Zoological Studies **39**(1): 1-12 (2000)

Zoological Studies

Review Article

Mitochondrial DNA Mutation and Depletion Are Associated with Decline of Fertility and Motility of Human Sperm

Yau-Huei Wei[1,2,*] and Shu-Huei Kao[1]

[1]*Department of Biochemistry*

[2]*Center for Cellular and Molecular Biology, National Yang-Ming University, Taipei, Taiwan 112, R.O.C.*

CONTENTS

ABSTRACT

Yau-Huei Wei and Shu-Huei Kao (2000) Mitochondrial DNA mutation and depletion are associated with decline of fertility and motility of human sperm. *Zoological Studies* **39**(1): 1-12. Sperm motility is one of the most important determinants of male fertility. In this review, we discuss recent findings that mutation and depletion of mitochondrial DNA (mtDNA) are associated with poor motility and diminished fertility in human sperm. The mtDNA mutations were identified and characterized by polymerase chain reaction (PCR) techniques and DNA sequencing, and the copy number of mtDNA was determined as the ratio between the amount of mtDNA and that of nuclear DNA in sperm. By use of these molecular techniques we first established that the common 4977-bp deletion and 2 novel deletions of 7345 bp and 7599 bp of mtDNA occur more frequently and in higher proportions in spermatozoa with poorer motility and lower fertility. On the other hand, we found that the copy number of mtDNA is lower for sperm with poorer motility. Scores of several motility parameters of sperm assessed by a computer-assisted semen analyzer are positively correlated with the copy number of mtDNA. Moreover, with a transmission electron microscope we examined spermatozoa with low levels of mtDNA from infertile patients. We found that more than 70% of spermatozoa exhibited normal helicoidal morphology of mitochondria packed in the midpiece. These results indicate that depletion of mtDNA in these spermatozoa is not due to a decrease in the number of mitochondria but is a result of the paucity of the mitochondrial genome per se. On the basis of these recent findings, we suggest that mutation and depletion of mtDNA in spermatozoa may play an important role in the pathophysiology of some male infertility.

Key words: Mitochondria, DNA mutation, Sperm motility, Infertility.

*To whom correspondence and reprint requests should be addressed. Tel: 886-2-28267118. Fax: 886-2-28264843. E-mail: joeman@mailsrv.ym.edu.tw

INTRODUCTION

Mitochondria are intracellular organelles responsible for energy metabolism in eukaryotic cells. As the major supplier of chemical energy in the form of adenosine triphosphate (ATP), mitochondria use more than 90% of the oxygen uptake of mammalian cells in the respiratory chain (Chance et al. 1979). Sperm require a great deal of ATP for the flagellum to move around in the early phase of fertilization (de Lamirande and Gagnon 1992). It has been generally accepted that there are 70-80 mitochondria in the midpiece of mammalian sperm (DeMartino et al. 1979, Michaels et al. 1982, Alcivar et al. 1989). The ATP generated in mitochondria is transported to the motility apparatus of the sperm tail via the creatine phosphate shuttle (Tombes and Shapiro 1985) or by radial and vertical diffusion (Cardullo and Baltz 1991).

Mammalian sperm, like somatic cells, contain multi-copies of mitochondrial DNA (mtDNA) in addition to the nuclear genome. Human mtDNA is a 16569-bp double-stranded circular DNA molecule that codes for 2 rRNAs, 22 tRNAs, and 13 polypeptides (Anderson et al. 1981) (Fig. 1). Each strand of DNA is transcribed as a single polycistronic transcript under the control of a strand-specific promoter (P_H and P_L), transcription factors, and specific regulatory proteins that are all encoded by nuclear DNA (Attardi and Schatz 1988, Poyton and McEwen 1996). Mitochondrial respiratory chain function depends on the coordinated gene expression of both the mitochondrial and nuclear genomes. A mutation in either genome may lead to defective respiratory function of mitochondria. Somatic mtDNA mutations have been proposed to be a major contributory factor for aging and age-related degenerative diseases (Linnane et al. 1989, Wei 1992). Several types of mtDNA mutations occur in high proportions in affected tissues of patients with overt mitochondrial diseases (Wallace 1994, Sherratt et al. 1997). Point mutation and large-scale deletion of mtDNA have been found in patients with mitochondrial myopathies and encephalomyopathies (Wallace 1992, Lestienne and Bataille 1994, Larsson and Clayton 1995), diabetes mellitus (Ballinger et al. 1994), and other multi-system disorders (Sherratt et al. 1997). In addition to mtDNA mutations, depletion of mtDNA has also been found in some patients with fatal infantile mitochondrial myopathy (Moraes et al. 1991, Mazziotta et al. 1992, Tritschler et al. 1992), spinal muscular atrophy (Pons et al. 1996), and fatal hepatic failure (Spelbrink et al. 1998).

It is long established that there is 1 copy of mtDNA in each mitochondrion in the midpiece of mammalian sperm (Michaels et al. 1982). During spermatogenesis, the mitochondria undergo drastic morphological changes and subcellular reorganization (Seitz et al. 1995). A reduction in the numbers of mitochondria and of the mitochondrial genome was demonstrated in maturation of mammalian sperm (Hecht 1984). In order to maintain a suitable number of mitochondria in mature sperm, replication and organization of mtDNA must be tightly controlled in the process of spermatogenesis. Since the bioenergetic function of mitochondria is crucial for sperm motility, any qualitative or quantitative aberrations in mtDNA may affect the cellular functioning of spermatozoa. In the past few years, several groups have demonstrated that large-scale deletions of mtDNA frequently occur in human sperm with low motility (Kao et al. 1995, Lestienne et al. 1997, Kao et al. 1998, Reynier et al. 1998). Recently, we found that the copy number of mtDNA is significantly reduced in sperm with poor motility or diminished fertility (Kao 1998). Since most mtDNA mutations exist at extremely low levels in sperm, several sophisticated PCR techniques have been developed for the analysis of mutations in mtDNA. We also employed the transmission electron microscopic method to visualize the assembly and morphology of sperm mitochondria. The results from these studies have led us to suggest that mutation and depletion of mtDNA play a role in the pathophysiology of infertility in some males.

MITOCHONDRIAL GENOME AND GENETICS

It was demonstrated more than 30 years ago that human and animal cells contain a 2nd genome in the mitochondria (Gray 1989). Each mammalian cell contains a few hundred to several thousand mitochondria, and each mitochondrion has 2 to 10 copies of mtDNA (Gray 1989, Veltri et al. 1990). The existence of this extra-chromosomal genetic system has conferred mitochondria with the ability to synthesize proteins in a semi-autonomous manner (Attardi et al. 1989). Mammalian mtDNA encodes 2 rRNAs, 22 tRNAs, and 13 polypeptides (Fig. 1), which are essential components of 4 respiratory enzyme complexes (Anderson et al. 1981, Attardi and Schatz 1988). The replication of mtDNA and gene expression of the mitochondrial genome are executed and regulated by enzymes and protein factors encoded by nuclear DNA (Larsson and Clayton 1995). Human mtDNA is maternally inherited (Giles et al. 1980) and is usually amplified during the maturation

process of oocytes from only a few precursor mtDNA molecules, a phenomenon leading to the establishment of current bottleneck theory (Jenuth et al. 1996, Reynier et al. 1998). Usually oocytes from healthy females have no mutated mtDNA molecules (a clean start). However, a wide spectrum of mutant mtDNA molecules occurs and accumulates in somatic cells in the aging process and in affected tissues of patients with mitochondrial diseases (Wallace 1992 1994 1999). Mutant mtDNAs are often randomly distributed at the tissue level and randomly segregated during cell division at the cellular level (Case and Wallace 1981, Huang et al. 1996, Huang et al. 1999).

