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Male Infertility: Role of Cellular, Biochemical and Molecular Events

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Abstract

Infertility is a unique category of human functional disability, where the fertility disturbances of one partner may only become evident through the other partner's problem, while optimal reproductive function in one partner may compensate for impaired function in the other. It is estimated that approximately 15% of married couples are infertile. Male factor infertility contributes to about 50% manifested as quantitative abnormality (azoospermia, cryptozoospermia and oligozoospermia), or as qualitative abnormality (asthenospermia, teratozoospermia and necrospermia) or both. Investigation of apoptosis in spermatogonia, spermatocytes and spermatids in the testis has been done extensively and many apoptotic factors have been identified. Testicular apoptosis has been reported in human specimens, but its correlation with serum gonadotropins and testosterone levels is not clear. It is assumed that somatic cells can die in the apoptotic, the autophagic, or the necrotic way but the mechanisms involved in the sperm death are obscure and the biological significance of apoptosis in ejaculated sperm is yet to be elucidated. Thus the goal of this article is to identify and discuss common themes in mitochondrial function and the apoptotic pathway related to mammalian reproduction. Apart from this finding the correlation of apoptosis with serum gonadotropins and testosterone levels will also help in correlating the processes taking place in spermatozoa responsible for male infertility.

Keywords: Apoptosis, spermatogenesis, gonadotropins, mitochondrial DNA

Introduction

Unwilling childlessness in the couples in spite of having regular unprotected sex within a certain period (12 months or more) of time can be defined as infertility.

Approximately 15% of sexually active couples, not using any contraception are unable to conceive. In about half of them male factor is the sole cause or contributes to the infertility problem. The main causes of this disorder for men are associated with various factors, among them are genetic, physiopathologic and anatomopathologic abnormalities, intense and prolonged physical exercises, aging, drugs, and even excessive time of sexual abstinence.

It was assumed earlier that most of genes regulating spermatogenesis are present on Y chromosome and major focus of infertility studies was Y chromosome. Eventually Y chromosomal aberrations have explained 15-20% cases of the infertile cases. The unexplained etiology in the remaining 80-85% of the cases provoked further research on the role of other genes in infertility. Involvement of autosomal genes in spermatogenesis is not surprising as it is not a mere play of spermatogenic genes on Y chromosome.

Spermatogenesis is a complex biological process which depends on a precisely controlled cascade of developmental genes orchestrating spermatogonial cell proliferation, chromosomal reduction divisions to produce a haploid genome in each daughter cell and finally, morphological differentiation of these latter cells into mature sperm. Mutations in any of the genes in this pathway can have a profound impact on the testis as a whole. Gene expression has been assessed for a limited number of genes. The expression of large number of these genes is developmentally regulated during spermatogenesis. Both transcriptional and translational control mechanism are responsible for temporal and stage specific expression pattern.

Apoptosis is a regulated active form of cell death that is of fundamental importance to the biology of various cellular systems it induces a series of cellular, morphological and biochemical alteration which leads to cell shrinkage, membrane blebbing and DNA fragmentation. In somatic cells, mitochondria are the prime regulator of apoptosis (programmed cell death), a process by initiating the activation of the mitochondrial permeability transition pore (mtPTP). Caspases, a family of cystiene aspartic acid proteases, are important in regulating apoptosis. Externalization of phosphatidyl serine to the sperm outer membrane leaflet is considered to mark terminal apoptosis. Activated caspases-3, loss of the mitochondrial membrane potential and DNA fragmentation are other markers of terminal apoptosis expressed by varying proportion of ejaculated sperm (Evenson *et al*., 1999; Paasch *et al*., 2004). Apoptosis plays an important role in regulating spermatogenesis of various mammalian species including human. Relatively high rates of apoptosis have been reported in testicular biopsies from infertile men with

the different degrees of testicular insufficiency (Lin *et al*., 1997). The portions of apoptotic sperm are reported to be higher in ejaculated semen samples from infertile men compared with healthy men. Moreover , sperm caspases become more activated in patients with infertility than in healthy men (Said *et al*., 2004; Taylor *et al*., 2004) There is an established consensus on the implication of apoptosis expressed in male infertility, however the exact mechanism of its involvement remain to be elucidated.

