

Myo-inositol as a male fertility molecule: speed them up!

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Abstract. – Myo-inositol (MYO) usually represents a therapeutic option for female infertility associated with insulin resistance. Recently, several evidences are accumulating about the potential use of MYO for the treatment of male infertility. This article summarizes the rationale for MYO in the treatment of male infertility. In particular, it illustrates the potential antioxidant and prokinetic role of MYO, and its importance for the modulation of hormonal regulation. In the final part of the manuscript has been added a proposal for a clinical algorithm reserved for patients with asthenozoospermia, where probably MYO could exert specific pharmacological effects.

Key Words

Sperm parameters, Male infertility, Myoinositol, Reactive oxygen species, ICSI

Male Infertility and Reactive Oxygen Species

Infertility is a worldwide problem with a negative medical and psychosocial impact on couples in reproductive age¹. The male partner, alone or in association with the female partner, contributes to about 50% of couple infertility². Conditions such as varicocele, cryptorchidism, and hypogonadism are among the many causes of male infertility. Nevertheless, a significant proportion of male infertility is undiagnosed despite an extensive diagnostic workout. This is referred to as idiopathic infertility which is characterized by sperm parameters below the World Health Organization (WHO) reference values³⁻⁵: i.e. oligozoospermia, asthenozoospermia and/or teratozoospermia (OAT). Idiopathic infertility is found in about 25-30% of infertile patients⁶.

One of the factors that may cause male infertility is the overproduction of reactive oxygen species (ROS). ROS are represented by a broad spectrum of molecules including: a) oxygen free radicals, such as superoxide anion (O₂⁻), hydroxyl radical (OH) and hyperoxyl radical (HOO); b) non-radical species, such as hypochlorous acid (HOCl) and hydrogen peroxide (H₂O₂); and c) reactive nitrogen species and free nitrogen radicals such as nitroxyl ion, nitrous oxide, peroxyxynitrite, etc.

Several conditions increase the oxidative stress in seminal fluid. These include pathological conditions involving the reproductive tract (varicocele, prostatitis)⁷⁻¹²; some life styles (smoking, alcohol abuse, drug addiction)¹³⁻¹⁶; environmental pollution (radiation, smog, industrial gasses) and nutritional errors (unbalanced hyperlipidic diet, etc.)¹⁷⁻¹⁹.

The role of ROS in the pathophysiology of human sperm function has been emphasized in recent years. The most prolific source of ROS in sperm suspensions is a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase located in leukocytes or in spermatozoa that produces superoxide that is further converted to peroxide by the action of superoxide dismutase. Hydrogen peroxide has been recognized as the most toxic oxidizing species for human spermatozoa, which are very sensitive to lipid peroxidation owing to the high content of polyunsaturated fatty acids in their plasma membrane, though this is not the sole mechanism through which sperm function might be impaired by ROS²⁰.

Spermatozoa are more susceptible than other cell types to the detrimental activity of these chemical compounds. In particular, ROS can affect motility, morphology and DNA stability of spermatozoa. Mitochondria are the organ-

elles producing the energy (adenosine triphosphate, ATP) required by spermatozoa for the performance of their function. ROS may induce changes in mitochondrial membranes and may thus compromise sperm function²¹. It has been shown that mitochondrial membrane potential (MMP) and ROS levels are inversely correlated. Such relationship could be due to two mutually interconnected phenomena: on the one hand, ROS causing damage to the mitochondrial membrane and, on the other hand, the damaged mitochondrial membrane causing increased ROS production²².

Spermatozoa, as well as critical phases of spermiogenesis, are particularly susceptible to ROS-induced damages for several reasons. These include: a) sperm chromatin condensation is a process that is very susceptible to increased oxidative stress; b) spermatozoa have very low DNA repair mechanisms; c) the sperm membrane contains high levels of poly unsaturated fatty acids; d) spermatozoa themselves produce ROS, especially during passage through epididymis; e) spermatozoa have low levels of cytoplasmic antioxidant enzymes (because most of the antioxidant enzymes are lost in spermiogenesis); and f) spermatozoa spend long periods as isolated cells in both male and female genital tracts²³⁻²⁶.