The mitochondrial genome is attached, at least transiently, to the inner membrane of mitochondria (Shearman and Kalf 1977) where reactive oxygen species (ROS) and free radicals (e.g., ubisemiquinone radicals) are continually generated by the respiratory chain (Chance et al. 1979, Richter et al. 1995). Mammalian mtDNA lacks the protection of histones and other DNA-binding proteins, and is replicated rapidly without efficient proofreading or a DNA repair system (Clayton et al. 1974, Tomkinson et al. 1990, Driggers et al. 1996, Beckman and Ames 1997, Croteau and Bohr 1997). Moreover, mtDNA has no introns, and both strands are transcribed to synthesize the 3 types of RNA required for the assembly of a functional protein synthesis machinery in the mitochondria. Thus random attacks on the naked mtDNA by ROS or free radicals will inevitably cause oxidative damage or mutation to the mitochondrial genome with pathological consequences.

On the other hand, mtDNA is replicated by a so-called “D-loop” mechanism, whereby a 3-strand intermediate is generated in the replication process (Clayton 1992). This replicating form of mtDNA is especially vulnerable to attack by ROS and free radicals. Moreover, mtDNA contains a great number of direct repeats of size from 4 to 17 bp, whereby some large-scale deletions may be induced by slippage mispairing during DNA replication (Shoffner et al. 1989). The peculiar structure and unique replication system of mtDNA and the highly oxidative environment in which it is located have caused it to mutate at a rate 10 to 20 times higher than that of nuclear DNA (Merriwether et al. 1991, Wallace 1994, Yakes and van Houten 1997). The mtDNA in somatic cells is thus subject to mutation at a high frequency in an individual’s life span. On the basis of the abundance of a number of point mutations in the skeletal muscle of elderly subjects (Zhang et al. 1998), it was calculated that in such tissues of 70-yr-old human subjects, each mtDNA molecule carries 50 base substitutions on average (Nagley and Wei 1998). The same tissues may also contain many different types of mtDNA deletions. Thus, it is quite common for mutant mtDNA molecules to coexist with wild-type mtDNA in the same cell, a condition termed “heteroplasmy” (Wallace 1992). Moreover, the random occurrence and distribution of mutant mtDNA molecules usually vary from tissue to tissue. This frequently results in the so-called “mosaic” defects in mitochondrial respiratory function, which may be revealed by histochemical or biochemical examinations of target tissues of subjects harboring such mtDNA mutations (Wallace 1994, Huang et al. 1996, Nagley and Wei 1998, Huang et al. 1999).

DISCOVERIES OF MITOCHONDRIAL DNA MUTATIONS

It was Piko and coworkers who first conducted a series of studies in the late 1970s which demonstrated that the frequency of rearrangement of mtDNA is increased in senescent animal tissues (Piko 1978). In addition, they found that the proportion of structurally altered mtDNA increased from about 0.1% in young adult animal tissues to approximately 2.0% in old mouse brain, 1.5% in old rat kidney, and 0.6% in heart muscle of old mice (Piko et al. 1984). They also noted that these aging-related mtDNA aberrations occur at variable sites, but that their distribution is nonrandom. From studies of sequence heterogeneity of mtDNA from rat and mouse tissues, Piko et al. (1988) concluded that the frequency of deletions and insertions of mtDNA increases with age. At about the same time, Holt and coworkers (1988) first demonstrated large-scale deletions of mtDNA in skeletal muscle of patients with mitochondrial myopathy. This type of DNA rearrangement was later shown to occur frequently in muscle of patients with chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), and Pearson’s marrow-pancreas syndrome (Zeviani et al. 1988, Shoffner et al. 1989, Rötig et al. 1990, Wallace 1992). The 4977-bp deletion, which occurs at the breakpoints with the 13-bp direct repeat of 5'-ACCTCCCTCACCA-3', has been established to be the most common mtDNA mutation in affected tissues of about 40% of patients with mitochondrial myopathy (Shoffner et al. 1989, Wallace 1992). This and other large-scale deletions (e.g., the 7436-bp deletion) of mtDNA have lately been found to occur frequently in aging human tissues (Yen et al. 1991, Wei 1992, Yang et al. 1995, Fahn et al. 1996, Wei 1998, Lu et al. 1999). Almost all large-scale deletions reported so far occur within the large arc be-

tween the 2 origins for replication of mtDNA (Fig. 1). Several types of duplication of mtDNA have also been found in muscle of a small number of patients with mitochondrial disorders (Poulton 1992, Poulton and Holt 1994, Li et al. 1996).

On the other hand, several point mutations of mtDNA have been detected in target tissues (mostly muscle) of patients with mitochondrial encephalomyopathies (Goto et al. 1990, Shoffner et al. 1990) and other neuromuscular disorders (Santorelli et al. 1993, Wallace 1994). It is now established that LHON, MERRF, MELAS, and Leigh syndromes are associated with mutations of G11778A, A8344G, A3243G, and T8993G (or T8993C), respectively, in mtDNA (Wallace et al. 1988, Wallace 1994, Sherratt et al. 1997, Wallace 1999). Some other minor point mutations of mtDNA have also been found in a smaller number of patients having these diseases, such as the T3271C mutation for the MELAS syndrome (Goto et al. 1991).

All of the aforementioned pathogenic mutations of mtDNA are usually present at high levels (> 70%) in affected tissues, such as muscle and brain, and are segregated with the diseases. Most of these mtDNA mutations have been shown to cause defects in protein synthesis and/or respiratory function of the mitochondria. The severity and onset age of the diseases usually correlate with the proportion of mutant mtDNA in the affected tissues. Recently, we detected low levels (< 5%) of large-scale deletions (mostly the 4977-bp deletion) of mtDNA in muscle of several patients with mitochondrial myopathy and encephalomyopathy; these patients have been proven to harbor high levels of a primary pathogenic mtDNA mutation (Shih 1996, Wei 1998, Pang et al. 1999). These less-abundant mutations of mtDNA may be elicited by the pre-existing primary mutation of the mitochondrial genome due to enhanced production of ROS and free radicals by the impaired respiratory function in affected tissues. Furthermore, some of these pathogenic mutations of mtDNA have also been found to occur at lower levels (< 1%) and to accumulate with age in various tissues of elderly subjects (Yen et al. 1991, Wei 1992, Yang et al. 1995, Fahn et al. 1996, Wei 1998, Wei et al. 1998). These mutant mtDNA molecules may be increased by amplification of pre-existing molecules in the oocyte from which the individual developed, or arise by de novo mutation of mtDNA in somatic tissues during the life span of that individual. Age-dependent increases in the frequency of occurrence and proportion of mutated mtDNA molecules have been proposed to be an important contributory factor in human aging and age-related neurodegenerative diseases (Wei 1992 1998). This age-related accumulation of mtDNA mutations may also play a role in the progression of clinical symptoms of mitochondrial diseases (Pang et al. 1999).

