European Review for Medical and Pharmacological Sciences 2017; 21 (2 Suppl): 4-9

# Contribution of electron microscopy to study *in vitro* inositol effects on human spermatozoa

M. COLONE[1], A. CALCABRINI[1], V. UNFER[2], A. STRINGARO[1]

[1]National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy
[2]Department of Obstetrics and Gynecology, Medicine and Psychology, Sapienza University of Rome, Rome, Italy

**Abstract. – Infertility is a worldwide problem and male partner contributes to almost 30% of cases of infertility. The term oligoasthenoteratospermia is related to defective spermatogenesis and is characterized by a reduction of motility and number of spermatozoa and a change in their morphology. Electron microscopes are frequently used in order to evaluate sperm pathology and overall to establish a correlation between structural and functional deficiencies of altered sperm. High levels of reactive oxygen species endanger sperm function and viability. The correlation between male infertility, reactive oxygen species levels and the innovative therapeutic strategy employing inositol has been highlighted through analysis of literature data.**

Key Words:
Male infertility, Oxidative stress, Sperm morphology, Electron microscopy, Inositol.

## Introduction

The process through which spermatozoa are produced from a no differentiated germ cell population in the testis is called spermatogenesis. In the multicellular epithelium of the seminiferous tubule, there are two cell types, the germ cells and the somatic Sertoli cells. The Sertoli cells create two compartments within the seminiferous epithelium. The barrier between these compartments is produced by the formation of specialized tight junctions between adjacent Sertoli cells[1].

It is a common assumption that infertility is primarily related to the woman. Indeed, only one-third of infertility cases are related to women alone. Statistically, one-third of infertility problems are related to men and the remaining one-third is a combination of fertility factors involving both partners or unknown causes. Up to 90% of man infertility is correlated to low sperm count or quality or both[2].

Oligoasthenoteratospermia (OAT) is characterized by the reduction of number, motility, and alterations of the morphology of spermatozoa; several are the therapies suggested for counteracting such a disease, however, they are often ineffective[3]. Much research remains to be performed on the topic of male infertility, as many cases still receive an "unknown cause" diagnosis. It usually occurs because of the abnormal size or inadequate number of spermatozoa, or because there are problems with ejaculation. One of the factors that may influence male infertility is the production of reactive oxygen species (ROS). Spermatozoa are more susceptible than other cell species to the harmful activity of ROS. In particular, they can affect motility, morphology and DNA stability of spermatozoa.

### Contribution of the Electron Microscopies

The electron microscope allowed overcome the physical limit of the resolving power of light microscopes (about 200 nm). There are mainly two types of electron microscopes: the transmission (TEM) and the scanning electron microscope (SEM). SEM was devised around the same year as TEM but was marketed only 25 years later (1965). In the field of biomedical research both TEM and SEM play the same important role, especially when they are used in association, yielding complementary morphological and ultrastructural information. TEM enters the cells and explores the ultrastructure of the intracellular components, SEM supplies information on the general morphology and surface architecture of biological samples. SEM has generally a poorer resolution than TEM, but it provides a three-dimensional representation of the sample[4,5].

During the 17$^{th}$ century, Antonie van Leeuwenhoek was the first to report the existence of spermatozoa, that he called *animalcules*, in the seminal fluid of animals and men[6]. Light microscopy (LM), as well as electron microscopy, has been largely employed to study sperm morphology that



*Corresponding Author:* Annarita Stringaro, Ph.D; e-mail: annarita.stringaro@iss.it