Spermatozoa acquire motility during the epididymal transit, becoming able to migrate from the vagina to the Fallopian tubes, to penetrate the cumulus oophorus and to carry out all those processes involved in fertilization²⁷. At first, after penetrating the cumulus oophorus of the ovum, the spermatozoon binds to the zona pellucida with its plasma membrane intact; then, it undergoes the acrosome reaction²⁸. This results in the release of hydrolytic enzymes that digest the zona pellucida, allowing to the spermatozoon to penetrate into the oocyte and to fertilize it²⁹. All these events are allowed by inositols and particularly by increasing intracellular Ca⁺⁺ release through inositol-gated channels because of the protein kinase PKC activation³⁰.

Myoinositol

Inositol is a polyalcohol, naturally occurring as nine stereoisomers, including D-chiro-inositol (DCI) and myo-inositol (MYO) that belongs to the vitamin B complex group. MYO plays a crucial role in cell morphogenesis and cytogenesis;

it is involved in cell membrane formation, lipid synthesis, cell growth and in several systemic processes and mechanisms of signal transduction in the plasma membrane as a precursor of second messengers³¹.

MYO is an important precursor for the phosphatidyl-inositol (PtdIns) signaling pathway. Inositol incorporated into PtdIns is converted successively to the polyphosphoinositides, PtdInsP and PtdIns(4,5)P₂. Under specific stimuli, PtdIns(4,5)P₂ is hydrolyzed to produce two second messengers, diacylglycerol (DAG) and Ins(1,4,5)P₃ which respectively modulate specific protein phosphorylation process and intracellular Ca⁺⁺ concentration³².

Despite their small size and structural simplicity, mounting evidence indicates that spermatozoa possess a sophisticated mechanism for regulation of cytoplasmic Ca⁺⁺ concentration^{33,34}. Recent studies speculate about the presence of two intracellular Ca⁺⁺ stores and one sperm-specific Ca⁺⁺-permeable channel (CatSper) in the plasma membrane of the flagellar principal piece³⁵⁻³⁹. One of the Ca⁺⁺-permeable channels present in the Ca⁺⁺ storage organelles of spermatozoa is the Ins(1,4,5)P₃-sensitive Ca⁺⁺ channel [commonly called Ins(1,4,5)P₃R] that has been studied extensively in a variety of cell types including sperm [40]. This channel binds the second messenger inositol 1,4,5-triphosphate [Ins(1,4,5)P₃], which leads to elevation of intracellular Ca⁺⁺ concentration⁴¹. Immunolocalization experiments showed that two Ins(1,4,5)P₃R-containing Ca⁺⁺ stores are present in the sperm, one in the acrosome and the other, a much smaller Ca⁺⁺ store, located within the redundant nuclear envelope at the back of the head^{36,42-44}.

Myoinositol and Male Reproduction

MYO concentration is significantly higher in the seminiferous tubules than in serum⁴⁵. In male reproductive organs, MYO is mainly produced by Sertoli cells in response to follicle-stimulating hormone (FSH) and is involved in processes that include the regulation of motility, capacitation and acrosome reaction of sperm cells⁴⁶.

It has been suggested that MYO may play a role in the osmoregulation of seminal fluid. Indeed, both hypo- and hyper-osmotic media have been found to significantly decrease sperm progressive motility and velocities⁴⁷. An

increased amount of a particular enzyme, Inositol-1 monophosphatase (IMPA-1), involved in the dephosphorylation of PtdIns, has been detected in asthenozoospermic patients⁴⁸. Therefore, it is possible that increased expression of IMPA-1 in asthenozoospermic patients could alter PtdIns signaling pathway and therefore induce a reduction in sperm motility. This sustains the role of signal transduction pathways induced by PtdIns in the regulation and maintenance of male germ cell motility⁴⁹.

Few studies have investigated the role of MYO as a possible antioxidant agent both for the systemic treatment of male infertility and for the improvement in the *in-vitro* quality of the sperm used for the fertilization applied to medically-assisted reproductive procedures. An initial study has shown that the spermatozoa of patients with OAT are covered by amorphous fibrous material that increases seminal fluid viscosity and reduces sperm motility. Furthermore, the mitochondria in the intermediate tract of spermatozoa of patients with OAT had damaged cristae. However, after incubation with inositol, the amorphous fibrous material disappeared, and cristae damage decreased⁵⁰.