MITOCHONDRIAL DNA MUTATIONS IN HUMAN SPERM

Spermatozoa need a great deal of energy to support their rapid movement after ejaculation. While producing ATP by normal aerobic metabolism, sperm mitochondria also generate ROS and free radicals as byproducts. These deleterious reactive compounds are usually disposed of by the coordinated functioning of enzymatic antioxidants, but a

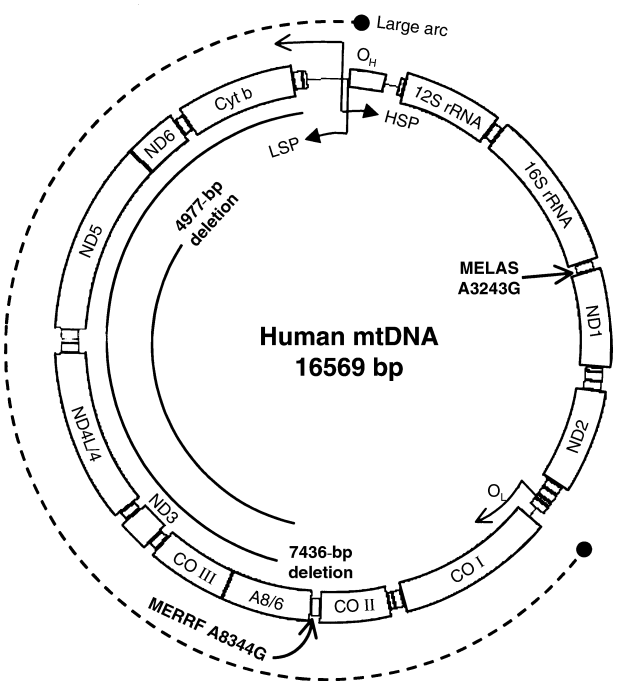

Fig. 1. Organization of genes and some mutations of the human mitochondrial genome. Human mtDNA encodes 13 polypeptides, 12S and 16S rRNAs, and a set of 22 tRNAs by transcription of both heavy (from HSP) and light strands (from LSP) in a coordinated manner. The 4977-bp and 7436-bp deletions, which occur at the hot regions within the large arc between the replication origins O_H and O_L, are the 2 most commonly found disease- and aging-associated large-scale deletions of human mitochondrial DNA (Wallace 1992, Wei 1992). The A3243G and A8344G transitions in the $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$ genes, respectively, are the 2 point mutations found in patients with MELAS and MERRF syndromes (Goto et al. 1990, Shoffner et al. 1990). Many other mutations, including point mutations, large-scale deletions, and duplications that have been found in affected tissues of patients with mitochondrial diseases, are not shown on the map. These mutations may occur alone or in combinations in various human tissues in aging or degenerative diseases.

certain fraction of them may escape the antioxidant defense system and cause transient or permanent damage to lipids, proteins, and nucleic acids (Aitken et al. 1989). Spermatozoa are especially vulnerable to peroxidative damage because their plasma membranes are rich in unsaturated fatty acids, and because they lack endogenous catalase and glutathione peroxidase (Aitken et al. 1992). Fraga and coworkers (1991) demonstrated that the content of 8-hydroxy 2'-deoxyguanosine (8-OH-dG), a marker of oxidative damage to DNA, in sperm is inversely related to the concentration of ascorbic acid in semen. It was pointed out that age-related increase of oxidative stress and oxidative damage to mtDNA is involved in male infertility (Cummins et al. 1994). We have thus hypothesized that mtDNA molecules in human spermatozoa are vulnerable to oxidative damage and mutation and that they play a role in male infertility.

To test this hypothesis, we used self-migration of sperm into 50%-80% Percoll gradients to fractionate spermatozoa with different motility scores (Fig. 2). The motility characteristics including motility (MOT), progressive motility (pMOT), average path velocity (VAP), straight line velocity (VSL), beat cross frequency (BCF), linearity (LIN), and amplitude of lateral head displacement (ALH), were immediately measured by a computer-assisted semen analyzer (CASA) (Fig. 3). The Percoll gradient fractionation was found to be efficient in separating spermatozoa with varying motilities (Kao et al. 1995). We then applied regular PCR and primer-shift PCR techniques to screen for large-scale deletions of mtDNA in spermatozoa with poor motility and those from patients with infertility and oligospermia or asthenospermia. Among the mtDNA deletions observed, the so-called

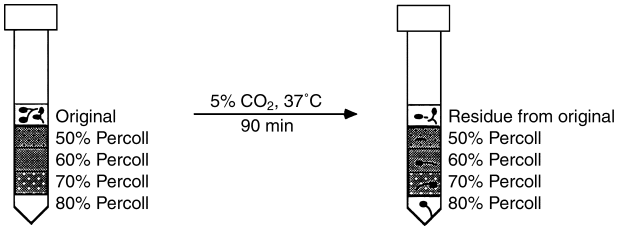

Fig. 2. Fractionation of human sperm by self-migration of spermatozoa into continuous Percoll gradients. The Percoll gradients were prepared by mixing 100% Percoll solution with Ham's F-10 medium at different volume ratios. Freshly ejaculated spermatozoa were gently washed and layered on top of the 50%-80% Percoll gradients, and incubated at 37 °C for 90 min in a humidified incubator maintained at 5% CO_2. At the end of incubation, the spermatozoa migrated according to their motilities into various Percoll gradients.

"common deletion" of 4977 bp was the most prevalent and abundant one (Kao et al. 1995). In addition, we identified 2 novel deletions of 7345 and 7599 bp of mtDNA in spermatozoa with poor motility (Kao et al. 1998). These 3 large-scale deletions all occur at the hot regions (Hou and Wei 1996) in the large arc between 2 replication origins of mtDNA (Fig. 1). Most importantly, they were found to occur more frequently in sperm with poorer motility, and their proportions were shown to correlate with motility in a negative manner (Fig. 4). Moreover, we demonstrated that the incidences of the 3 types of mtDNA deletions in spermatozoa from patients with primary infertility and oligospermia or asthenospermia are significantly higher than those of sperm of fertile males (Kao et al. 1998). Lestienne and coworkers (1997) used PCR techniques to detect multiple deletions of mtDNA in a patient with oligoasthenozoospermia. It is important to note that other mtDNA mutations in sperm may exist that were not revealed by the primers and PCR techniques used. Indeed, in the spermatozoa with low motility and those from in-

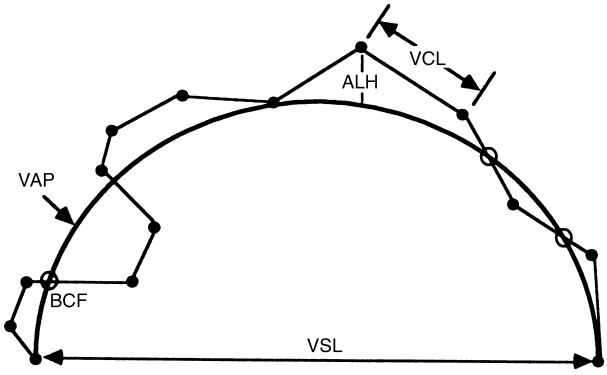

Fig. 3. Assessment of motility characteristics of human spermatozoa by a computer-assisted semen analyzer. A 10 μl aliquot of human sperm suspension obtained from Percoll gradient fractionation was placed in a Makler chamber (Self-Medical Inc., Haifa, Israel) and assessed for motility characteristics in a computer-assisted semen analyzer (CASA, Hamilton Thorn Research Inc., Danvers, MA, USA). The parameters assessed include average path velocity (VAP: the average velocity traveled by a sperm through 5 points in the path of movement), percentage motile (MOT: percentage of spermatozoa exhibiting a VAP > 10 μm/s), progressive motility (pMOT), straight-line velocity (VSL: straight-line distance traveled by the sperm head in unit time), curvilinear velocity (VCL: the 2-dimensional track described by the sperm head in unit time), amplitude of lateral head displacement (ALH: the amplitude of the lateral sperm head displacement in micrometers), linearity (LIN), and beat-cross frequency (BCF). It was found that spermatozoa in the higher Percoll gradients have higher scores in almost all the above motility parameters (Kao et al. 1995), which indicates that the self-migration method is valid for separation of human spermatozoa with different motilities.

fertile patients, we frequently observed some less abundant deletions of mtDNA. These minor deletions and the 3 aforementioned major deletions may exist alone or in different combinations with varying proportions in spermatozoa. It remains to be investigated whether these mtDNA deletions are localized in the same mitochondria (intra-mitochondrial heteroplasmy) or in different organelles (inter-mitochondrial heteroplasmy) of spermatozoa harboring mutated mtDNA molecules.