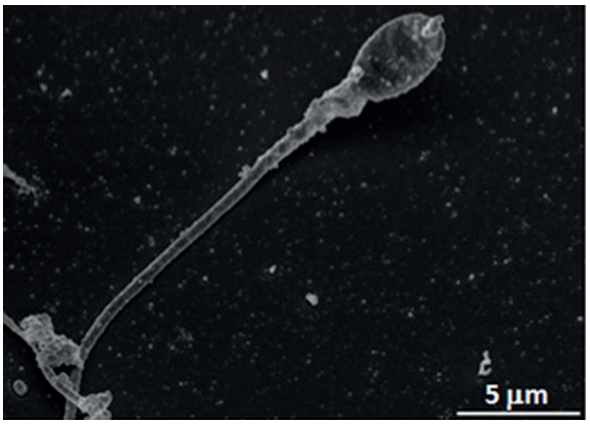


**Figure 1.** Scanning electron microscopy imaging of control spermatozoa cell surface.

represents an important factor in determining the fertilizing properties of spermatozoa[7]. LM serves as the routine method for morphological examination of spermatozoa, whereas through TEM single sections of sperm cells can be easily analyzed. SEM is usually employed to study the spermatozoa surface morphology obtaining three-dimension images[5] (Figure 1). Also, microscopic studies are often correlated with molecular biology, proteomic and genomics to understand the male infertility causes and to establish a correlation between structural and functional deficiencies of altered sperm[8-11]. Sperm motility and morphology have long been recognized as indicators of the fertility potential of human spermatozoa. The recent introduction of micro-fertilization techniques provides access to the structural and functional features of spermatozoa that are being used for fertilization[12]. Overall, TEM is an important adjunct to the traditional methods of semen analysis. Indeed, LM is not able to provide the detailed information required to characterize the morphological and functional state of the mitochondria, dense fibers, fibrous sheath and axoneme. Detailed morphological information regarding sperm flagellum or tail spermatozoa, nuclear and acrosomal structures has been obtained through ultrastructural studies[10]. Furthermore, SEM analysis contributed to demonstrate a significant reduction in the length and/or important morphological alterations of the midpieces associated with multiple ultrastructural abnormalities in the sperm[5] (Figure 2).

### *Oxidative Stress and Sperm's Damages*

Oxidative stress is defined as an imbalance between ROS generation and antioxidant capacity of the mammalian cells. In the past few years, the scientific community studying male infertility addressed the research towards oxidative stress field, since it had been observed that one of the factors which may influence this pathology is linked to the production of ROS by morphologically altered spermatozoa[3,13-16]. It is well known that human spermatozoa contain especially high levels of polyunsaturated fatty acids that are very sensible to oxidative stress. Moreover, these cells discharge their cytoplasm during maturation, so they do not contain high levels of antioxidant enzymes that protect from ROS