Mitochondria, DNA spans about 16,500 DNA base pairs, and it represents a fraction of the total DNA in cells. It encodes 37 genes, which are closely packed with no introns, these are needed for normal functioning of mitochondria. Some of these genes are responsible for synthesis of enzymes of oxidative phosphorylation which is a major source of energy. The remaining genes are responsible for synthesizing molecules like transfer RNA (tRNA) and ribosomal RNA (rRNA), which help in synthesizing functional proteins by assembling amino acids. Mitochondrial rRNAs are smaller than the 18s and 25 S cytoplasmic rRNAs, they also have quite distinct sequences. The mRNA translation in mitochondria is also different from the universal genetic code. Mitochondria have a profound effect on all biochemical pathways including one that drives sperm motility. Few studies have suggested that mutations in polymerase gamma, enzyme involved in proofreading activity of human mitochondrial DNA could cause male infertility. The gene family mtND 1 - 4 is involved in synthesis of essential subunits of mitochondrial respiratory chain and mtND 4 mutations have been implicated in mitochondrial diseases. Mutations in the mtDNA of sperm result in either functionless or malfunctioning proteins, hence affecting sperm motility (Folgero *et al*., 1995; Kao *et al*., 1998).

Mitochondria are multitasking organelles involved in ATP synthesis, reactive oxygen species (ROS) production, calcium signaling and apoptosis; and mitochondrial defects are known to cause physiological dysfunction, including infertility. The interrelated mitochondrial systems are assembled from roughly 1000 genes distributed between the two very different genetic systems of the mammalian cell: the nuclear genome and the mitochondrial genome. Hence, the complexities of mitochondrial disease reflect the intricacies of both the physiology and the genetics of the mitochondrion.

Spermatozoa of infertile men have reported presence of nuclear aberrations, including abnormal chromatin structure and possible DNA strand breaks (Sun *et al.*, 1997; Donnelly *et al.*, 2000; Gandini *et al.*, 2000; Irvine *et al.*, 2000; Oehninger, 2000; Zini *et al.*, 2001; Sakkas *et al.*, 2002; Henkel *et al.*, 2004). DNA damage could be attributed to direct oxidative damage (freeradical induced damage) and

could be a consequence of apoptosis that could disrupt the functional and genomic integrity of human sperm (Barroso *et al.*,2000; Oehninger, 2000; Evenson *et al.*, 2002; Oehninger *et al.*, 2003; Moustafa *et al.*, 2004). The excessive generation of reactive oxygen species (ROS) is associated with perioxidative damage to the sperm plasma membrane and the stimulation of DNA fragmentation possibly associated with poor semen quality and male factor infertility (Aitken *et al.*, 1998; Aitken, 1999; Aitken and Krausz, 2001). Another possible theory to explain the origin of DNA damage in sperm is as a result of improper DNA packaging during spermiogenesis (Gorczyca *et al*., 1993; Sailer *et al*., 1995; Manicardi *et al*., 1998; Sakkas *et al*., 1999 a, b; Barroso *et al*., 2000; Sakkas *et al*., 2002; Oehninger *et al*., 2003). Another theory that has been proposed is the "abortive apoptosis" theory (Sakkas *et al.*, 1999 a, b; 2002). Apoptosis in mature sperm is initiated during spermatogenesis in which some cells, earmarked for elimination may escape the removal mechanism contributing to poor quality sperm. "Abortive apoptosis" may fail in the total clearance of sperm earmarked for elimination by apoptosis and therefore ejaculate sperm with apoptotic markers appear to have escaped programmed cell death and reflect the failure of sperm to complete apoptosis. Three hypotheses exist for possible DNA damage in sperm cells and these include damage during the transition of histone to protamine complex during spermiogenesis (McPherson and Longo, 1993; Sun *et al.*, 1997; Sakkas *et al.*, 1999 a, b). Sakkas *et al.* (2002) proposed that the presence of DNA damage in sperm is not directly linked to an apoptotic process in spermatozoa, but arises due to problems with the nuclear remodelling process. Sun *et al.* (1997) demonstrated that the exposure to environmental toxins is positively correlated to DNA damage in sperm. It was found that DNA strand breaks in sperm correlated negatively with semen quality parameters including concentration, morphology and motility (Sun *et al.*, 1997; Sakkas *et al.*, 1999a; Irvine *et al.*, 2000). The assessment of the chromatin status of sperm is important when evaluating the ability of sperm to fertilize the oocyte and should be routinely performed during assisted reproduction.