By using the PCR-RFLP (restriction fragment length polymorphism) techniques, we screened for several commonly found pathogenic point mutations of mtDNA in spermatozoa with poor motility or in spermatozoa from patients with infertility (Kao 1998). Among these mutations, only very low levels (< 0.1%) of the MELAS-specific A3243G mutation of mtDNA were found in some spermatozoa with poor motility (Kao and Wei, unpubl. observ.). However, we cannot rule out the possibility that some novel point mutations of mtDNA are present in some of the spermatozoa with poor motility or in spermatozoa of infertile males. It is worth mentioning that we detected about 38% of mtDNA with the A3243G mutation in one of the asymptomatic maternal relatives of a male patient with full-blown MELAS syndrome (Huang et al. 1994). This adult male patient harbored about 90% of mtDNA with the A3243G mutation in muscle and had defective gonad function and impaired spermatogenesis. This may be one of the muscular dystrophy-related secondary clinical manifestations of the MELAS syndrome and other mitochondrial myopathies.

MITOCHONDRIAL DNA DEPLETION IN HUMAN SPERM

In addition to qualitative changes (e.g., mutation), a reduction in the copy number of mtDNA (a condition termed mtDNA depletion) has also been observed in affected tissues of some patients having distinct mitochondrial diseases. Moraes et al. (1991) reported that mtDNA was depleted to varying degrees in different tissues of a patient with fatal mitochondrial disease in early infancy. In the following year, Mazziotta et al. (1992) demonstrated in another patient that mtDNA depletion is associated with fatal infantile liver failure. Tritschler and coworkers (1992) also showed that in some patients mitochondrial myopathy can be caused by, or is associated with, mtDNA depletion. Macmillan and Shoubridge (1996) reported that mtDNA depletion is prevalent in a Canadian pediatric population referred to them for neurologic evaluation. No pathogenic mtDNA mutations were found in the patients, and there was evidence of autosomal recessive inheritance of the diseases in some families. These observations have led to the suggestion that the gene(s) responsible for mtDNA depletion is encoded by the nuclear genome (Taanman et al. 1997, Zeviani et al. 1997).

Since spermatozoa need to have a suitable number of functioning mitochondria to supply energy for motility and fertilization capability, we have recently investigated whether mtDNA depletion is associated with decreased motility and diminished fertility of human sperm. By using a hot-start concurrent PCR technique, we determined the ratio between the DNA band intensity of the PCR product of the ND1 gene of mtDNA and that of the β-actin gene of nuclear DNA for each sperm sample (Kao 1998). This ratio is an index of the relative amount (copy number) of mtDNA with respect to nuclear DNA. The results showed that there is a positive correlation between the mtDNA copy number and sperm motility determined by the CASA system (Kao et al. 2000). When the sperm samples from 85 male subjects were divided into 4 groups with sperm motilities of < 20%, 21%-40%, 41%-60%, and > 60%, we found that the average copy numbers of mtDNA per spermatozoon were 7.2 ± 1.3, 32.4 ± 1.4, 48.4 ± 1.2, and 74.1 ± 2.0, respectively. Moreover, the scores of

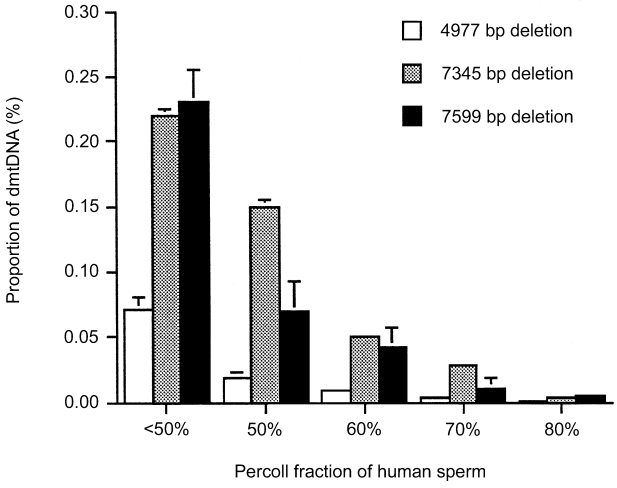

Fig. 4. Proportions of mtDNA molecules with 3 large-scale deletions in different sperm fractions separated by Percoll gradients. The average proportions of the 4977 bp-deleted, 7345 bp-deleted, and 7599 bp-deleted mtDNAs of spermatozoa in each of the 5 fractions are presented by blank bars, dotted bars, and black bars, respectively. Spermatozoa were fractionated by the self-migation method as described in figure 2, and the proportions of deleted mtDNAs were determined by the semi-quantitative PCR method as previously described (Kao et al. 1998).

a number of motility parameters, including pMOT, BCF, LIN, and ALH, were also found to correlate positively with the copy number of mtDNA in spermatozoa (Kao 1998, Kao et al. 2000). We also noted that spermatozoa from infertile or subfertile patients had significantly lower copy numbers of mtDNA as compared with fertile males.

In order to determine whether the decline in the copy number of mtDNA is caused by a decrease in the number of mitochondria, we used a transmission electron microscope to examine the longitudinal ultrastructure of a small number of spermatozoa from several patients with asthenospermia. The results showed that the subcellular structures and morphologies of intracellular organelles including acrosomes, nuclei, mitochondria, and axonemes were apparently normal. Upon electron microscopic examination of representative spermatozoa from several patients with asthenospermia, we found that the average copy number of mtDNA was about 0.9 per spermatozoon (close to the normal value of 1.0). These findings suggest that depletion of mtDNA in spermatozoa with poor motility and diminished fertility is not due to a paucity of mitochondria but is caused by a lack of the mitochondrial genome per se.

Although the mechanism of mtDNA depletion is unclear, it is thought that mtDNA depletion is caused by mutations in the mitochondrial replication machinery. However, no mutations have been found in the D-loop region of mtDNA, which controls the replication of mtDNA, in patients with the mtDNA depletion syndrome. In addition, mitochondrial transcription factor A (mtTFA), mitochondrial single-strand DNA-binding protein, endonuclease G, and DNA polymerase γ have been demonstrated to be involved in the regulation of mtDNA replication. These proteins or enzymes have been proposed to play a role in controlling the copy number of mtDNA (Spelbrink et al. 1998). Nuclear involvement in mtDNA depletion was established by observations that mtDNA populations can be restored in vitro by fusion of ρ° cells (mtDNA-less cells) with enucleated cultured cells from patients with mtDNA depletion syndrome (Bentlage and Attardi 1996, Taanman et al. 1997). Reduction in the level of mtTFA was observed in muscle fibers of patients with mtDNA depletion syndrome (Larsson et al. 1994, Poulton et al. 1994). MtTFA has been suggested to play an important role in the regulation of replication and transcription of mtDNA, affecting the tight coupling between DNA replication and gene expression in mitochondria. Moreover, it was demonstrated that the expression of mtTFA is down-regulated during spermatogenesis in humans (Larsson et al. 1997). Depletion of mtDNA may arise spontaneously under genetic pressure during spermatogenesis by defective replication of mtDNA resulting from a paucity of mtTFA or poor communication between the nuclear and mitochondrial genomes.

