire, F.H., A.B. Christophe, A.A. Zalata, W.S. Dhooge, A.M. Mahmoud and C.E. Depuydt, 2000. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins Leukot. Essent. FattyAcids, 63: 159-165.

Costa, M., D. Canale, M. Filicori, S. Dlddio and A. Lenzi, 1994. L-carnitine in idiopathic astheno zoospermia: A multicenter study. Andrologia, 26: 155-159.

Dega, H., J.L. Laporte, C. Frances, S. Herson and O. Chosidow, 1996. Ginseng as a cause for Stevens- Johnson syndrome? Lancet, 347: 1344-1344.

Diez-Sanchez, C., E. Ruiz-Pesini, A.C. Lapena, J. Montoya, A. Perez-Martos, J.A. Enriquez and M.J. Lopez-Perez, 2003. Mitochondrial DNA content of human spermatozoa. Biol. Reprod., 68: 180-185.

Donnelly, E.T., N. McClure and S.E. Lewis, 1999. The effect of ascorbate and alpha- Tocopherol supplementation *in vitro* on DNA integrity and hydrogen peroxide–induced DNA damage in human spermatozoa. Mutagenesis, 14: 505-512.

Donnelly, E.T., M. Connell, N. McClure and S.E. Lewis, 2000. Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. Hum. Reprod., 15: 1552-1561.

Duda, R.B., B. Taback, B. Kessel, D.D. Dooley and H. Yang *et al*., 1996. pS2 expression induced by American ginseng in MCF-7 breast cancer cells. Ann. Surgical Oncol., 3: 515-520.

Emiliani, S., M. Van den Bergh, A.S. Vannin, J. Biramane, M. Verdoot and Y. Englert, 2001. Evidence of reduced single-stranded testicular sperm DNA from obstructive azoospermic men after 3 days of *in-vitro* culture. Hum. Reprod., 16: 1200-1203.

Erenpreiss, J., M. Spano, J. Erenpreisa, M. Bungum and A. Giwercman, 2006. Sperm chromatin structure and male fertility: Biological and clinical aspects. Asian J. Androl., 8: 11-29.

Feng, H.L., 2003. Molecular biology of male infertility. Arch. Androl., 49: 19-27.

Ford, W.C.L. and K. Whittington, 1998. Antioxidant treatment for male subfertility: A promise that remains unfulfilled. Hum. Reprod., 13: 1416-1419.

Fraga, C.G., P.A. Motchnik, M.K. Shigenaga, H.J. Helbock and R.A. Jacob, 1991. Ames BN. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc. Nat. Acad. Sci. USA., 88: 11003-11006.

Geva, E., J.B. Lessing, L. Lerner-Geva and A. Amit, 1998. Free radicals, antioxidants and human spermatozoa: Clinical implications. Hum. Reprod., 13: 1422-1424.

Greco, E., M. Iacobelli, L. Rienzi, F. Ubaldi, S. Ferrero and J. Tesarik, 2005. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J. Androl., 26: 349-353.

Hammond, T.G. and J.A. Whitworth, 1981. Adverse reactions to ginseng. Med. J. Aust., 1: 492-492.

Hawkes, W.C. and P.J. Turek, 2001. Effects of dietary selenium on sperm motility in healthy men. J. Androl., 22: 764-772.

Hayashida, K., K. Omagari, J.I. Masuda, H. Hazama, Y. Kadokawa, K. Ohba and S. Kohno, 2005. The sperm mitochondria-specific translocator has a key role in maternal mitochondrial inheritance. Cell. Biol. Int., 29: 472-481.

Henkel, R., M. Hajimohammad, T. Stalf, C. Hoogendijk and C. Mehnert *et al.*, 2004. Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertility Sterility, 81: 965-972.

Huszar, G., C. Celik-Ozenci, S. Cayli, Z. Zavaczki, E. Hansch and L. Vigue, 2003. Hyaluronic acid binding by human sperm indicates cellular maturity, viability and unreacted acrosomal status. Fertility Sterility, 9: 1616-1624.

Huszar, G., S. Ozkavukcu, A. Jakab, C. Celik-Ozenci, G.L. Sati and S. Cayli, 2006. Hyaluronic acid binding ability of human sperm reflects cellular maturity and fertilizing potential: Selection of sperm for intracytoplasmic sperm injection. Curr. Opin. Obstetrics Gynecol., 18: 260-267.

Jakab, A., D. Sakkas, E. Delpiano, S. Cayli and E. Kovanci *et al.*, 2005. Intracytoplasmic sperm injection: A novel selection method for sperm with normal frequency of chromosomal aneuploidies. Fertility Sterility, 84: 1665-1673.