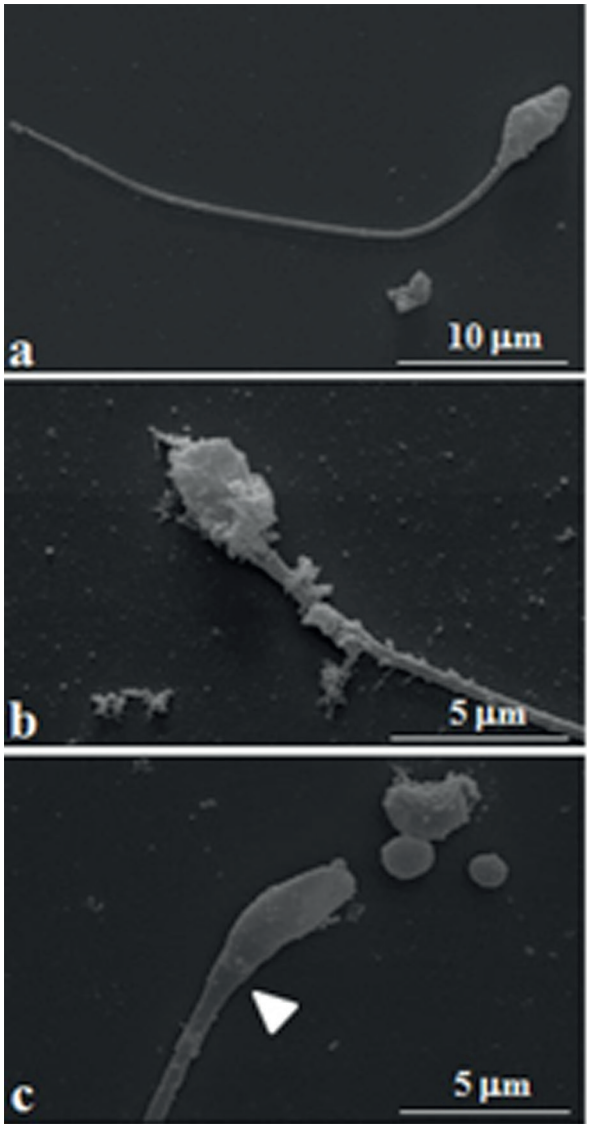


**Figure 2.** Scanning electron microscopy imaging: ***a***, control specimen; ***b***, oligoasthenoteratospermia specimen covered with amorphous material; ***c***, pathological spermatic cell following 2 hours of treatment with inositol (2 mg/ml). Arrowhead points out intermediate tract thickening.

attack (glutathione peroxidase, catalase and superoxide dismutase)[17,18].

Oxidative stress can induce DNA damage such as nucleotide modifications, base loss, deletions, frame-shits mutations, DNA cross-links and chromosomal rearrangements. These aberrations in the chromatin structure can expose the genome to further oxidative insult[19]. DNA damage correlates with the lower reproductive potential of sperm, and the assessment of this parameter has a better predictive score than conventional semen analysis. A recent meta-analysis suggests that antioxidant therapy improves male fertility[17,20-27].

A substantial number (up to 40%) of males diagnosed with semen disorders shows high levels of ROS[28]. Furthermore, human spermatozoa can undergo the effects of ROS released by white blood cells, such as neutrophils, that infiltrate the male tract as a consequence of infection[20]. Consequently, the presence of free-radical generating leucocytes in human sperm suspensions prepared for Assisted Reproductive Therapy represents a highly significant determinant of fertilization success *in vitro*[29].

Moreover, Najafi et al[30] have demonstrated that changes in the redox status can induce apoptosis. Apoptosis is a physiological phenomenon characterized by cellular morphological and biochemical modifications that cause cells to die in a controlled manner. There are two main stages of apoptotic cell death. The first one involves the interaction of a death receptor, such as the TNF receptor-1 or the Fas receptor, with its ligand and the subsequent one depends on the signaling molecule cytochrome c, which triggers caspases, such as caspases 3 and 9[30]. Apoptosis is activated when the intracellular status becomes more oxidized and caspases, belonging to cysteine protease family, are sensitive to redox state[30-32].

The rationale for treating infertile men with oral antioxidants is based on the premise that seminal oxidative stress (common in infertile men) is due in part to a deficiency in seminal antioxidants. However, the levels of semen ROS should not be entirely suppressed (by oral antioxidants) as this may impair normal sperm functions (e.g., sperm capacitation and hyperactivation) that normally require low levels of ROS[33]. Consequently, there is great interest in evaluating antioxidant agents that could protect spermatozoa from ROS. Some studies have reported a dose-response relationship between the daily intake of antioxidant nutrients (carotenoids, lycopene and lutein) and semen quality[34,35]. Recently, resveratrol, a natural phytoalexin, has been studied for its antioxidant properties. It is widely consumed in the Mediterranean diet in the form of peanuts, grapes and wine[36-38].

Another possible antioxidant agent is represented by inositol. It is a carbocyclic polyol with 9 different stereoisomers synthesized from glucose-6-phosphate, the first product of glycolysis, and is eliminated by the kidney. Inositol is a precursor of secondary messengers, such as diacylglycerol and inositol triphosphate, which are involved in the cellular signal transduction system and the regulation of calcium intracellular concentration[39,40]. It is widely distributed in nature and is involved in many systemic mechanisms of signal transduction in the plasma membrane. Inositol is synthesized by 2 enzymes present in high concentrations in the testes, which synthesize inositol from glucose-6-phosphate previously transported into cells by a sodium/inositol cotransport protein, whose expression is sensitive to osmolar changes[40].