OTHER ABERRATIONS OF MITOCHONDRIAL DNA IN HUMAN SPERM

Since the reported mtDNA mutations in spermatozoa are mostly, if not exclusively, those detected by PCR using primers specifying a segment of mtDNA encompassing only a small number of putative deletions or point mutations, some other mutations may exist that have been undetected. Recently, Reynier et al. (1998) screened for large-scale deletions of mtDNA in 94 sperm samples with the long PCR technique followed by Southern hybridization. They found that about 85% of sperm samples contained large-scale mtDNA deletions of variable sizes, and that most spermatozoa had 2 to 7 deletions of mtDNA. Furthermore, they noted that these mtDNA deletions are similar to those observed in skeletal muscle, myocardium, and other tissues of aged individuals. The mtDNA mutations detected so far may just represent the “tip of the iceberg” of all possible mutations in spermatozoa. Interestingly, these investigators detected a smaller number of distinctive mtDNA deletions in mature oocytes. Moreover, different oocytes from the same donor were found to harbor different sets of mtDNA deletions. These findings suggest that pre-existing mtDNA molecules with large-scale deletions are preferentially retained or selected after the reduction/amplification stage of mtDNA in early oogenesis. They concluded that mtDNA deletions occur more frequently in human spermatozoa than in oocytes.

It is possible that mtDNA mutations are elicited during the process of spermatogenesis. During cell division, mtDNA molecules must be amplified and randomly segregated into daughter cells. As discussed above, during replication mtDNA exists transiently as a three-strand intermediate that is prone to large-scale deletions via slippage-mispairing (Shoffner et al. 1989, Larsson and Clayton 1995). At this stage, the mitochondrial genome is also exposed to ROS, and mutation may be induced if intracellular levels of antioxidants are insufficient for protection of spermatozoa from oxidative damage (Cummins et al. 1994). Indeed, we found that the intracellular content of 8-OH-dG in spermatozoa with poor motility was significantly higher than that in mo-

tile spermatozoa (Kao 1998). The ratios between 8-OH-dG and dG (in units of 10^{-3}%) of DNA in spermatozoa with motility scores of 0%-30%, 31%-60%, and 61%-90% were determined to be 9.60 ± 0.01 (n = 12), 6.25 ± 0.42 (n = 19), and 4.63 ± 0.49 (n = 7), respectively. In addition, the level of 8-OH-dG in total DNA (composed of mtDNA and nuclear DNA) was found to correlate with lipid peroxide content in seminal plasma or spermatozoa (Kao 1998). These findings suggest that spermatozoa with poor motility have been more severely damaged by ROS, free radicals, or lipid peroxides before or after ejaculation. This notion is supported by our recent finding that concentrations of ascorbate in seminal plasma and α-tocopherol in spermatozoa and seminal plasma are positively correlated with sperm motility and are negatively correlated with 8-OH-dG content of spermatozoa (Kao 1998). On the other hand, we also noted a positive correlation between the level of mtDNA with the 4977-bp deletion and the specific content of 8-OH-dG in spermatozoa. This indicates that ROS-elicited oxidative damage to DNA may be fixed as large-scale deletions of mtDNA in sperm mitochondria. This is consistent with our recent findings that large-scale deletions of mtDNA occur much more frequently in the hot regions with bent or antibent DNA structures (Hou and Wei 1996 1998). However, it remains to be elucidated how oxidative damage to mtDNA in sperm leads to large-scale deletions of the mitochondrial genome in vivo.

DISCUSSION AND CONCLUSIONS

Since sperm require a substantial amount of energy to swim fast enough to reach the oviduct during fertilization, the appropriate bioenergetic function of mitochondria is critical for male fertility (Tombes and Shapiro 1985, de Lamirande and Gagnon 1992). In the active respiration state, as much as 5% of the oxygen utilized by mitochondria is converted to superoxide anions and other ROS. Upon enzymatic action of superoxide dismutase, superoxide anions are converted to hydrogen peroxide (H_2O_2), which cannot be further disposed of by spermatozoa due to a lack of catalase and glutathione peroxidase (Aitken et al. 1992). Thus, H_2O_2 accumulates and is easily converted to hydroxyl radicals in the presence of divalent metal ions via the Fenton reaction. MtDNA molecules, which are transiently attached to the inner membrane of mitochondria, are thus extremely vulnerable to oxidative damage under active respiration of spermatozoa (Fraga et al. 1996). Aitken and coworkers (1989) showed that excess amounts of ROS and free radicals in spermatozoa and seminal plasma have adverse effects on sperm motility and fertility. We have also demonstrated that oxidative damage to lipids and DNA of spermatozoa is associated with declining motility and diminishing fertility of human sperm (Chen et al. 1997, Kao 1998). Oxidative damage to mtDNA has been shown to be much higher than that to nuclear DNA in human and animal tissues (Richter et al. 1988, Yakes and van Houten 1997). As mtDNA is extensively damaged by ROS or free radicals, DNA strand breaks and large-scale deletions may be induced (Nagley and Wei 1998, Lu et al. 1999). Indeed, we detected the common 4977-bp deletion and 2 novel deletions of 7345 bp and 7599 bp in mtDNA from spermatozoa with poor motility and diminished fertility (Kao et al. 1998). These large-scale deletions result in complete removal or truncation of some structural genes and tRNA genes of mtDNA. The defective protein subunits encoded by such kinds of mutated mtDNA are assembled with nuclear DNA-encoded subunits to yield impaired respiratory enzymes. Spermatozoa containing defective mitochondria not only produce ATP less efficiently but also generate more ROS and free radicals, which may further damage mitochondria and mtDNA leading to an ultimate energy crisis and decline of motility and fertility (Fig. 5).

Previous studies have shown that mtDNA and mitochondria are more abundant in tissues with higher energy demand, such as heart and skeletal muscle (Robin and Wong 1990, Veltri et al. 1990). The change of sperm mtDNA copy number is a very important event throughout spermatogenesis, fertilization, and embryogenesis. Recently, it was demonstrated that expression of mtTFA is reduced in male germ cells during spermatogenesis in human, and sperm mtDNA is subsequently reduced (Larsson et al. 1997). However, over-reduction in the number of sperm mtDNA during or after spermatogenesis may be detrimental to spermatozoa. We have demonstrated for the first time that depletion of sperm mtDNA is an important contributory factor in diminished sperm motility and fertility. However, the number of mitochondria per se was found to be maintained within a normal range in spermatozoa with reduced mtDNA copy number (Kao et al. 2000). This finding indicates that mtDNA depletion is not caused by a defect in mitochondrial biogenesis. This is in agreement with the observation that the number of mitochondria remains constant but the number of mtDNA molecules per mitochondrion is altered under different pathophysiological conditions (Larsson et al. 1994, Bentlage and Attardi 1996).

A number of studies have demonstrated that

mtDNA mutations (deletions and rearrangements) occur frequently in sperm with poor motility (Kao et al. 1995, Lestienne et al. 1997, Kao et al. 1998). This may possibly be related to changes of the microenvironment (e.g., free radical levels, oxygen pressure, and toxic metabolites of xenobiotics or cigarette smoke) of the germ cells during or after spermatogenesis. It is noteworthy that exogenous sources of ROS and free radicals can aggravate oxidative damage and mutations of mtDNA in human tissues (Yang et al. 1995, Fahn et al. 1998). On the other hand, mutated mtDNA molecules may be propagated by defective nuclear factors from paternal or maternal germ cells, be clonally expanded during the maturation of oocytes, and be localized in primordial germ cytoplasm during embryogenesis (Jenuth et al. 1996, Poulton et al. 1998). It is also possible that mtDNA molecules with oxidative damage or mutation are unstable, and are quickly degraded resulting in a decrease of mtDNA copy number in spermatozoa with poor motility. This notion is supported by recent findings that the relative content of mtDNA in lung tissue is significantly reduced in heavy smokers (Lee et al. 1998). In conclusion, recent studies from this and other laboratories have clearly demonstrated that mutation and depletion of mtDNA do play important roles in the diminution of fertility and decline of various motility parameters that are important determinants of male fertility.