Kao, S.H., H.T. Chao and Y.H. Wei, 1998. Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. Mol. Hum. Reprod., 4: 657-666.

Kao, S.H., H.T. Chao, H.W. Liu, T.L. Liao and Y.H. Wei, 2004. Sperm mitochondrial DNA depletion in men with asthenospermia. Fertility Sterility, 82: 66-73.

Keskes-Ammar, L., N. Feki-Chakroun, T. Rebai, Z. Sahnoun and H. Ghozzi *et al.*, 2003. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch. Androl., 49: 83-94.

Kessopoulou, E., H.J. Powers, K.K. Sharma, M.J. Pearson, J.M. Russell, I.D. Cooke and C.L. Barratt, 1995. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated with male infertility. Fertility Sterility, 64: 825-831.

Kodama, H., R. Yamaguchi, J. Fukuda, H. Kasai and T. Tanaka, 1997. Increased oxi-dative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertility Sterility, 68: 519-524.

Lenzi, A., F. Culasso, L. Gandini, F. Lombardo and F. Dondero, 1993. Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male infertility. Hum. Reprod., 8: 1657-1662.

Lenzi, A., F. Lombardo, P. Sgro, P. Salacone, L. Caponecchia, F. Dondero and L. Gandini, 2003. Use of carnitine therapy in selected cases of male factor in fertility: A double-blind cross over trial. Fertility Sterility, 79: 292-300.

Lenzi, A., L. Gandini and M. Picardo, 1998. A rationale for glutathione therapy. Hum. Reprod., 13: 1419-1421.

Lewis, S.E.M and R.J. Aitken, 2005. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res., 322: 33-41.

Love, C.C. and R.M. Kenney, 1999. Scrotal heat stress induces altered sperm chromatin structure associated with a decrease in protamines disulfide bonding in the stallion. Biol. Reprod., 60: 615-620.

Marchetti, C., G. Obert, A. Deffosez, P. Formstecher and P. Marchetti, 2002. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum. Reprod., 17: 1257-1265.

Martinez, F., L. Rienzi, M. Iacobelli, F. Ubaldi and C. Mendoza *et al.*, 2002. Caspase activity in preimplantation human embryos is not associated with apoptosis. Hum. Reprod., 17: 1584-1590.

May-Panloup, P., M.F. Chretien, F. Savagner, C. Vasseur and M. Jean *et al.*, 2003. Increased sperm mitochondrial DNA content in male infertility. Hum. Reprod., 18: 550-556.

Mishra, S., P.R. Somanath, Z. Huang and S. Vijayraghavan, 2003. Binding and inactivation of the germ cell-specific protein phosphatase PP1Y2 by sds22 during epididymal sperm maturation. Binding and inactivation of the germ cell-specific protein phosphatase PP1ã2 by sds22 during epididymal sperm maturation. Biol. Reprod., 69: 1572-1579.

Mishra, S., R.B. Singh, S.P. Dwivedi, F. de Meester and R. Rybar *et al.*, 2009. Effects of nutraceuticals on genetic expressions. Open Nutraceuticals J., 2: 70-80.

Moncada, M.L., E. Vicari, C. Cimino, A.E. Calogero, A. Mongioi, R. Agata, 1992. Effect of acetylcarnitine treatment in oligoasthenospermic patients. Acta Eur. Fertil., 23: 221-224.

Moriyama, H., K. Nakamura, N. Sanda, E. Fujiwara and S. Seko *et al.*, 1987. Studies on the usefulness of a long term, high-dose treatment of methylcobalamin in patients with oligozoospermia. Hinyokika Kiyo, 33: 151-156.

Nanda, S., S. Mishra, V.P. Varshney and R.B. Singh, 2010a. A biotechnological approach to apoptosis of somatic and germ cells in living organisms. Open Nutraceuticals J., 3: 81-93.

Nanda, S., V.P. Varshney and S. Mishra, 2010b. Biochemical characterization of the mechanism of binding of a regulatory protein to the spermatozoa-specific protein phosphatase in *Swiss albino* rat. J. Applied Biosci., 27: 1715-1726.

Nasr-Esfahani, M.H., S. Razavi, A.A. Vahdati, F. Fathi and M. Tavalaee, 2008. Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. J. Assist. Reprod. Genet., 25: 197-203.

Oliva, R., 2006. Protamines and male infertility. Human Reprod. Update, 12: 417-435.

O'Connell, M., N. McClure and S.E.M. Lewis, 2002a. A comparison of mitochondrial and nuclear DNA status in testicular sperm from fertile men and those with obstructive azoospermia. Hum. Reprod., 17: 1571-1577.

O'Connell, M., N. McClure and S.E.M. Lewis, 2002b. Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal human sperm. Hum. Reprod., 17: 1565-1570.

