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REVIEW ARTICLE

Myo-inositol in health and disease: its impact on semen parameters and male fertility

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ABSTRACT

Background: Myo-inositol (cis-1,2,3,5-trans-4,6-cyclohexanehexol; MI) is the most prominent of nine inositol stereoisomers. MI, its phosphate derivatives, and associated lipids are widely found in vegetables and animal tissues and are known to participate in numerous biological processes.

Objectives: To perform a review analysis on MI presence, functions, and impact in male fertility.

Materials and Methods: A thorough search of listed publications in PubMed on MI and its derivatives was done.

Results: Published information was found and compiled on MI identification, natural dietary sources and absorption, biosynthesis, concentrations, as well as MI as its derivatives (PI, PIP, GPI, IPG) roles in several human tissues and body fluids in health and disease. A section was focused on MI presence, biosynthesis, and functions in the mammalian male genital tract and in spermatozoa, and summarized reports describing the impact of *in vivo* and *in vitro* MI supplementation on human semen quality and fertility. Studies reported a discrete improvement in sperm motility in fresh and frozen-thawed semen, and a better sperm performance in natural and assisted fertility.

Discussion and Conclusion: MI was reported as an effective supplement for sperm quality. In any case, several study designs lack appropriate controls or data analysis to confirm the relevance of the findings. While promising, larger prospective randomized controlled studies will be required to confirm the positive effect of MI supplementation in male infertility management. Moreover, further investigations are encouraged to unravel MI roles in sperm physiology and the underlying molecular mechanisms.

INOSITOL SOURCES, BIOSYNTHESIS, AND ABSORPTION

Inositol is a sixfold alcohol (polyol) of cyclohexane, also called cyclohexane-1,2,3,4,5,6-hexol, with five equatorial and one axial hydroxyl (OH) groups. Its formula is $C_6H_{12}O_6$ or $(\text{-CHOH-})_6$ (Fig. 1). Inositol exists in nine stereoisomers, resulting from epimerization of the six OH- groups (cis-, epi-, allo-, myo-, neo-, scyllo-, L-*ch*iro-, D-chiro-, and muco-inositol; Murthy, 2006; Thomas *et al.*, 2016). The most prominent form widely occurring in nature is cis-1,2,3,5-trans-4,6-cyclohexanehexol, or myo-inositol (MI[1]) (former name 'meso-inositol'), followed by D-chiro-inositol (DCI) (Posternak, 1942; Fig. 1).

MI was first isolated in 1850 from Liebig's meat extracts by J.J. Scherer, who also determined its empirical formula (Scherer, 1850). Scherer named MI from the ancient Greek stem of ἰς (is, in-, 'sinew, fiber'), -ose (indicating 'a carbohydrate'), -ite ('ester'), and -ol ('an alcohol'). In the following years, other investigators further evaluated MI presence in animals and plants, reported several methods to extract it, and evaluated its importance in nature (Cloëtta, 1856; Müller, 1857; Copper-Lane, 1861; Marmé, 1864). Based on its empirical formula, these reports related MI to sugars; in fact, it was named the 'muscle-sugar', since muscle was one of its main sources. However, Maquenne showed in 1887 that MI is not a sugar but a cyclic hexamethylene compound, or hexahydroxyhydrobenzene; it also established its cyclohexanol structure, and reported its purification from leaves (Maquenne, 1887a,b,c). Around 30 years later,