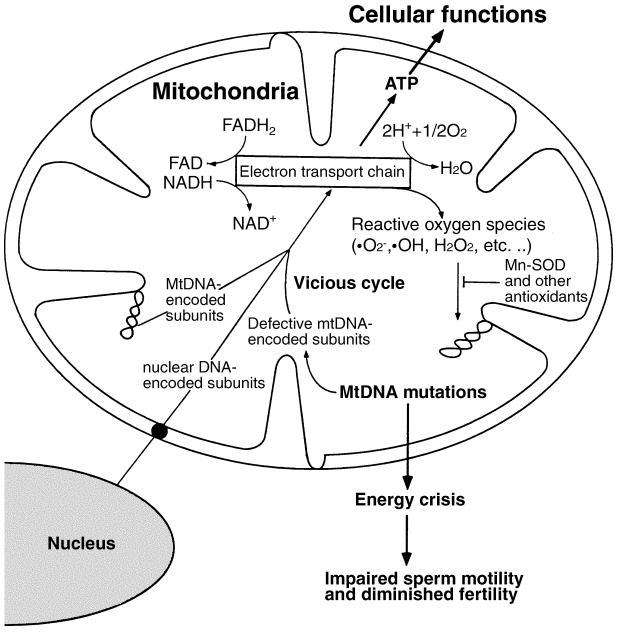

Fig. 5. A vicious cycle of mitochondrial DNA mutations and its ultimate effect on sperm motility and fertility. The electron transport chain (ETC), which is composed of mtDNA-encoded and nuclear DNA-encoded protein subunits, is actively involved in oxidative phosphorylation during fast movement of spermatozoa. About 1%-5% of the oxygen consumed in this process is converted to ROS, which are usually disposed of by Mn-SOD and other antioxidant systems. However, a certain fraction of the ROS may escape the defense system, a result which is more pronounced in defective spermatozoa, and cause oxidative damage to lipids, proteins, and nucleic acids in mitochondria. The oxidatively modified and/or mutated mtDNAs are transcribed and translated to produce defective protein subunits that may assemble to form defective respiratory enzymes. The impaired ETC system not only produces ATP less efficiently but also generates more ROS, which further enhance the oxidative stress and elicit damage to mitochondria. This vicious cycle is accelerated when oxygen consumption is increased, and if the ROS are not efficiently disposed of during active respiration, mutations and oxidative damages may accumulate to a point that the energy metabolism of mitochondria is insufficient to maintain normal functioning of spermatozoa. Under such conditions, sperm motility will significantly decline and fertility will diminish.

Acknowledgments: The authors would like to express their gratitude to the National Science Council of the Republic of China for generous support of research work on sperm mitochondrial DNA mutation and depletion in male infertility.

REFERENCES

Aitken RJ, DW Buckingham, KM West. 1992. Reactive oxygen species and human spermatozoa: analysis of the cellular mechanisms involved in luminol- and lucigenin-dependent chemiluminescence. J. Cell Physiol. **151:** 466-477.

Aitken RJ, JS Clarkson, S Fisfel. 1989. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol. Reprod. **40:** 183-197.

Alcivar AA, LE Hake, CF Millette, JM Transler, NB Hecht. 1989. Mitochondrial gene expression in male germ cells of the mouse. Dev. Biol. **135:** 263-271.

Anderson S, AT Bankier, BG Barrell, MHL de Bruijn, AR Coulson, J Drouin, IC Eperon, DP Nierlich, BA Roe, F Sanger, PH Schreier, AJH Smith, R Staden, IG Young. 1981. Sequence and organization of the human mitochondrial genome. Nature **290:** 457-465.

Attardi G, A Chomyn, MP King, B Kruse, PL Polosa, NN Murdter. 1989. Regulation of mitochondrial gene expression in mammalian cells. Biochem. Soc. Trans. **18:** 509-513.

Attardi G, G Schatz. 1988. Biogenesis of mitochondria. Annu. Rev. Cell Biol. **4:** 289-333.

Ballinger SW, JM Shoffner, S Gebhart, DA Koontz, DC Wallace. 1994. Mitochondrial diabetes revisited. Nature Genet. **7:** 458-459.

Beckman KB, BN Ames. 1997. Oxidative decay of DNA. J. Biol. Chem. **272:** 19633-19636.

Bentlage HACM, G Attardi. 1996. Relationship of genotype to phenotype in fibroblast-derived transmitochondrial cell lines carrying the 3243 mutation associated with the MELAS encephalomyopathy: shift towards mutant genotype and role of mtDNA copy number. Hum. Mol. Genet. **5:** 197-205.

Cardullo RA, JM Baltz. 1991. Metabolic regulation in mammalian

sperm: mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motil. Cytoskel. **10:** 180-188.

Case JT, DC Wallace. 1981. Maternal inheritance of mitochondrial DNA polymorphisms in cultural human fibroblasts. Somat. Cell Mol. Genet. **7:** 103-108.

Chance B, H Sies, A Boveris. 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. **59:** 527-603.

Chen CS, HT Chao, RL Pan, YH Wei. 1997. Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm. Biochem. Mol. Biol. Int. **43:** 291-303.

Clayton DA. 1992. Transcription and replication of animal mitochondrial DNA. Int. Rev. Cytol. **141:** 217-232.

Clayton DA, JN Doda, EC Friedberg. 1974. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. Proc. Natl. Acad. Sci. USA **71:** 2777-2781.

Croteau DL, VA Bohr. 1997. Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. J. Biol. Chem. **272:** 25409-25412.

Cummins JM, AM Jequier, R Kan. 1994. Molecular biology of human male infertility: links with ageing, mitochondrial genetics, and oxidative stress? Mol. Reprod. Dev. **37:** 345-362.

de Lamirande E, C Gagnon. 1992. Reactive oxygen species and human spermatozoa II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J. Androl. **13:** 379-377.

DeMartino C, A Florido, ML Marcante, W Malorni, S Barcellona, M Belloci, B Silvestrini. 1979. Morphological, histochemical and biochemical studies on germ cell mitochondria of normal rats. Cell Tissue Res. **196:** 1-22.

Driggers WJ, VI Grishko, SP LeDoux, GL Wilson. 1996. Defective repair of oxidative damage in the mitochondrial DNA of a *Xeroderma pigmentosum* group A cell line. Cancer Res. **56:** 1262-1266.

Fahn HJ, LS Wang, RH Hsieh, SC Chang, SH Kao, MH Huang, YH Wei. 1996. Age-related 4977 bp deletion in human lung mitochondrial DNA. Am. J. Respir. Crit. Care Med. **154:** 1141-1145.

Fahn HJ, LS Wang, SH Kao, SC Chang, MH Huang, YH Wei. 1998. Smoking-associated mitochondrial DNA mutations and lipid peroxidation in human lung tissues. Am. J. Respir. Cell Mol. Biol. **19:** 901-909.

Fraga CG, PA Motchnik, MK Shigenaga, HJ Helbock, RA Jacob, BN Ames. 1991. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc. Natl. Acad. Sci. USA **88:** 11003-11006.

Fraga CG, PA Motchnik, AJ Wyrobek, DM Rempel, BN Ames. 1996. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. Mutat. Res. **351:** 199-203.

Giles RE, H Blanc, HM Cann, DC Wallace. 1980. Maternal inheritance of human mitochondrial DNA. Proc. Natl. Acad. Sci. USA **77:** 6715-6719.

Goto Y, I Nonaka, S Horai. 1990. A mutation in the transfer $RNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathy. Nature **348:** 651-653.

Goto Y, I Nonaka, S Horai. 1991. A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). Biochim. Biophys. Acta **1097:** 238-240.

Gray MW. 1989. Origin and evolution of mitochondrial DNA. Annu. Rev. Cell Biol. **5:** 25-50.

Hecht N, H Liem, KC Kleene, RJ Distel, SM Ho. 1984. Maternal inheritance of the mouse mitochondrial genome is not mediated by a loss or gross alteration of the paternal mitochondrial DNA or by methylation of the oocyte mitochondrial DNA. Dev. Biol. **102:** 452-461.