Apoptosis is an evolutionary-conserved regulatory physiological mechanism that allows eukaryotes to eliminate unneeded, senescent, or aberrant cells (Kerr *et al*., 1972). A cell will undergo apoptosis as a result of information received from its environment interpreted in the context of internal information, such as its cell type, state of maturity and developmental history pathways to activate apoptosis are different in various cell types; however, the mechanism of death itself may be the same, that is, a final common pathway (Cohen, 1993). Before entering in to apoptotic pathway, cells are generally encountered by a signal to activate the particular genetic machinery (Afford and Randhawa, 2000). A stimulus, which

originates from outside the cell exists which can advance or delay apoptotic cell death. Two distinct pathways exist for the initiation of apoptosis: extrinsic or receptor-linked apoptosis and intrinsic or mitochondria-mediated apoptosis (Bantel *et al.*, 1998; Joza *et al.*, 2002; Kiechle and Zhang, 2002; Turk *et al.*, 2002; Vaughan *et al.*, 2002; LaCasse *et al.*, 2004). Apoptotic-initiating pathways and will be briefly outlined below.

Extrinsic or Receptor-Linked apoptotic pathway

The induction of apoptosis occurs via death receptors (cell surface receptors) that transmit apoptosis signals initiated by specific ligands (Green and Kroemer, 1998; Sinha Hikim and Swerdloff, 1999; Slee *et al.*, 1999; Scheider and Tschopp, 2000; Joza *et al.*, 2002; Turke *et al.*, 2002; Sinha Hikim *et al.*, 2003; LaCasse *et al.*, 2004; Fadeel and Orrenius, 2005; Jin and El-Deiry, 2005). Apoptosis controlled by cytokines; specifically Fas ligand (FasL) and tumour necrosis factor receptor 1 (TNF) have been studied extensively.The binding of FasL to Fas forms a cluster and a complex beneath the cell membrane with a number of cytosolic proteins to form the active "death domain", Fas-associating protein with death domain (FADD) or MORT1 (Nagata, 1997; Ormerod, 1998; Nagata, 2000; Joza *et al.,* 2002; Van Cruchten and Van den Broeck, 2002; Wajant, 2002; LaCasse *et al.*, 2004). This receptor-ligand complex then becomes the recognition molecule for a precursor enzyme, caspase- 8 (FLICE/MACH), which in turn may induce self-activation of the protease domain. The recruitment of caspase- 8 may result in the self-activation of proteolytic activity and trigger the interleukin-1ß-converting enzyme (ICE) protease cascade. Caspase-8 is the major initiator caspase identified in death receptor signalling, while caspase-10 may be involved in initiation (Chen and Wang, 2002). Caspase-2, an initiator caspase has been shown to associate with the cytoplasmic regions of death receptors in response to apoptotic stimuli (Bantel *et al.*, 1998; Joza *et al*., 2002; Said *et al.*, 2004). The activated ICE members can cleave various substrates and cause morphological changes to the cells and nuclei. Presently, 14 known caspase family members exist which form an intracellular proteolytic cascade that fine-tune many cellular events in the events of programmed cell death (Afford and Randhawa, 2000). The binding of TNF to TNF receptor (TNFR1) recruits TRADD via interactions between death domains. The death domain of TRADD then recruits FADD/MORT1 in a pathway to activate caspase- 8 and induce apoptosis (Cohen, 1997; Nagata, 1997; Nagata, 2000; Joza *et al.*, 2002; LaCasse *et al.*, 2004). In another pathway, receptor interacting protein (RIP) binds to TRADD and transduces an apoptotic signal through death domain. RIP together with TNF receptor-associated factor (TRAF2)

activates NF-kB, which may induce the expression of survival genes. The Fas receptor and tumour necrosis factor receptor 1 (TNFR1) both trigger apoptosis via FADD (Fas-associating protein with death domain), which results in a sequence of events leading to apoptotic death of the cell. The stimulation of some cell surface molecules, such as Fas ligand (FasL) and tumour necrosis factor (TNF) can often induce cell death by apoptosis, thereby killing the cell within hours (Williams and Smith, 1993; Nagata, 1997; Nagata, 2000). Other death receptors include: TNF receptor-related-apoptosis mediated-protein (TRAMP) and two receptors which bind TNF-related apoptosis-inducing ligand (TRAIL), called TRAIL-R1 and TRAIL-R2 (Bantel *et al.*, 1998).