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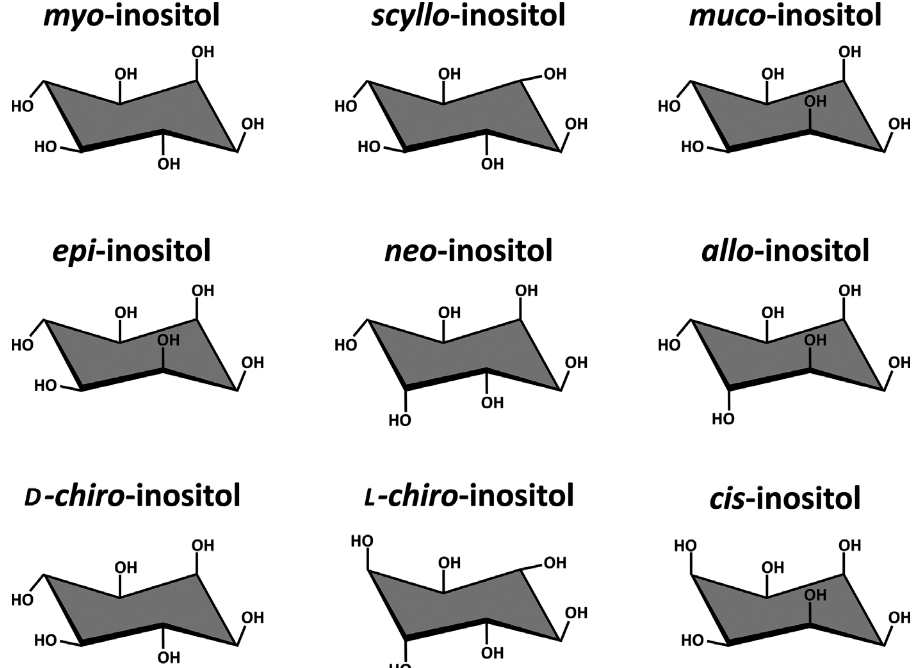
Figure 1 Inositol stereoisomers. Chair conformation of the nine stereoisomers of inositol: *myo-, scyllo-, muco-, epi-, neo-, allo-, D-chiro-, L-chiro-, and cis-*inositol.



Needham described a procedure to quantify MI in animal tissues and determined its levels in a set of rabbit tissues; moreover, it showed identical characteristics between muscle MI and phytin (Needham, 1923).

It has been established that MI, its phosphate derivatives, and associated lipids are present in different food types (corns, beans, fruits, nuts) and are widely distributed in several animal tissues (Clements & Darnell, 1980; Schlemmer *et al.*, 2009; Dinicola *et al.*, 2017). MI is mainly present in animals in its free form or as phosphatidylinositol (PI); in breast milk, is found in its free form and as 6-β-galactinol (Naccarato & Wells, 1974). In vegetables, MI is found as phytic acid (inositol hexakisphosphate, inositol-P6), which contains six phosphate groups partially ionized at physiological pH (Holub, 1986). Phytic acid is the phosphorus main form stored in many plant tissues, especially bran, seeds, legumes, and oil, and has several functions with significant nutritional roles (Dinicola *et al.*, 2017). Western diets contain less phytate because of massive refinement of grains and rice; for this reason, MI diet contribution is approximately 1 g/day (Goodhart, 1973). Despite the presence of plant food phytases, dietary phytic acid is converted, after oral ingestion, to free MI, orthophosphate, and intermediate forms (mono-, di-, tri-, tetra-, and penta-phosphate esters of inositol) by bacterial phytases and phosphatases during food processing in the large intestine. These enzymes are homologous to mammalian inositol-P6 phosphatase (MINPP) and are responsible for dietary inositol-P6 digestion (Sandberg & Anderson, 1988; Schlemmer *et al.*, 2001; Schlemmer *et al.*, 2009; Stentz *et al.*, 2014). MI is absorbed by the apical membrane of intestinal epithelial cells and reabsorbed in the renal proximal tubes (Bissonnette *et al.*, 2004). Several molecules, such as glucose, modulate MI uptake (Haneda *et al.*, 1990; Cammarata *et al.*, 1992); moreover, *in vitro* and *in vivo* results support an increased MI intestinal absorption when combined with alpha-lactalbumin administration (Monastra *et al.*, 2018).

In addition to dietary ingestion, MI is synthesized by mammalian tissues, including brain, liver, kidney, mammary gland, and testis. The ability of mammals to convert D-glucose to MI was first reported in studies done in inositol-dependent (human: KB and HeLa) and inositol-independent (mouse L-929) cell cultures (Eagle *et al.*, 1960). Later, it was found that brain, liver, and kidney slices could convert glucose to MI (Hauser & Finelli, 1963). MI biosynthesis starts with glucose 6-phosphate, the first glycolysis product, and comprises an isomerization reaction to inositol-3-phosphate (Ins3P) by the inositol-3-phosphate synthase (EC:5.5.1.4; IPS[2]). IPS requires NAD^+ and involves a two-step reaction of oxidation and reduction. Presently, the genomes of over one hundred organisms, including humans, contain annotated orthologs that encode IPS. Ins3P is then dephosphorylated into inositol by the inositol monophosphatase 1 (EC:3.1.3.25; IMP 1 or IMPase 1; Fig. 2). IPS is encoded by the *ISYNA1* gene and IMP 1 by the *IMPA1* gene; their transcripts are expressed in numerous tissues, as reported in a panel of the Human Protein Atlas (Fig. 3A, 3B). In addition to IMPA1, another IMPA transcript was first identified in humans, named IMPA2, showing 54% amino acid identity to human *IMPA1*, and homology to IMPAs of other organisms; it is located on chromosome 18p11.2, a region related to susceptibility for bipolar disorders by linkage analysis (Yoshikawa *et al.*, 1997). MI may be also obtained by recycling inositol phosphates (Benjamin *et al.*, 2014).