Inositol, due to the antioxidant activity, could preferentially influence the mitochondria functionality. Mitochondria are sub-cellular organelles present in the cell cytoplasm. They have an outer and an inner membrane separated by an inter-membrane space. The inner membrane forms numerous folds inside mitochondrial matrix (cristae)[41-51]. An apoptotic stimulus induces the depolarization of mitochondrial membrane potential (MMP), an early and reversible event, following the opening of mitochondrial permeability transition pore. Therefore, the permeability of the mitochondrial membrane is altered leading to the release of cytochrome c and other apoptogenic factors into the cytosol and loss of oxidative phosphorylation.

Sperm mitochondria, as well as the somatic ones, are the location of the oxidative phosphorylation process, which is necessary for the production of metabolic energy in the form of adenosine triphosphate (ATP). Sperms use ATP to sustain their motility, which is one of the major factors to determine male fertility. The role of these organelles in sperm motility was investigated thoroughly, mainly because the causes of fertility-impairing pathologies linked to a pronounced reduction of sperm motility are largely unknown[52]. Mitochondria are thought to be the most important organelles for the evaluation of sperm quality, and this may be due to the fact that mitochondria contain their own DNA and membrane potential which can easily be examined *in*

*vitro* to reflect sperm DNA integrity and motility, respectively[53,54]. Several studies demonstrated the important role of mitochondria in spermatogenesis and fertility, even though a conclusive evidence stating their exact involvement still lacks[51]. The functionality of sperm mitochondria has been investigated using different technical approaches, such as cytofluorimetric methods and ultrastructural analysis[55,56].

In these studies, a positive correlation between the mitochondrial functionality and sperm motility or, in general, the quality of semen samples, was found to be reproducible. Moreover, biochemical analyses showed that inositol ameliorates sperm mitochondrial activities.

MMP directly reflects the energy generation in terms of ATP. Healthy mitochondria have high MMP values and facilitate the adequate synthesis of ATP to sustain the sperm motility[57]. MMP evaluation, therefore, can be used to evaluate sperm quality. It has been shown that men with normal sperm parameters have a significantly greater percentage of spermatozoa with high MMP compared with patients with abnormal parameters.

Polyol myo-inositol is a compound having an unusually high concentration in the seminiferous tubule fluid. It is known that myo-inositol (MYO) is a compatible osmolyte, and it is postulated that the molecule can help in maintaining an ideal osmotic state when a cell is placed in a hypertonic environment. MYO enters the cell along with sodium to offset the change in osmolarity generated by the loss of water molecules. High concentrations of MYO have been demonstrated to be important for osmoregulation in the seminiferous epithelium[1]. Condorelli et al[58] showed that MYO significantly increased the number of spermatozoa with high MMP and significantly decreased the number of those with low MMP in patients suffering oligo-astheno-teratozoospermia. No effect of MYO was observed on phosphatidylserine externalization and chromatin compactness (two parameters of apoptosis induction) in normozoospermic men and patients with OAT[58]. The results of this study showed that MYO ameliorates sperm mitochondrial function, thereby improving semen motility in patients with altered sperm parameters. In addition, MYO increased the total number of spermatozoa after swim-up in normozoospermic men and, more importantly, in patients with abnormal sperm parameters. These findings suggest that MYO could be used *in vitro* in assisted reproductive techniques, both to increase the number of spermatozoa to be used for intrauterine insemination and to ameliorate the sperm quality to be used for *in vitro*-assisted reproductive techniques[58,59].

## Concluding Remarks

Male partner contributes to about 30% of case of infertility[3]. Oxidative stress plays a fundamental role in infertility development[60,61]. Sperm cells are very susceptible to ROS action as their plasmatic membrane is particularly rich in polyunsaturated fatty acids; in addition, during their maturation process, they lost some of the cytoplasmic antioxidant enzymes. It is well known that high concentrations of ROS in the reproductive apparatus and the seminal fluid are associated with important dysfunctions and cellular damages involving spermatozoa.