Holt IJ, AE Harding, A Morgan-Hughes. 1988. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature **331:** 717-719.

Hou JH, YH Wei. 1996. The unusual DNA structures of the hot-regions flanking large-scale deletions in human mitochondrial DNA. Biochem. J. **318:** 1065-1070.

Hou JH, YH Wei. 1998. AT-rich sequences flanking the 5'-end breakpoint of the 4977-bp deletion of human mitochondrial DNA are located between two bent-inducing DNA sequences that assume distorted structure in organello. Mutat. Res. **403:** 75-84.

Huang CC, RS Chen, CM Chen, HS Wang, CC Lee, CY Pang, HS Hsu, HC Lee, YH Wei. 1994. MELAS syndrome with mitochondrial $tRNA^{Leu(UUR)}$ gene mutation in a Chinese family. J. Neurol. Neurosurg. Psychiatry **57:** 586-589.

Huang CC, RS Chen, NS Chu, CY Pang, YH Wei. 1996. Random mitotic segregation of mitochondrial DNA in MELAS syndrome. Acta Neurol. Scand. **93:** 198-202.

Huang CC, CC Chu, CY Pang, YH Wei. 1999. Tissue mosaicism in the skeletal muscle and sural nerve biopsies in the MELAS syndrome. Acta Neurol. Scand. **99:** 125-129.

Jenuth J, A Peterson, K Fu, E Shoubridge. 1996. Random genetic drift in female germline explains the rapid segregation of mammalian mitochondrial DNA. Nature Genet. **14:** 146-151.

Kao SH. 1998. Mitochondrial DNA mutation and depletion in human sperm with declined motility and fertility. Ph.D. dissertation, Institute of Biochemistry, National Yang-Ming Univ., Taipei.

Kao SH, HT Chao, HW Liu, YH Wei. 2000. Mitochondrial deoxyribonucleic acid depletion is associated with the decline of fertility and motility of human sperm. Biol. Reprod., (revised).

Kao SH, HT Chao, YH Wei. 1995. Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm. Biol. Reprod. **52:** 729-736.

Kao SH, HT Chao, YH 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.

Larsson NG, DA Clayton. 1995. Molecular genetic aspects of human mitochondrial disorders. Annu. Rev. Genet. **29:** 151-178.

Larsson NG, A Oldfors, JD Garman, GS Barsh, DA Clayton. 1997. Down-regulation of mitochondrial transcription factor A during spermatogenesis in humans. Hum. Mol. Genet. **6:** 185-191.

Larsson NG, A Oldfors, E Holme, DA Clayton. 1994. Low levels of mitochondrial transcription factor A in mitochondrial DNA depletion. Biochem. Biophys. Res. Commun. **200:** 1374-1381.

Lee HC, CY Lu, HJ Fahn, YH Wei. 1998. Aging- and smoking-associated alteration in the relative content of mitochondrial DNA in human lung. FEBS Lett. **441:** 292-296.

Lestienne P, N Bataille. 1994. Mitochondrial DNA alterations and genetic diseases: a review. Biomed. Pharmacother. **48:** 199-214.

Lestienne P, P Reynier, MF Chretien, I Penisson-Besnier, Y Malthiery, V Rohmer. 1997. Oligoasthenospermia associated with multiple mitochondrial DNA rearrangements.

Mol. Hum. Reprod. **3:** 811-814.

Li JY, KW Kong, MH Chang, SC Cheung, HC Lee, CY Pang, YH Wei. 1996. MELAS syndrome associated with a tandem duplication in the D-loop of mitochondrial DNA. Acta Neurol. Scand. **93:** 450-455.

Linnane AW, S Marzuki, T Ozawa, M Tanaka. 1989. Mitochondrial DNA mutation as an important contributor to ageing and degenerative diseases. Lancet **i:** 642-645.

Lu CY, HC Lee, HJ Fahn, YH Wei. 1999. Oxidative damage elicited by imbalance of free radical scavenging enzymes is associated with large-scale mtDNA deletions in aging human skin. Mutat. Res. **423:** 11-21.

Macmillan CJ, EA Shoubridge. 1996. Mitochondrial DNA depletion: prevalence in a pediatric population referred for neurologic evaluation. Pediatr. Neurol. **14:** 203-210.

Mazziotta M, E Ricci, E Bertini, CD Vici, S Sewidei, AB Burlina, G Sabetta, A Bartuli, G Manfredi, G Silvestri. 1992. Fatal infantile liver failure associated with mitochondrial DNA depletion. J. Pediatr. **121:** 896-901.

Merriwether DA, AG Clark, SW Ballinger, TG Schurr, H Soodyall, T Jenkins, ST Sheny, DC Wallace. 1991. The structure of human mitochondrial DNA variation. J. Mol. Evol. **33:** 543-555.

Michaels GS, WW Hauswirth, PJ Laipis. 1982. Mitochondrial DNA copy number in bovine oocytes and somatic cells. Dev. Biol. **94:** 246-251.

Moraes CT, S Shanske, HJ Tritschler, JR Aprille, F Andreetta, E Bonilla, EA Schon, S DiMauro. 1991. MtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. Am. J. Hum. Genet. **48:** 492-501.

Nagley P, YH Wei. 1998. Ageing and mammalian mitochondrial genetics. Trends Genet. **14:** 513-517.

Pang CY, CC Huang, MY Yen, EK Wang, KP Kao, SS Chen, YH Wei. 1999. Molecular epidemiological study of mitochondrial DNA mutations in patients with mitochondrial diseases in Taiwan. J. Formos. Med. Assoc. **98:** 326-334.

Piko L, KJ Bulpitt, R Meyer. 1984. Structural and replicative forms of mitochondrial DNA in tissues from adult and senescent Blab/c mice and Fischer 344 rats. Mech. Ageing Dev. **26:** 113-131.

Piko L, AJ Hougham, KJ Bulpitt. 1988. Studies of sequence heterogeneity of mitochondrial DNA from rat and mouse tissues: evidence for an increased frequency of deletions/additions with aging. Mech. Ageing Dev. **43:** 279-293.

Piko L, R Meyer, J Eipe, N Costea. 1978. Structural and replicative forms of mitochondrial DNA from human leukocytes in relation to age. Mech. Ageing Dev. **7:** 351-365.

Pons R, F Andretta, CH Wang, TH Vu, E Bonilla, S DiMauro, DC DeVivo. 1996. Mitochondrial myopathy stimulating spinal muscular atrophy. Pediatr. Neurol. **15:** 153-158.

Poulton J. 1992. Duplications of mitochondrial DNA: implications for pathogenesis. J. Inherit. Metab. Dis. **15:** 487-498.

Poulton J, IJ Holt. 1994. Mitochondrial DNA: does more lead to less? Nature Genet. **8:** 313-314.

Poulton J, V Macaulay, DR Marchington. 1998. Mitochondrial genetics '98: Is the bottleneck cracked? Am. J. Hum. Genet. **62:** 752-757.

Poulton J, K Morten, C Freeman-Emmerson, C Potter, C Sewry, V Dibowitz, H Kidd, J Stephenson, W Whitehouse, FJ Hansen, M Parisi, G Brown. 1994. Deficiency of the human mitochondrial transcription factor h-mtTFA in infantile mitochondrial myopathy is associated with mtDNA depletion. Hum. Mol. Genet. **3:** 1763-1769.

Poyton RO, JE McEwen. 1996. Crosstalk between nuclear and mitochondrial genomes. Annu. Rev. Biochem. **65:** 563-607.

Reynier P, MF Chrétien, F Savagner, G Larcher, V Rohmer, P Barriere, Y Malthiery. 1998. Long PCR analysis of human gamete mtDNA suggests defective mitochondrial maintenance in spermatozoa and supports the bottleneck theory for oocytes. Biochem. Biophys. Res. Commun. **252:** 373-377.