At a functional level, MYO acts directly on mitochondria increasing the membrane potential⁵¹. MMP is an apoptotic marker clearly related to the functional parameters of the sperm cells, including motility, the capacity of fertilization and embryo quality, and is therefore used as an index of fertility^{52,53}. High values of MMP indicate the integrity of this structure with optimal levels of activity and are associated with high cell viability.

These morpho-functional data have recently been confirmed by evidence suggest that spermatozoa from patients with OAT incubated with MYO have a significantly higher sperm motility and a higher MMP, probably increasing cytosolic Ca⁺⁺ and consequently inner mitochondrial Ca⁺⁺. In particular, a high percentage of sperm with low MMP represents a very important index of impaired sperm mitochondrial function, and MYO decreases this percentage. Moreover, when comparing patients with OAT and healthy subjects, it was found that MYO might have a stimulatory function in OAT sperm and a protective function in normal semen. These findings suggest that MYO could be used *in-vitro* in assisted reproductive techniques (ART), both to increase the number of spermatozoa to be used for intrauterine in-

semination and to ameliorate the sperm quality to be used for *in-vitro* ART^{54,55}.

As a consequence, MYO treatment of spermatozoa with different MMP level might have produced different results in fertilization rate following *in-vitro* fertilization (IVF)^{52,53,56}.

Rubino et al⁵⁷ reported that the oocyte fertilization rate after Intracytoplasmic Sperm Injection (ICSI) may be significantly increased when spermatozoa are treated and prepared with MYO compared with placebo supplemented media. They have also observed a statistically significant higher percentage of grade A embryos on day 3 in the MYO group. Furthermore, the efficacy and safety of MYO were investigated in a double-blind, randomized, placebo-controlled trial on 194 patients with idiopathic infertility. After 3 months of treatment, the results of this study showed that MYO is a safe supplement able to significantly rebalance serum gonadotropin and inhibin B levels and to increase sperm parameters in patients with idiopathic infertility⁵⁸. However, a recent *in vivo* study has shown that exogenous administration of MYO significantly improves sperm parameters (seminal fluid volume and sperm number) both in patients with oligo-asthenozoospermia and in normal fertile men but there was no motility improvement in both groups after treatment⁵⁹.

MYO in the Andrological Clinical Practice (Therapeutic Aspects)

Based on the evidences illustrated in this article, we suggest a specific use of MYO in infertile patients with asthenozoospermia⁵⁴. In this case, it is proper to distinguish between patients with absolute asthenozoospermia, who are candidate to ICSI, and patients with relative asthenozoospermia, who need to undergo an accurate diagnostic workup⁶⁰. This is aimed at excluding the following pathological factors: inflammation of the male accessory glands or leukocytospermia⁶¹, papillomavirus infection⁶², increased seminal fluid viscosity⁶³, significant decrease of the testicular volume or of the hormonal serum levels and altered accessory gland secretory function^{61,64}. Once having excluded all these, we suggest to proceed carrying on the evaluation of the MMP and to prescribe treatment with MYO to those patients who have low sperm MMP. This algorithm is illustrated in Figure 1.