Recent scientific evidence indicates that the use of antioxidants, both through the systemic route and as a supplement employed during *in vitro* techniques for the preparation of seminal fluid, appears to be effective in order to induce a significant reduction of the high ROS levels. Then, MYO represents a promising therapeutic strategy against male infertility.

### Acknowledgments

The content of this paper was created and enriched solely by the authors through a process of the ongoing literature searches

### Conflict of interest

The authors declare no conflicts of interest

## References

1) Chauvin TR, Griswold MD. Characterization of the expression and regulation of genes necessary for myo-inositol biosynthesis and transport in the seminiferous epithelium. Biol Reprod 2004; 70: 744-751.
2) Leaver B. Male infertility: an overview of causes and treatment options. Br J Nurs 2016; 25: 35-40.
3) Alam N. Male factor infertility-basics revisited. J Coll Physicians Surg Pak 2009; 19: 205-206.
4) Condello M, Caraglia M, Castellano M, Arancia G, Meschini S. Structural and functional alterations of cellular components as revealed by electron microscopy. Microsc Res Tech 2013; 76: 1057-1069.
5) Colone M, Marelli G, Unfer V, Bozzuto G, Molinari A, Stringaro A. Inositol activity in oligoasthenoteratospermia-an *in vitro* study. Eur Rev Med Pharmacol Sci 2010; 14: 891-896.

6) Chemes HE, Rawe VY. Sperm pathology: a step beyond descriptive morphology. Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. Hum Reprod Update 2003; 9: 405-428.

7) Mortimer D, Menkveld R. Sperm morphology assessment-historical perspectives and current opinions. J Androl 2001; 22: 192-205.

8) Pesch S, Bergmann M. Structure of mammalian spermatozoa in respect to viability, fertility and cryopreservation. Review Micron 2006; 37: 597-612.

9) Fisch C, Dupuis-Williams P. Ultrastructure of cilia and flagella - back to the future! Review Biol Cell 2011; 103: 249-270.

10) Mobberley MA. Electron microscopy in the investigation of asthenozoospermia. Br J Biomed Sci 2010; 67: 92-100.

11) Aitken RJ. Sperm function tests and fertility. Int J Androl 2006; 29: 69-75.

12) Cooper TG. Cytoplasmic droplets: the good, the bad or just confusing?. Hum Reprod 2005; 20: 9-11.

13) Tunc O, Tremellen K. Oxidative DNA damage impairs global sperm DNA methylation in infertile men. J Assist Reprod Gen 2009; 26: 537-544.

14) Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil 1987; 81: 459-469.

15) Aitken RJ, Baker MA. Oxidative stress and male reproductive biology. Reprod Fertil Develop 2004; 16: 581-588.

16) Fingerova H, Oborna I, Novotny J, Svobodova M, Brezinova J, Radova L. The measurement of reactive oxygen species in human neat semen and in suspended spermatozoa: a comparison. Reprod Biol Endocrinol 2009; 7: 118-123.

17) Aitken RJ, Curry BJ. Redox regulation of human sperm function: From the physiological control of sperm capacitation to the etiology of infertility and DNA damage in the germ line. Antioxid Redox Signal 2011; 14: 367-381.

18) Rhemrev JP, van Overveld FW, Haenen GR, Teerlink T, Bast A, Vermeiden JP. Quantification of the non-enzymatic fast and slow TRAP in a postaddition assay in human seminal plasma and the antioxidant contributions of various seminal compounds. J Androl 2000; 21: 913-920.

19) Shamsi MB, Imam SN, Dada R. Sperm DNA integrity assays: diagnostic and prognostic challenges and implications in management of infertility. J Assist Reprod Genet 2011; 28: 1073-85.