O'Connell, M., N. McClure, L.A. Powell, E.K. Steele and S.E.M. Lewis, 2003. Differences in mitochondrial and nuclear DNA status of highdensity and low-density sperm fractions after density gradient preparation. Fertility Sterility, 79: 754-762.

Pryor, J.P., J.P. Blandy, P. Evans, C.D.M. De Saintonge and M. Usherwood, 1978. Controlled clinical trial of arginine for infertile men with oligozoospermia. Br. J. Urol., 50: 47-50.

Quintana-Murci, L., A. Rotig, A. Munnich, P. Rustin and T. Bourgeron, 2001. Mitochondrial DNA inheritance in patients with deleted mtDNA. J. Med. Genet., 38: e28-e28.

Rodrigo, L., C. Rubio, E. Mateu, C. Simon, J. Remohi, A. Pellicer and M. Gil-Salom, 2004. Analysis of chromosomal abnormalities in testicular and epididymal spermatozoa from azoospermic ICSI patients by fluorescence in-situ hybridization. Hum Reprod., 19: 118-123.

Rolf, C., T.G. Cooper, C.H. Yeung and E. Nieschlag, 1999. Antioxidant treatment of patients with astheno zoospermia or moderate oligo astheno zoospermia with high-dose vitamin C and vitamin E: A randomized, placebo-controlled study. Hum. Reprod., 14: 1028-1033.

Ruiz-Pesini, E., C. Diez, A.C. Lapena, A. Perez-Martos and J. Montoya *et al.*, 1998. Correlation of sperm motility with mitochondrial enzymatic activities. Clin. Chem., 44: 1616-1620.

Sakkas, D., E. Mariethoz, G. Manicardi, D. Bizzaro, P.G. Bianchi and U. Bianchi, 1999. Origin of DNA damage in ejaculated human spermatozoa. Rev. Reprod., 4: 31-37.

Sakkas, D., O. Moffatt, G.C. Manicardi, E. Mariethoz, N. Tarozzi and D. Bizzaro, 2002. Nature of DNA damage in ejaculated human spermatozoa and the possible involvement of apoptosis. Biol. Reprod., 66: 1061-1067.

Saleh, R., A. Agarwal, R. Sharma, T. Said, S. Sikka and A. Jr. Thomas, 2003. Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. Fertil. Steril., 80: 1431-1436.

Salvati, G., G. Genovesi, L. Marcellini, P. Paolini and I. De Nuccio *et al.*, 1996. Effects of panax ginseng C.A. meyer saponins on male fertility. Panmineva Medica, 38: 249-254.

Sanchez-Pena, L.C., B.E. Reyes, L. Lopez-Carrillo, R. Recio and J. Moran-Martinez *et al.*, 2004. Organophosphorous pesticide exposure alters sperm chromatin structure in Mexican agricultural workers. Toxicol. Applied Pharm., 196: 108-113.

Schwartz, M. and J. Vissing, 2002. Paternal inheritance of mitochondrial DNA. New Engl. J. Med., 347: 576-580.

Scott, R., A. Mac Pherson, R.W. Yates, B. Hussain and J. Dixon, 1998. The effect of oral selenium supplementation on human sperm motility. Br. J. Urol., 82: 76-80.

Seli, E., D.K. Gardner, W.B. Schoolcraft, O. Moffatt and D. Sakkas, 2004. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertility Sterility, 82: 378-383.

Seshagiri, P.B., 2001. Molecular insight into the causes of male infertility. J. Biosci., 24: 429-435.

Shafik, A., A.A. Shafik, I. Shafik and O. El-Sibai, 2006. Sperm DNA fragmentation. Syst. Biol. Reprod. Med., 52: 197-208.

Sharma, R.K., T. Said and A. Agarwal, 2004. Sperm DNA damage and its clinical relevance in assessing reproductive outcome. Asian J. Androl., 6: 139-148.

Sharma, V., S. Kalim, M.K. Srivastava, S. Nanda and S. Mishra, 2009. Oxidative stress and coxsackievirus infections act as mediators of beta cell damage: A review. Scientific Res. Essay, 4: 42-58.

Sheynkin, Y., M. Jung, P. Yoo, D. Schulsinger and E. Komaroff, 2005. Increase in scrotal temperature in laptop computer users. Hum. Reprod., 20: 452-455.

Sikka, S.C., M. Rajasekaran and W.J.G. Hellstrom, 1995. Role of oxidative stress and antioxidants in male infertility. J. Androl., 16: 464-481.

Sinclair, S., 2000. Male infertility: Nutritional and environmental considerations. Altern. Med. Rev., 5: 28-38.