Intrinsic or mitochondria-mediated apoptotic pathway

Mitochondria play an important role in the promotion of apoptosis through the release of cytochrome c (Halestrap *et al.*, 2000). The intrinsic pathway is triggered by stress stimuli, including growth factor deprivation and DNA damage (Schuler and Green, 2001). This pathway involves the release of an extrinsic protein, cytochrome c on the outer surface of the inner mitochondrial membrane from the mitochondria during apoptosis (Bantel *et al.*, 1998; Green and Kroemer, 1998; Nuñez, *et al.*, 1998; Wilson, 1998; Budihardjo *et al.*, 1999; Porter and Jänicke, 1999; Van der Heiden and Thompson, 1999; Joza *et al.*, 2002; Kiechle and Zhang, 2002; Van Cruchten and Van den Broeck, 2002; Sinha Hikim *et al.*, 2003; LaCasse *et al.*, 2004; Fadeel and Orrenius, 2005). The other mitochondrial proteins released from the mitochondrial intermembrane space during apoptosis are Smac/DIABLO and HtrA2/Omi (Joza *et al.*, 2002; Turke *et al.*, 2002; LaCasse *et al.*, 2004). They promote cell death by inactivating inhibitors of apoptosis proteins (IAPs), thereby releasing caspases from inhibition by IAPs. Cytochrome c is released due to a decrease in the inner mitochondrial transmembrane potential. Caspase-2 engages the mitochondria-dependant apoptotic pathway by inducing the release of cytochrome c and other apoptogenic factors (Smac and HtrA2) in the cell cytoplasm (Guo *et al*., 2002; Zhivotosky and Orrenius, 2005). Proteins belonging to the Bcl-2 family appear to regulate the membrane permeability to ions and possibly to cytochrome c (Burlacu, 2003). The regulation of apoptosis by Bcl-2 will be briefly reviewed below. Once released, cytochrome c forms a subunit with other cytosolic protein factors, apoptotic protease activation factor-1 (Apaf-1), procaspase- 9 and either ATP or dATP to activate caspase-3 .The action of cytochrome c and dATP causes a conformational structural change of the Apaf-1 protein to form an apoptosome. Caspase-9 is the initiator caspase for mitochondrion-dependant apoptosis (Chen and Wang, 2002). Apaf-1 binds to ATP/dATP and hydrolyses it to ADP or dADP,

respectively resulting in the formation of Apaf-1/cytochrome c complex. A conformational change occurs in Apaf-1 and a region, the caspase recruitment domain (CARD) is exposed allowing for the homophilic binding of caspase-9.

Thus, this complex recruits and activates procaspase-9 and activated caspase-9 is released from the complex to cleave and activate downstream caspases, caspase-3, -6 and –7. The induction of apoptosis via the intrinsic or extrinsic apoptotic pathways results in the activation of an initiator caspase, which activates a cascade of happenings to the activation of effector caspases, responsible for the cleavage of key cellular proteins that lead to the typical morphological changes observed in cells undergoing apoptosis. Caspase-8 and caspase-10 are initiator caspases in death receptor-mediated apoptosis, while caspase-9 is the initiator caspase in mitochondrion-dependent apoptosis (Chen and Wang, 2002). These pathways differ in one fundamental aspect: one is "external" in that it is promoted by a series of specific external ligands operating through defined transmembrane receptors; the other is an internal system where activation of the effector enzymes is induced by intracellular changes, involving the mitochondria (Garland, 2000; Joza *et al.*, 2002; Turke *et al.*, 2002). Despite the difference in the initiation manner, the extrinsic and intrinsic pathways merge at the level of caspase-3 and 7 and once activated, these cleave intracellular targets ultimately leading to the manifestations of apoptosis

Regulators of Apoptosis

The activation of the apoptosis-signalling pathway occurs in response to various regulatory factors. There are a number of regulators of cell death and the effects appear to depend on the cell type and the stimulus for apoptosis. The bcl-2 family of proteins plays a crucial role in the regulation of apoptosis.

Bcl-2

A key component of apoptosis activation involves the activation of the caspases that participates in a cascade of events, which ultimately cause disassembly of the cell. The initiation of the caspases proteolytic cascade is regulated by proteins of which the Bcl-2 family plays a pivotal role in the control of apoptosis (Van der Heiden and Thompson, 1999; Joza *et al.*, 2002; Burlacu, 2003). The Bcl-2 protein is a membrane protease that is localised to the outer mitochondria, endoplasmic reticulum membrane, and nuclear envelope (Van der Heiden and Thompson, 1999; Burlacu, 2003). Eighteen members for the Bcl-2 family have been identified and divided into a subgroup of anti-apoptotic members, such as (Bcl-2, Bcl-XL,