20) Aitken RJ, Buckingham DW, Brindle J, Gomez E, Baker HW, Irvine DS. Analysis of sperm movement in relation to the oxidative stress created by leukocytes in washed sperm preparations and seminal plasma. Hum Reprod 1995; 10: 2061-2071.

21) Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 1987; 8: 338-348.

22) Twigg J, Fulton N, Gomez E,. Irvine DS, Aitken RJ. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. Hum Reprod 1998; 13: 1429-1436.

23) Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod 1989; 41: 183-197.

24) Aitken RJ, Baker HW. Seminal leukocytes: passengers, terrorists or good samaritans?. Hum Reprod 1995; 10: 1736-1739.

25) Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database Syst Rev 2014; 12: 1-188.

26) Anderson D, Schmid TE, Baumgartner A, Cemeli-Carratala E, Brinkworth MH, Wood JM. Oestrogenic compounds and oxidative stress (in human sperm and lymphocytes in the Comet assay). Mutat Res 2003; 544: 173-178.

27) Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, Irvine DS. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 1998; 59: 1037-1046.

28) Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril 1992; 57: 409-416.

29) Aitken RJ, Muscio L, Whiting S, Connaughton HS, Fraser BA, Nixon B, Smith ND, De Iuliis GN. Analysis of the effects of polyphenols on human spermatozoa reveals unexpected impacts on mitochondrial membrane potential, oxidative stress and DNA integrity; implications for assisted reproductive technology. Biochem Pharmacol 2016; 121: 78-96.

30) Najafi TF, Hejazi M, Esnaashari FF, Sabaghiyan E, Hajibaba-kashani S, Yadegar Z. Assessment of Sperm Apoptosis and Semen Quality in Infertile Men-Meta Analysis. Iran Red Crescent Med J 2012; 14: 182-183.

31) Bansal AK, Bilaspuri GS. Impacts of oxidative stress and antioxidants on semen functions. Vet Med Int 2011; 2011: 1-7.

32) Aitken RJ, M. Baker A. Oxidative stress, sperm survival and fertility control. Mol Cell Endocrinol 2006; 250: 66-69.

33) Aitken RJ, Paterson M, Fisher H, Buckingham DW, van Duin M. Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. J Cell Sci 1995; 108: 2017-2025.

34) de Lamirande E, Jiang H, A. Zini, Kodama H, Gagnon C. Reactive oxygen species and sperm physiology. Rev Reprod 1997; 2: 48-54.

35) Zini A, de Lamirande E, Gagnon C. Low levels of nitric oxide promote human sperm capacitation in vitro. J Androl 1995; 16: 424-431.

36) Mínguez-Alarcón L, Mendiola J, López-Espín J J, Sarabia-Cos L, Vivero-Salmerón G, Vioque J. Dietary intake of antioxidant nutrients is associated with semen quality in young university students. Hum Reprod 2012; 27: 2807-2814.

37) Zareba P, Colaci DS, Afeiche M, Gaskins AJ, Jørgensen N, Mendiola J, Swan SH, Chavarro JE. Semen quality in relation to antioxidant intake in a healthy male population. Fertil Steril 2013; 100: 1572-1579.

38) Kovacic P, Somanathan R. Multifaceted approach to resveratrol bioactivity: Focus on antioxidant action, cell signaling and safety. Oxid Med Cell Longev 2010; 3: 86-100.

39) Eisenberg FJr, Parthasarathy R. Measurement of biosynthesis of myo-inositol from glucose 6-phosphate. Methods Enzymol 1987; 141: 127-143.

40) Ford WC. Glycolysis and sperm motility: does a spoonful of sugar help the flagellum go round? Hum Reprod 2006; 12: 269-274.

41) Ruiz-Pesini E, Díez-Sánchez C, López-Pérez MJ, Enrìquez J. The role of the mitochondrion in sperm function: is there a place for oxidative phosphorylation or is this a purely glycolytic process? Curr Top Dev Biol 2007; 77: 3-19.