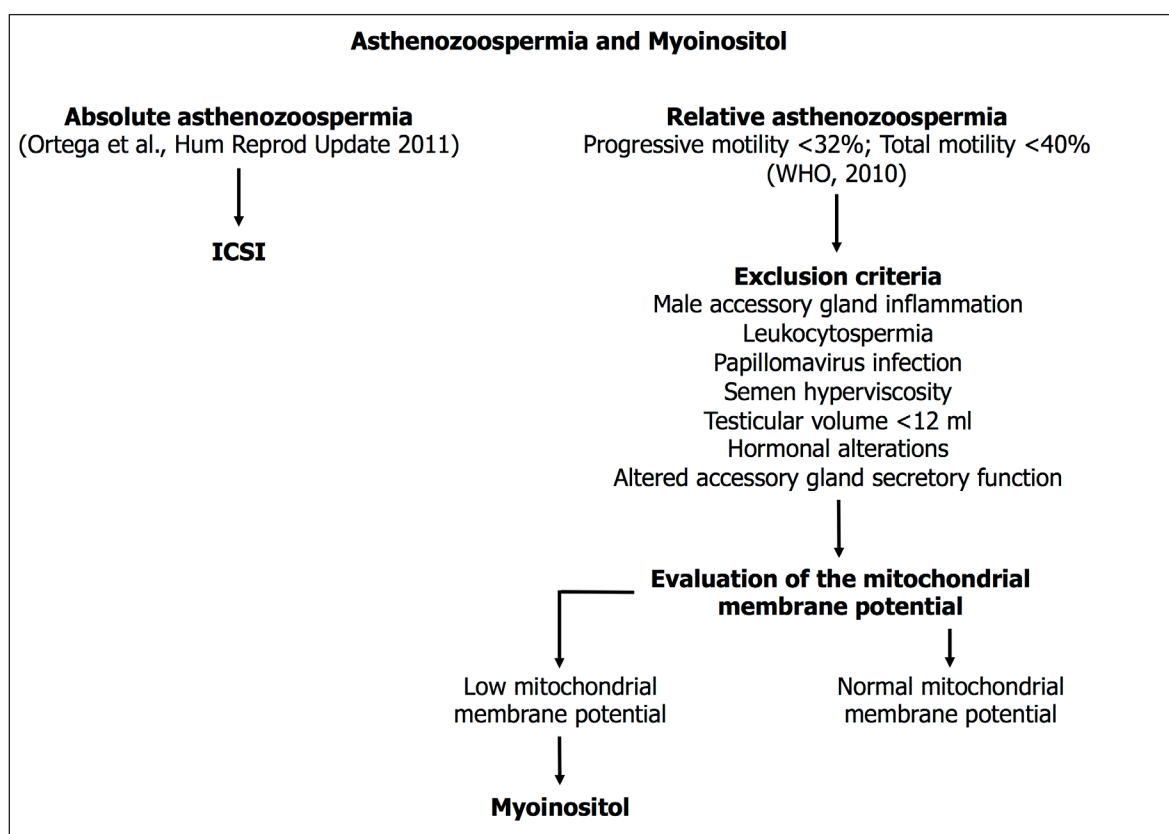


Figure 1. Possible use of MYO it patients with asthenozoospermia.

Conclusions

These data suggest that inositol is able to improve the sperm mitochondrial function, thereby improving sperm motility in patients with altered sperm parameters. Hence, patients with idiopathic infertility could take advantage by MYO supplementation. There is a need to encourage further research on all the possible benefits of MYO supplementation of culture media for human IVF procedures because the improvement of culture conditions still represents one of the main goals of the human ART research.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) FISHER JR, HAMMARBERG K. Psychological and social aspects of infertility in men: an overview of the evidence and implications for psychologically informed clinical care and future research. *Asian J Androl* 2012; 14: 121-129.
- 2) AGARWAL A, MULGUND A, HAMADA A, CHYATTE MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* 2015; 13: 37.
- 3) SIDDIQ FM, SIGMAN M. A new look at the medical management of infertility. *Urol Clin North Am* 2002; 29: 949-963.
- 4) DENG Y, ZHANG W, SU D, YANG Y, MA Y, ZHANG H, ZHANG. Some single nucleotide polymorphisms of MSY2 gene might contribute to susceptibility to spermatogenic impairment in idiopathic infertile men. *Urology* 2008; 71: 878-882.
- 5) WORLD HEALTH ORGANIZATION. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edn. World Health Organization, Geneva, Switzerland, 2010.
- 6) QUAAS A, DOKRAS A. Diagnosis and treatment of unexplained infertility. *Rev Obstet Gynecol* 2008; 1: 69-76.
- 7) PASQUALOTTO FF, SUNDARAM A, SHARMA RK, BORGES E JR, PASQUALOTTO EB, AGARWAL A. Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. *Fertil Steril* 2008; 89: 602-607.
- 8) HENDIN BN, KOLETTIS PN, SHARMA RK, THOMAS AJ JR, AGARWAL A. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J Urol* 1999; 161: 1831-1834.
- 9) POTTS JM, PASQUALOTTO FF. Seminal oxidative stress in patients with chronic prostatitis. *Andrologia* 2003; 35: 304-308.