Mcl-1, A1, Bcl-W) and a large group of pro- 27 apoptotic members, such as (Bax, Bak, Bok, Bad, Bid, Bim, Blk, BNIP and *egl-1*) (Goss *et al.*, 1999; Pepper and Bentley, 2000; Joza *et al.*, 2002; Burlacu, 2003). The balance between the anti-apoptotic and the pro-apoptotic members are critical in determining whether a cell undergoes apoptosis. These proteins regulate apoptosis in part by affecting the mitochondria compartmentalization of cytochrome c (Van der Heiden and Thompson, 1999). The Bcl-2 family, the most prominent regulators of apoptosis exert their apoptosis-regulatory effects primarily by regulating mitochondrial alterations that precede the activation of caspases and nucleases (Pepper and Bentley, 2000; Joza *et al.*, 2002). The pro-apoptotic bcl-2 proteins are generally found in the cytosol where they can sense stress. In the event of cellular stress they shift to the surface of the mitochondria where the antiapoptotic proteins are present. This interaction between pro- and antiapoptotic proteins disorganize the normal function of the anti-apoptotic bcl-2 proteins and can progress to the formation of pores in the mitochondria leading to release of cytochrome c and other pro-apoptotic molecules from the intermembrane space of mitochondria (Goss *et al.*, 1999; Van der Heiden and Thompson, 1999; Burlacu, 2003). On binding to Apaf-1, these mediators activate caspase-9, which triggers the cascade of effector caspases ultimately resulting in irreversible cell death.

p53

Intrinsic stresses such as oncoproteins, damage in DNA, severe deficiency of oxygen and survival factor deprivation, can activate the intrinsic apoptotic pathway (Agarwal *et al.*, 1998; Schuler and Green, 2001; Gewies, 2003). p53, a tumour suppressor protein is present in the mitochondria and appears to contribute to the process of apoptosis (Choisy-Rossi *et al.*, 1999; Chang, 2002). p53 initiates apoptosis by transcriptionally activating pro-apoptotic Bcl-2 family members (bax and bak) and repressing antiapoptotic proteins (bcl-XL and bcl-2). The regulated release of proapoptotic factors from the mitochondria initiates the release of cytochrome c from the mitochondria. A study by Deng and Wu (2000) found that the subcellular localization of Bax protein plays a key role in deciding the fate of the cell, as the translocation of Bax from the cytosol to the mitochondria is a critical step in p53-mediated apoptosis. Shortly after p53 accumulation on the mitochondrial membrane the release of cytochrome c takes place which induces the formation of the apoptosome. Procaspase-9 is activated, which binds to the apoptosome and further mediates the activation of effector caspases (Schuler and Green, 2001; Gewies, 2003). The execution of p53-mediated apoptosis proceeds through the effector caspases, their mechanism of action depends on

the experimental context (Schuler and Green, 2001). Thus, it has been proposed that the intrinsic pathway is necessary for both stress induced and p53-dependant caspase activation. The p53 gene is highly expressed in the testis and is known to be involved in apoptosis, suggesting that it is one of the major causes of germ cell loss in the testis (Ohta *et al.*, 2003). The findings of a study suggested that p53 mediates spontaneous testicular germ cell apoptosis in mice and failure to remove defective germ cells by this mechanism results in the increased percentages of abnormal sperm and reduced fertility (Yin *et al.*, 1998). It has been postulated that p53 is not active in normal adult human testis, indicating that there are p53-independent mechanisms of spontaneous germ cell apoptosis (Print and Loveland, 2000; Oldereid *et al.*, 2001).

Germ Cell Apoptosis

Spermatogenesis, the dynamic process of cell differentiation is a precisely controlled and cyclical timed process comprising of proliferation of spermatogonia, meiotic divisions of spermatocytes, and the differentiation of spermatids into spermatozoa (Blanco-Rodrĩguez, 1998; Blanco-Rodrĩguez and Martĩnez-Garcĩa, 1999; Fujisawa *et al.*, 1999; Print and Loveland, 2000; Kierszenbaum, 2001; 2001; Kim *et al.*, 2002; Sinha Hikim *et al.*, 2003). Sertoli cells in close contact with the germ cells facilitate spermatogenesis by providing structural and nutritional support for the neighbouring germ cells. The proliferation and differentiation of germ cells depends on the release of two hormones from the anterior pituitary gland, namely follicle-stimulating hormone (FSH) and luitenizing hormone (LH) (Sassone-Corsi, 1997). The removal of these hormones induces germ cell apoptosis through indirect effects (Print and Loveland, 2000). Spermatogenesis is accompanied by germ cell apoptosis in the seminiferous epithelium and can also be triggered by a variety of stimuli (Billig *et al.*, 1996; Blanco-Rodrĩguez, 1998; Fujisawa *et al.*, 1999; Sinha Hikim and Swerdloff, 1999; Kierszenbaum, 2001; Sinha Hikim *et al.*, 2003). Kim *et al.* (2002) proposed four possible functional roles for the presence of apoptosis during spermatogenesis. He postulated that germ cell apoptosis is necessary to maintain an optimal germ cell to Sertoli cell ratio, to eliminate any abnormal germ cells, the formation of the blood-testis barrier requires the elimination of excessive germ cells and an apoptotic surge of germ cell apoptosis occurs prior to puberty regulating the ratio of germ cells to Sertoli cells. Approximately 75% of the spermatogonia die in the process of programmed cell death before reaching maturity (Print and Loveland, 2000; Matinčič *et al.*, 2001). A dramatic increase in germ cell apoptosis occurs in some pathological conditions, which include idiopathic infertility in males (Sassone-Corsi, 1997). It has been