Richter C, V Gogvadze, R Laffranchi, R Schlapbach, M Schnizer, M Suter, P Walter, M Yaffee. 1995. Oxidants in mitochondria: from physiology to disease. Biochim. Biophys. Acta **1271:** 67-74.

Richter C, JW Park, BN Ames. 1988. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc. Natl. Acad. Sci. USA **85:** 6465-6467.

Robin ED, R Wong. 1990. Mitochondrial DNA molecules and virtual number of mitochondria per cells in mammalian cells. J. Cell. Physiol. **136:** 507-513.

Rötig A, V Cormier, S Blanche, JP Bonnefont, F Ledeist, N Romero, J Schmitz, P Rustin, A Fischer, JM Saudubray, A Munnich. 1990. Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. J. Clin. Invest. **86:** 1601-1608.

Santorelli FM, S Shanske, A Macaya, DC DeVivo, S DiMauro. 1993. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. Ann. Neurol. **34:** 827-834.

Seitz J, J Mobius, M Bergmann, A Meinhardt. 1995. Mitochondrial differentiation during meiosis of male germ cells. Int. J. Androl. **18:** 7-11.

Shearman CW, GF Kalf. 1977. DNA replication by a membrane DNA complex from rat liver mitochondria. Arch. Biochem. Biophys. **182:** 573-586.

Sherratt EJ, AW Thomas, JC Alcolado. 1997. Mitochondrial DNA defects: a widening clinical spectrum of disorders. Clin. Sci. **92:** 225-235.

Shih KD. 1996. Studies on the major and minor mitochondrial DNA mutations associated with MERRF disease. Ph.D. dissertation, Institute of Biochemistry, National Yang-Ming Univ., Taipei.

Shoffner JM, MT Lott, AMS Lezza, P Seibel, SW Ballinger, DC Wallace. 1990. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. Cell **61:** 931-937.

Shoffner JM, MT Lott, AS Voljavec, SA Soueidan, DA Costigan, DC Wallace. 1989. Spontaneous Kearns-Sayre/chronic progressive external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. Proc. Natl. Acad. Sci. USA **86:** 7952-7956.

Spelbrink JN, MJ van Galen, R Zwart, HD Bakker, A Rovio, HT Jacobs, C van den Bogert. 1998. Familial mitochondrial DNA depletion in liver: haplotype analysis of candidate genes. Hum. Genet. **102:** 327-331.

Taanman JW, AG Bodnar, JM Cooper, AAM Morris, PT Clayton, JV Leonard, AHV Schapira. 1997. Molecular mechanisms in mitochondrial DNA depletion syndrome. Hum. Mol. Genet. **6:** 935-942.

Tombes RM, BM Shapiro. 1985. Metabolite channeling: a phosphoryl-creatine shuttle to mediate high energy phosphate transport between sperm mitochondrion and tail. Cell **41:** 325-334.

Tomkinson AE, RT Bonk, J Kim, N Bartfel, S Linn. 1990. Mammalian mitochondrial endonuclease activities specific for

ultraviolet-irradiated DNA. Nucleic Acids Res. **18:** 929-935.
Tritschler HJ, F Andretta, CT Moraes, E Bonilla, E Arnaudo, MJ Danon, S Glass, BM Zelaya, E Vamos, N Telerman-Toppet, S Shanske, B Kadenbach, S DiMauro, EA Schon. 1992. Mitochondrial myopathy of childhood associated with depletion of mitochondrial DNA. Neurology **42:** 209-217.
Veltri KL, M Espiritu, G Singh. 1990. Distinct genomic copy number in mitochondria of different mammalian organs. J. Cell. Physiol. **143:** 160-164.
Wallace DC. 1992. Disease of the mitochondrial DNA. Annu. Rev. Biochem. **61:** 1175-1212.
Wallace DC. 1994. Mitochondrial DNA sequence variation in human evolution and disease. Proc. Natl. Acad. Sci. USA **91:** 8739-8746.
Wallace DC. 1999. Mitochondrial diseases in man and mouse. Science **283:** 1482-1488.
Wallace DC, G Singh, MT Lott, JM Shoffner, JA Hodge, TG Schurr, AMS Lezza, LJ Elsas II, EK Nikoskelainen. 1988. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science **242:** 1427-1430.
Wei YH. 1992. Mitochondrial DNA alterations as ageing-associated molecular events. Mutat. Res. **275:** 145-155.
Wei YH. 1998. Oxidative stress and mitochondrial DNA mutations in human aging. Proc. Soc. Exp. Biol. Med. **217:** 53-63.
Wei YH, CY Lu, HC Lee, CY Pang, YS Ma. 1998. Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. Ann. NY Acad. Sci. **854:** 155-170.
Yakes FM, B van Houten. 1997. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc. Natl. Acad. Sci. USA **94:** 514-519.
Yang JH, HC Lee, YH Wei. 1995. Photoageing-associated mitochondrial DNA length mutations in human skin. Arch. Dermatol. Res. **287:** 641-648.
Yen TC, JH Su, KL King, YH Wei. 1991. Ageing-associated 5 kb deletion in human liver mitochondrial DNA. Biochem. Biophys. Res. Commun. **178:** 124-131.
Zeviani M, P Fernandez-Silva, V Tiranti. 1997. Disorders of mitochondria and related metabolism. Curr. Opin. Neurol. **10:** 160-167.
Zeviani M, CT Moraes, S DiMauro, H Nakase, E Bonilla, EA Schon, LP Rowland. 1988. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. Neurology **38:** 1339-1346.
Zhang C, VWS Liu, CL Addessi, DA Sheffield, AW Linnane, P Nagley. 1998. Differential occurrence of mutations in mitochondrial DNA of human skeletal muscle during aging. Hum. Mutat. **11:** 360-371.

與人類精子運動活力及授孕力低落相關之粒線體 DNA 突變與減量

魏耀揮[1,2] 高淑慧[1]

精子運動活力是決定男性生殖力的重要因子。在這一篇回顧性論文中，我們對最近發現之粒線體 DNA 突變和減量與精子運動活力及授孕力低落的相關性作一討論。我們是以聚合酵素鏈鎖反應(PCR)技術及 DNA 定序法偵測並確立精子的粒線體 DNA 突變，而粒線體 DNA 的拷貝數則以粒線體 DNA 和核 DNA 量的比值計算之。運用這些分子生物學技術，我們率先於運動活力及授孕力低落的精子細胞發現一個常見的 4977 bp 粒線體 DNA 缺失突變和兩個新的 7345 bp 及 7599 bp 粒線體 DNA 缺失突變。這三種粒線體 DNA 缺失突變的發生頻率及含量在運動活力及授孕力低落的精子細胞中有明顯增加的現象。另一方面，我們發現運動活力較差的精子細胞其所含的粒線體 DNA 量較少。以電腦輔助分析儀測得之數種精子運動活力指標分數也與粒線體 DNA 的拷貝數呈正相關。再者，我們以穿透式電子顯微鏡觀察來自不孕症男性之含粒線體 DNA 量較少的精子細胞。我們發現超過百分之七十的精子細胞擁有正常螺旋柱狀粒線體的中段結構。這些結果顯示粒線體 DNA 減量的精子細胞並不是含較少的粒線體，而是各個粒線體所含之粒線體 DNA 分子的數目較少。基於以上這些新發現，我們認為精子細胞的粒線體 DNA 突變和減量可能在某些男性不孕症的致病機轉扮演一重要的角色。

關鍵詞：粒線體， DNA 突變，精子運動活力，不孕症。

[1] 國立陽明大學生物化學研究所
[2] 國立陽明大學細胞暨分子生物學研究中心