Free MI plasma concentration in adult humans is 24.5 μM; in contrast, millimolar levels are detected in other mammalian tissues (Dawson & Freinkel, 1961; Hinton *et al.*, 1980; Holub, 1986; Michaelis *et al.*, 1993). MI is transported from the extracellular fluid to the cell in a temperature-, pH-, and energy-sensitive process (Schneider, 2015). There are three different active cotransporters that use Na^+ or H^+ as co-solutes: SMIT1 (solute carrier family 5 member, third member of the solute

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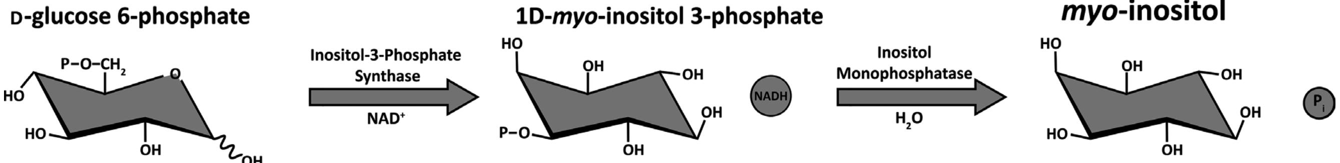
Figure 2 Inositol biosynthesis from D-glucose 6-phosphate. MI biosynthesis starts with isomerization of D-glucose 6-phosphate to 1D-*myo*-inositol 3-phosphate (Ins3P) by the inositol-3-phosphate synthase (IPS), which requires NAD + and involves a two-step oxidation/reduction reaction. The Ins3P is dephosphorylated by inositol monophosphatase (IMP 1) into *myo*-inositol (MI).



carrier family[3]) is a high-affinity, Na^+-dependent cotransporter (Kwon *et al.*, 1992; Matskevitch *et al.*, 1998), SMIT2 is kinetically similar but pharmacologically different (Coady *et al.*, 2002), and HMIT/SLC2A13 is the lower-affinity H^+-dependent transporter (Uldry *et al.*, 2001). SMIT1 is an integral membrane protein that transports 1 inositol with 2 Na^+; it was the first inositol transport system identified (Hager *et al.*, 1995), and it is encoded by the *SLC5A3* gene (Kwon *et al.*, 1992; Fig. 3C). It structurally resembles glucose transporters, characterized by inverted-repeat domains with many transmembrane segments (Gamba, 2001; Abramson & Wright, 2009). In mammals, both SMIT1 and SMIT2 react to osmotic imbalances (Isaacks *et al.*, 1994; Bissonnette *et al.*, 2008; Klaus *et al.*, 2008), but no osmoregulatory functions have been found in HMIT/SLC2A13. Mice deficient in *SLC5A3* ($SLC5A3^{-/-}$) have an inositol uptake reduction of 96%, embryo developmental abnormalities, and perinatal mortality, unless animals are fed with MI from birth (Buccafusca *et al.*, 2008). In line with these findings, increased MI levels, reported in different tissues under extracellular hypertonic conditions (Thurston *et al.*, 1989; Strange *et al.*, 1991; Trachtman *et al.*, 1991; Handler & Kwon, 1996), have been attributed to increased Na^+-dependent transport activity (Strange *et al.*, 1991; Handler & Kwon, 1996; Matsuoka *et al.*, 1999). Osmoregulatory elements have been reported to control *SLC5A3* gene transcriptional activity (Trachtman *et al.*, 1991; Handler & Kwon, 1996; Neuhofer *et al.*, 2002); specifically, expression of TonEBP (tonicity-responsive enhancer binding protein) transcription factor is upregulated under hypertonic conditions, and it is translocated to the cell nucleus.