42) Storey BT. Mammalian sperm metabolism: oxygen and sugar, friend and foe. Int J Dev Biol 2008; 52: 427-437.

43) Piomboni P, Focarelli R, Stendardi A, Ferramosca A, Zara V. The role of mitochondria in energy production for human sperm motility. Int J Androl 2012; 35: 109-124.

44) Ross A, Christie S, Kerr MG. An electron microscope study of a tail abnormality in spermatozoa from a subfertile man. J Reprod Fertil 1971; 24: 99-103.

45) Evenson DP, Darzynkiewicz Z, Melamed MR. Simultaneous measurement by flow cytometry of sperm cell viability and mitochondrial membrane potential related to cell motility. J Histochem Cytochem 1982; 30: 279-280.

46) Ruiz-Pesini E, Lapeña AC, Díez C, Alvarez E, Enrìquez JA, López-Pérez MJ. Seminal quality correlates with mitochondrial functionality. Clin Chim Acta 2000; 300: 97-105.

47) Rawe VY, Galaverna GD, Acosta AA, Olmedo SB, Chemes HE. Incidence of tail structure distortions associated with dysplasia of the fibrous sheath in human spermatozoa. Hum Reprod 2001; 16: 879-886.

48) Sutovsky P, Terada Y, Schatten G. Ubiquitin-based sperm assay for the diagnosis of male factor infertility. Hum Reprod 2001; 16: 250-258.

49) Rawe V Y, Olmedo S B, Benmusa A, Shiigi S M, Chemes H E, Sutovsky P. Sperm ubiquitination in patients with dysplasia of the fibrous sheath. Hum Reprod 2002; 17: 2119-2127.

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

51) Rajender S, Rahul P, Mahdi AA. Mitochondria, spermatogenesis and male infertility. Mitochondrion 2010; 10: 419-428.

52) Turner RM. Moving to the beat: a review of mammalian sperm motility regulation. Reprod Fertil Develop 2006; 18: 25-38.

53) Carra E, Sangiorgi D, Gattuccio F, Rinaldi AM. Male infertility and mitochondrial DNA. Biochem Biophys Res Commun 2004; 322: 333-339.

54) Amaral A, Ramalho-Santos J. Assessment of mitochondrial potential: implications for the correct monitoring of human sperm function. Int J Androl 2010; 33: 180-186.

55) Baccetti B, Bruni E, Gambera L, Moretti E, Piomboni P. An ultrastructural and immunocytochemical study of a rare genetic sperm tail defect that causes infertility in humans. Fertil Steril 2004; 82: 463-468.

56) Pelliccione F, Micillo A, Cordeschi G, D'Angeli A, Necozione S, Gandini L, Lenzi A, Francavilla F, Francavilla S. Altered ultrastructure of mitochondrial membranes is strongly associated with unexplained asthenozoospermia. Fertil Steril 2011; 95: 641-646.

57) Agnihotri SK, Agrawal AK, Hakim BA, Vishwakarma AL, Narender T, Sachan R, Sachdev M. Mitochondrial membrane potential (MMP) regulates sperm motility. In Vitro Cell Dev Biol Anim 2016; 52: 953-960.

58) Condorelli RA, La Vignera S, Di Bari F, Unfer V, Calogero AE. Effects of myoinositol on sperm mitochondrial function in-vitro. Eur Rev Med Pharmacol Sci 2011; 15: 129-134.

59) Condorelli RA, La Vignera S, Bellanca S, Vicari E, Calogero AE. Myoinositol: does it improve sperm mitochondrial function and sperm motility? Urology 2012; 79: 1290-1295.

60) Saleh RA, Agarval A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, Nelson DR, Thomas AJ. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 2003; 79: 1597-1605.

61) Wang X, Sharma RK, Sikka SC, Thomas AJ Jr, Falcone T, Agarwal A. Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility. Fertil Steril 2003; 80: 531-535.