shown that spontaneous apoptosis has been observed frequently in spermatocytes, less frequently in spermatogonia and seldom in spermatids (Sasagawa *et al.*, 2001). The presence of apoptosis in human germ cells in the testis specifically in spermatocytes has been reported by Fujisawa *et al.* (1999). They found that the rate of apoptosis decreased in the testis of infertile men; however, the mechanism remains to be elucidated. The exact incidence of male adult germ cell apoptosis remains unclear, since not all degenerating germ cells display the classical morphology of apoptosis (Print and Loveland, 2000; Kierszenbaum, 2001). The Fas-system has been shown to participate in the regulation of the spontaneous germ cell apoptosis (Richburg, 2000; Riccioli *et al.*, 2003). It has been hypothesized that in the normal state, Sertoli cells express Fas ligand and this triggers apoptosis of a few Fas-positive germ cells revealing a paracrine control between the Sertoli and germ cells (Lee *et al.*, 1997; 1999; Pentikäinen *et al.*, 1999; Kierszenbaum, 2001). They concluded that this death-delivering process ensures testicular homeostasis by the elimination of ill-supported germ cells. The development of mature sperm is the product of a precisely regulated sequence of events in which germ cell proliferation, differentiation, self-renewal and apoptosis is controlled.

Sperm Apoptosis

The debate regarding sperm apoptosis remains a controversial issue as uncertainty exists as to whether apoptotic sperm are sperm with poor functional activity or in fact indicative of sperm that have failed to complete maturation during spermiogenesis. Several investigations demonstrated the occurrence of possible apoptosis in ejaculated human sperm (Baccetti *et al.*, 1996; Blanc-Layrac *et al.*, 2000; Oosterhuis *et al.*, 2000; Shen *et al.*, 2002; McVicar *et al.*, 2004) and in bull sperm (Anzar *et al.*, 2002). A study conducted by Lachaud *et al.* (2004) examined the ability of human spermatozoa to initiate apoptosis *in vitro* and their findings suggested that these cells lack the capacity to initiate the apoptotic pathway of cell death.

Mitochondrial DNA

Mitochondria have been shown to play a pivotal role in apoptotic signaling in various cell types. It has been recently reported that in lymphocytes the voltage-gated potassium channel Kv1.3, known to reside in the plasma membrane, is active also in the inner mitochondrial membrane, suggesting that this channel might play a role in the apoptotic signaling not only in lymphocytes but also in other cells (focus on spermatozoa). Changes in mitochondrial DNA disrupt the mitochondria's ability to efficiently generate energy for the cell The mature

spermatozoon contains 72 -100 mitochondria and it is heavily dependent on the respiratory chain for motility. Association of Mitochondrial mutations with infertility cannot be ruled out. Recent studies have shown that mutations in the mitochondrial genome are associated with poor semen parameters, such as sperm maturation (Holyoake *et al*., 2001), sperm motility (Folgero *et al*., 1995; Kao *et al*., 1998), and other related disorders (St John *et al*., 2000). A correlation of the asthenozoospermia and oligoasthenozoospermia with the typical mitochondrial DNA diseases involving point mutation or multiple deletions has been reported (Sampson *et al*., 2001)

Conclusion

In view of the rising prevalence of male infertility and growing interest towards designing of a functional male contraceptive and treatment of infertility, knowledge of the cellular, biochemical and molecular events that regulate the fertilizing potential of human spermatozoa is essential. The correlation of apoptosis with serum gonadotropins and testosterone levels become also pertinent to find out the causes of male infertility.

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