- 10) LA VIGNERA S, CALOGERO AE, CANNIZZARO MA, VICARI E. Mono or bilateral inflammatory postmicrobial prostatitis-epididymitis: differences in semen parameters and reactive oxygen species production. *Minerva Endocrinol* 2006; 31: 263-272.
- 11) LA VIGNERA S, CONDORELLI RA, VICARI E, TUMINO D, MORGIA G, FAVILLA V, CIMINO S, CALOGERO AE. Markers of semen inflammation: supplementary semen analysis? *J Reprod Immunol* 2013; 100: 2-10.
- 12) CASTIGLIONE R, SALEMI M, VICARI LO, VICARI E. Relationship of semen hyperviscosity with IL-6, TNF- α , IL-10 and ROS production in seminal plasma of infertile patients with prostatitis and prostatitis-epididymitis. *Andrologia* 2014; 46: 1148-1155.
- 13) KIZILER AR, AYDEMIR B, ONARAN I, ALICI B, OZKARA H, GULYASAR T, AKYOLCU MC. High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biol Trace Elem Res* 2007; 120: 82-91.
- 14) SALEH RA, AGARWAL A, SHARMA RK, NELSON DR, THOMAS AJ JR. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril* 2002; 78: 491-499.
- 15) KOCH OR, PANI G, BORRELLO S, COLAVITTI R, CRAVERO A, FARRÈ S, GALEOTTI T. Oxidative stress and antioxidant defenses in ethanol-induced cell injury. *Mol Aspects Med* 2004; 25: 191-198.
- 16) WU D, CEDERBAUM AI. Alcohol, oxidative stress, and free radical damage. *Alcohol Res Health* 2003; 27: 277-284.
- 17) CHITRA KC, SUJATHA R, LATCHOUMYANDANE C, MATHUR PP. Effect of lindane on antioxidant enzymes in epididymis and epididymal sperm of adult rats. *Asian J Androl* 2001; 3: 205-208.
- 18) LATCHOUMYANDANE C, MATHUR PP. Induction of oxidative stress in the rat testis after short-term exposure to the organochlorine pesticide methoxychlor. *Arch Toxicol* 2002; 76: 692-698.
- 19) ESKENAZI B, KIDD SA, MARKS AR, SLOTER E, BLOCK G, WYROBEK AJ. Antioxidant intake is associated with semen quality in healthy men. *Hum Reprod* 2005; 20: 1006-1012.
- 20) GRIVEAU JF, LE LANNOU D. Reactive oxygen species and human spermatozoa: physiology and pathology. *Int J Androl* 1997; 20: 61-69.
- 21) ARMSTRONG JS, RAJASEKARAN M, CHAMULITRAT W, GATTI P, HELLSTROM WJ, SIKKA SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. *Free Radic Biol Med* 1999; 26: 869-880.
- 22) WANG X, SHARMA RK, GUPTA A, GEORGE V, THOMAS AJ, FALCONE T, AGARWAL A. Alterations in mitochondrial membrane potential and oxidative stress in infertile men: a prospective observational study. *Fertil Steril* 2003; 80: 844-850.
- 23) AGARWAL A, MAKKER K, SHARMA R. Clinical relevance of oxidative stress in male factor infertility: an update. *Am J Reprod Immunol* 2008; 59: 2-11.
- 24) LEWIS S, AITKEN R. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue Res* 2005; 322: 33-41.
- 25) CHENOWETH P. Influence of the male on embryo quality. *Theriogenology* 2007; 68: 308-315.
- 26) PRAKASH S, PRITHIVIRAJ E, SURESH S, LAKSHMI NV, GANESH MK, ANURADHA M, GANESH L, DINESH P. Morphological diversity of sperm: a mini review. *Iran J Reprod Med* 2014; 12: 239-242.