MI biosynthesis may be subjected to positive and negative modulators; among them, the glycogen synthase kinase 3 (GSK3) is a positive regulator for optimal IPS activity, finding an inositol decrease when GSK3 activity is lost (Azab *et al.*, 2007). On the other hand, gender and epigenetic modulation by tissue-specific methylation (Seelan *et al.*, 2011) may act as negative modulators, as well as *ISYNA1* alternative splicing, which leads to the expression of an isoform that impairs its activity (Seelan *et al.*, 2009).

The amount of MI biosynthesized has been estimated in 4 g (22 nmol)/day in the adult kidney (Clements & Diethelm, 1979), far higher than dietary intake. It is found in large concentrations in thyroid, testis, liver, brain, spleen, pituitary gland, and kidney. Its excess is catabolically broken down in the kidney to yield O-glucuronic acid and oxylulose-5-phosphate that enter the pentose phosphate cycle, to finally be eliminated in the urine. In this regard, renal failure has been associated with abnormalities in MI metabolism and increased plasma levels (Pitkänen, 1976). An adequate MI supply is expected from biosynthesis and dietary intake, but MI may be altered due to food processing, biosynthesis impairment, absorption reduction, and excretion increase, as summarized by Dr. Dinicola and collaborators (Dinicola *et al.*, 2017).

INOSITOL DERIVATIVES

MI is the precursor of biosynthesis of phospholipids (phosphatidylinositol, PI) and phosphatidylinositides (phosphatidylinositol-phosphates, PIPs). In addition, it is involved in the synthesis of water-soluble inositol phosphates (IPs) (Fig. 4). Some PI is incorporated to PI glycan structures in the endoplasmic reticulum to form glycosylphosphatidylinositol (GPI) anchors, tethering many proteins to the plasma membrane, together with many other derivatives as inositol phosphoglycans (IPG) and inositol ethers and esters (Livermore *et al.*, 2016; Fig. 4).

PI is biosynthesized *de novo* by an enzymatic reaction mediated by the inositol phosphatidyl-transferase (PI synthetase, PIS) (Michell, 2008); then, PI may be phosphorylated to form phosphatidylinositol-4-phosphate (PIP), phosphatidylinositol-biphosphate (PIP2), and phosphatidylinositol-trisphosphate (PIP3). These phosphorylated PI-based lipids, collectively known as inositides or PIPs, are produced by a set of specific phosphoinositide kinases (PIKs), including phosphatidylinositol-5-phosphate 4-kinase (PIP4K), phosphatidylinositol-4-phosphate 5-kinase (PIPK1), and phosphatidylinositol-3-kinase (PI3K) (Schoepp, 1985). Since steric hindrance has been considered to hamper hydroxyl groups 2 and 6 to be phosphorylated, PIPs may be found in seven different combinations resulting from phosphorylation of 3, 4, and 5 hydroxyl groups in the MI ring. However, there are reports suggesting the presence of a larger molecular species array that differ by the acyl chain type attached to the glycerol backbone (Leevers *et al.*, 1999). Then, PI (3,4,5)P3 acts as second messenger, by recruiting Akt serine–threonine kinase (AKT) and PI-dependent kinase 1 (PDK1) to the plasma membrane. AKT is activated to pAKT by mTORC2 phosphorylation (Traynor-Kaplan *et al.*, 2017); the active form participates in a large and highly relevant number of cellular events.

Regarding inositol phosphate production, plants are able to phosphorylate inositol by means of an inositol kinase that yields IP3 (Ins-(3,4,5)-P3) (Sarbassov *et al.*, 2005). In humans, inositol phosphates are generated from phosphorylated forms by specific phosphatases, as well as from phosphoinositide hydrolysis (Dietz & Albersheim, 1965). Phospholipase C (PLC) metabolizes PIP2 (specifically PI-(4,5)-P2) into the intracellular second messengers 1,2-inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Thomas & Potter, 2014). In particular, binding of IP3 ligands to specific receptors (IP3R, type I-III isoforms) induces Ca^{2+} release from the endoplasmic reticulum to the cytoplasm (Nishizuka, 1986; Irvine, 1990). Then, Ins(1,4,5)P3 is rapidly