Spano, M., E. Seli, D. Bizzaro, G.C. Manicardi and D. Sakkas, 2005. The significance of sperm nuclear DNA strand breaks on reproductive outcome. Curr. Opin. Obstetrics Gynecol., 17: 255-260.

St. John, J., D. Sakkas, K. Dimitriadi, A. Barnes and V. Maclin *et al.*, 2000a. Failure of elimination of paternal mitochondrial DNA in abnormal embryos. Lancet, 355: 200-200.

St. John, J.C., D. Sakkas and C.L. Barratt, 2000b. A role for mitochondrial DNA and sperm survival. J. Androl., 21: 189-199.

St. John, J.C., R.P. Jokhi and C.L. Barratt, 2001. Men with oligoasthenoteratozoospermia harbour higher numbers of multiple mitochondrial DNA deletions in their spermatozoa, but individual deletions are not indicative of overall aetology. Mol. Hum. Reprod., 7: 103-111.

Suleiman, S.A., M.E. Ali, Z.M. Zaki, E.M. El-Malik and M.A. Nasir, 1996. Lipid peroxi- dation and human sperm motility: Protective role of vitamin E. J. Androl., 17: 530-537.

Sutovsky, P., K. Van Leyen, T. McCauley, B.N. Day and M. Sutovsky, 2004. Degradation of paternal mitochondria after fertilization: Implications for heteroplasmy, assisted reproductive technologies and mtDNA inheritance. Reprod. Biomed. Online, 8: 24-33.

Sutovsky, P., R.D. Moreno, J. Ramalho-Santos, T. Dominko, C. Simerly and G. Schatten, 2000. Ubiquitinated sperm mitochondria, selective proteolysis and the regulation of mitochondrial inheritance in mammalian embryos. Biol. Reprod., 63: 582-590.

Tarin, J.J., J. Brines and A. Cano, 1998. Antioxidants may protect against infertility. Hum. Reprod., 13: 1415-1416.

Tesarik, J., C. Mendoza and E. Greco, 2002. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. Hum. Reprod., 17: 184-189.

Tesarik, J., E. Greco and C. Mendoza, 2004. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum. Reprod., 19: 611-615.

Tesarik, J., R. Mendoza-Tesarik and C. Mendoza, 2006. Sperm nuclear DNA damage: Update on the mechanism, diagnosis and treatment. Reprod. Biomed. Online, 12: 715-721.

Tesarik, J. and C. Mendoza, 2007. Treatment of severe male infertility by micromanipulation-assisted fertilization: An update. Frontiers Biosci., 12: 105-114.

Thangaraj, K., M.B. Joshi, A.G. Reddy, A.A. Rasalkar and L. Singh, 2003. Sperm mitochondrial mutations as a cause of low sperm motility. J. Androl., 24: 388-392.

Torregrosa, N., D. Dominguez-Fandos, M.I. Camejo, C.R. Shirley and M.L. Meistrich *et al.*, 2006. Protamine 2 precursors, protamine 1/protamine 2 ratio, DNA integrity and other sperm parameters in infertile patients. Hum. Reprod., 21: 2084-2089.

Tseden, K., O. Topaloglu, A. Meinhardt, A. Dev and I. Adham *et al.*, 2007. Premature translation of transition protein 2 mRNA causes sperm abnormalities and male infertility. Mol. Reprod. Dev., 74: 273-279.

Vernet, P., N. Fulton, C. Wallace and R.J. Aitken, 2001. Analysis of reactive oxygen species generating systems in rat epididymal spermatozoa. Biol. Reprod., 65: 1102-1113.

Vicari, E. and A.E. Calogero, 2001. Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. Hum. Reprod., 16: 2338-2342.

Vitali, G., R. Parente and C. Melotti, 1995. Carnitine supplementation in human idiopathic asthenospermia: Clinical results. Drugs Exp. Clin. Res., 21: 157-159.

Vogt, P. 2004. Molecular genetics of human male infertility: From genes to new therapeutic perspectives. Curr. Pharm. Design, 10: 471-500.

Wong, W.Y., H.M. Merkus, C.M. Thomas, R. Menkveld, G.A. Zielhuis and R.P. Steegers-Theunissen, 2002. Effects of folic acid and zinc sulfate on male factor subfertility: A double-blind, randomized, placebo-controlled trial. Fertility Sterility, 77: 491-498.

Ye, H., G. Huang, Y. Y. Gao and D.Y. Liu, 2006. Relationship between human spermhyaluronan binding assay and fertilization rate in conventional *in vitro* fertilization. Hum. Reprod., 21: 1545-1550.

Zini, A. and J. Libman, 2006. Sperm DNA damage: Clinical significance in the era of assisted reproduction. Can. Med. Assocc. J., 175: 495-500.