- 27) BEAUCHAMP PJ, GALLE PC, BLASCO L. Human sperm velocity and post insemination cervical mucus test in the evaluation of the infertile couple. *Arch Androl* 1984; 13: 107-112.
- 28) KOPF GS, GERTON GL. The mammalian sperm acrosome and the acrosome reaction. In: *Elements of Mammalian Fertilization* (eds PM Wasserman) CRC Press, Boston, MA, 1991; pp. 153-203.
- 29) BREITBART H, SPUNGIN B. The biochemistry of the acrosome reaction. *Mol Hum Reprod* 1997; 3: 195-202.
- 30) ROLDAN ER, SHI QX. Sperm phospholipases and acrosomal exocytosis. *Front Biosci* 2007; 12: 89-104.
- 31) MARAT AL, HAUCKE V. Phosphatidylinositol 3-phosphates-at the interface between cell signalling and membrane traffic. *Embo J* 2016; 35: 561-579.
- 32) BERRIDGE MJ. Inositol lipids and cell proliferation. *Biochim Biophys Acta* 1987; 907: 33-45.
- 33) JIMENEZ-GONZALEZ C, MICHELANGELI F, HARPER CV, BARRATT CL, PUBLICOVER SJ. Calcium signalling in human spermatozoa: a specialized 'toolkit' of channels, transporters and stores. *Hum Reprod Update* 2006; 12: 253-267.
- 34) ALASMARI W, BARRATT CL, PUBLICOVER SJ, WHALLEY KM, FOSTER E, KAY V, MARTINS DA SILVA S, OXENHAM SK. The clinical significance of calcium-signalling pathways mediating human sperm hyperactivation. *Hum Reprod* 2013; 28: 866-876.
- 35) HO HC, SUAREZ SS. An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca²⁺ store is involved in regulating sperm hyperactivated motility. *Biol Reprod* 2001; 65: 1606-1615.
- 36) HO HC, SUAREZ SS. Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. *Biol Reprod* 2003; 68: 1590-1596.
- 37) HARPER CV, BARRATT CL, PUBLICOVER SJ. Stimulation of human spermatozoa with progesterone gradients to simulate approach to the oocyte. Induction of [Ca²⁺]_i oscillations and cyclical transitions in flagellar beating. *J Biol Chem* 2004; 279: 46315-46325.
- 38) COSTELLO S, MICHELANGELI F, NASH K, LEFIEVRE L, MORRIS J, MACHADO-OLIVEIRA G, BARRATT C, KIRKMAN-BROWN J, PUBLICOVER S. Ca²⁺ stores in sperm: their identities and functions. *Reproduction* 2009; 138: 425-437.
- 39) LISHKO PV, KIRICHOK Y, REN D, NAVARRO B, CHUNG JJ, CLAPHAM DE. The control of male fertility by spermatozoan ion channels. *Annu Rev Physiol* 2012; 74: 453-475.
- 40) VERMASSEN E, PARYS JB, MAUGER JP. Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants. *Biol Cell* 2004; 96: 3-17.
- 41) MICHELANGELI F, MEZNA M, TOVEY S, SAYERS LG. Pharmacological modulators of the inositol 1,4,5-trisphosphate receptor. *Neuropharmacology* 1995; 34: 1111-1122.
- 42) WALENSKY LD, SNYDER SH. Inositol 1,4,5-trisphosphate receptors selectively localized to the acrosomes of mammalian sperm. *J Cell Biol* 1995; 130: 857-869.
- 43) KURODA Y, KANEKO S, YOSHIMURA Y, NOZAWA S, MIKOSHIBA K. Are there inositol 1,4,5-triphosphate (IP₃) receptors in human sperm? *Life Sci* 1999; 65: 135-143.

- 44) NAABY-HANSEN S, WOLKOWICZ MJ, KLOTZ K, BUSH LA, WESTBROOK VA, SHIBAHARA H, SHETTY J, COONROD SA, REDDI PP, SHANNON J, KINTER M, SHERMAN NE, FOX J, FLICKINGER CJ, HERR JC. Co-localization of the inositol 1,4,5-trisphosphate receptor and calreticulin in the equatorial segment and in membrane bounded vesicles in the cytoplasmic droplet of human spermatozoa. *Mol Hum Reprod* 2001; 7: 923-933.
- 45) CHAUVIN TR, GRISWOLD MD. Characterization of the expression and regulation of genes necessary for MYO biosynthesis and transport in the seminiferous epithelium. *Biol Reprod* 2004; 70: 744-751.
- 46) BEVILACQUA A, CARLOMAGNO G, GERLI S, MONTANINO OLIVA M, DEVROEY P, LANZONE A, SOULANGE C, FACCHINETTI F, CARLO DI RENZO G, BIZZARRI M, HOD M, CAVALLI P, D'ANNA R, BENVENGA S, CHIU TT, KAMENOV ZA. Results from the International Consensus Conference on myo-inositol and D-chiro-inositol in Obstetrics and Gynecology--assisted reproduction technology. *Gynecol Endocrinol* 2015; 31: 441-446.
- 47) LIU DY, CLARKE GN, BAKER HW. Hyper-osmotic condition enhances protein tyrosine phosphorylation and zona pellucida binding capacity of human sperm. *Hum Reprod* 2006; 21: 745-752.
- 48) CRYNS K, SHAMIR A, VAN ACKER N, LEVI I, DANEELS G, GORIS I, BOUWKNECHT JA, ANDRIES L, KASS S, AGAM G, BELMAKER H, BERSUDSKY Y, STECKLER T, MOECHARS D. IMPA1 is essential for embryonic development and lithium-like pilocarpine sensitivity. *Neuropsychopharmacology* 2008; 33: 674-684.
- 49) MARTINEZ-HEREDIA J, DE MATEO S, VIDAL-TABOADA JM, BALLESCA JL, OLIVA R. Identification of proteomic differences in asthenozoospermic sperm samples. *Hum Reprod* 2008; 23: 783-791.
- 50) 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.
- 51) 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.
- 52) MARCHETTI C, JOUY N, LEROY-MARTIN B, DEFOSSEZ A, FORMSTECHEP P, MARCHETTI P. Comparison of four fluorochromes for the detection of the inner mitochondrial membrane potential in human spermatozoa and their correlation with sperm motility. *Hum Reprod* 2004; 19: 2267-2276.
- 53) MARCHETTI P, BALLOT C, JOUY N, THOMAS P, MARCHETTI C. Influence of mitochondrial membrane potential of spermatozoa on in vitro fertilisation outcome. *Andrologia* 2012; 44: 136-141.
- 54) 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.
- 55) ARTINI PG, CASAROSA E, CARLETTI E, MONTELEONE P, DI NOIA A, DI BERARDINO OM. In vitro effect of myo-inositol on sperm motility in normal and oligoasthenospermia patients undergoing in vitro fertilization. *Gynecol Endocrinol* 2016; 2: 1-4.
- 56) MARCHETTI C, OBERT G, DEFFOSEZ A, FORMSTECHEP 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.
- 57) RUBINO P, PALINI S, CHIGIONI S, CARLOMAGNO G, QUAGLIARIELLO A, DE STEFANI S, BAGLIONI A, BULLETTI C. Improving fertilization rate in ICSI cycles by adding myoinositol to the semen preparation procedures: a prospective, bicentric, randomized trial on sibling oocytes. *J Assist Reprod Genet* 2015; 32: 387-394.
- 58) CALOGERO AE, GULLO G, LA VIGNERA S, CONDORELLI RA, VAIARELLI A. Myoinositol improves sperm parameters and serum reproductive hormones in patients with idiopathic infertility: a prospective double-blind randomized placebo-controlled study. *Andrology* 2015; 3: 491-495.
- 59) GULINO FA, LEONARDI E, MARILLI I, MUSMECI G, VITALE SG, LEANZA V, PALUMBO MA. Effect of treatment with myo-inositol on semen parameters of patients undergoing an IVF cycle: in vivo study. *Gynecol Endocrinol* 2016; 32: 65-68.
- 60) ORTEGA C, VERHEYEN G, RAICK D, CAMUS M, DEVROEY P, TOURNAYE H. Absolute asthenozoospermia and ICSI: what are the options? *Hum Reprod Update* 2011; 17: 684-692.
- 61) LA VIGNERA S, VICARI E, CONDORELLI RA, D'AGATA R, CALOGERO AE. Male accessory gland infection and sperm parameters (review). *Int J Androl* 2011; 34: 330-347.
- 62) LA VIGNERA S, VICARI E, CONDORELLI RA, FRANCHINA C, SCALIA G, MORGIA G, PERINO A, SCHILLACI R, CALOGERO AE. Prevalence of human papilloma virus infection in patients with male accessory gland infection. *Reprod Biomed Online* 2015; 30: 385-391.
- 63) LA VIGNERA S, CONDORELLI RA, VICARI E, D'AGATA R, SALEMI M, CALOGERO AE. Hyperviscosity of semen in patients with male accessory gland infection: direct measurement with quantitative viscosimeter. *Andrologia* 2012; 44: 556-559.
- 64) CONDORELLI R, CALOGERO AE, LA VIGNERA S. Relationship between testicular volume and conventional or nonconventional sperm parameters. *Int J Endocrinol* 2013; 2013: 145792.