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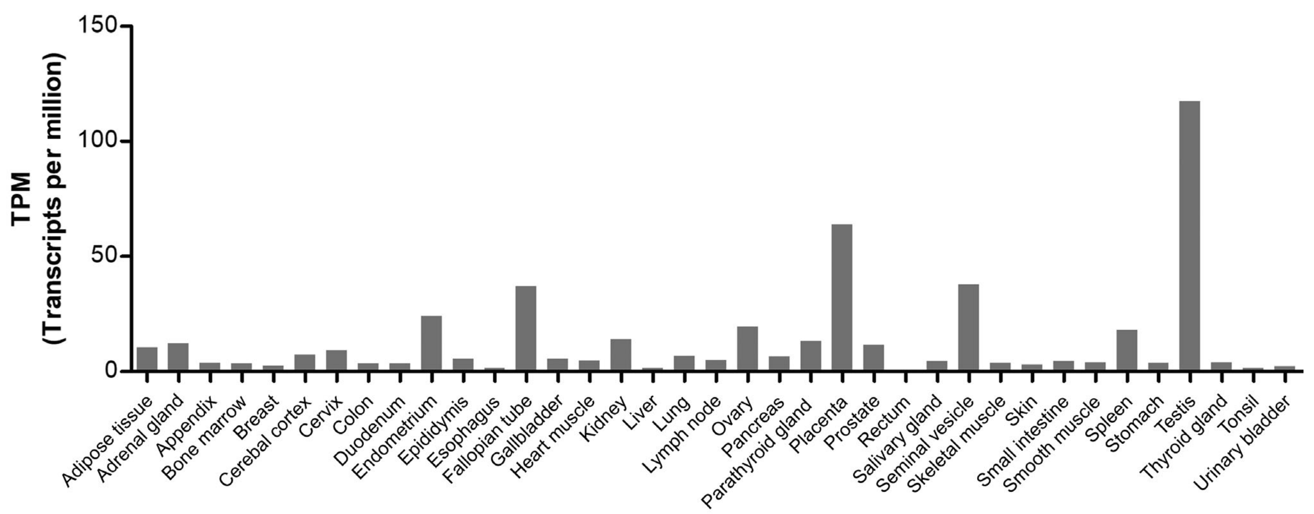
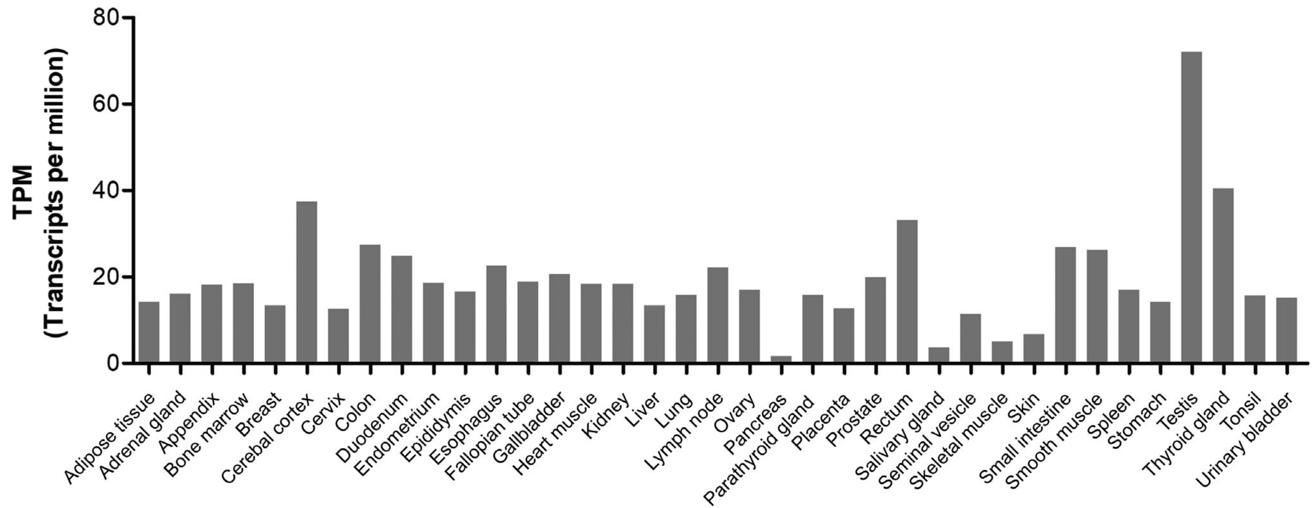
Figure 3 IMPA1, ISYNA1, and SLC5A3 expression in human tissues. RNA expression analysis of IMPA1 (top), ISYNA1 (middle), and SLC5A3 (bottom) in a large panel of human tissues (Data retrieved from Human Protein Atlas v18.1, https://www.proteinatlas.org/).





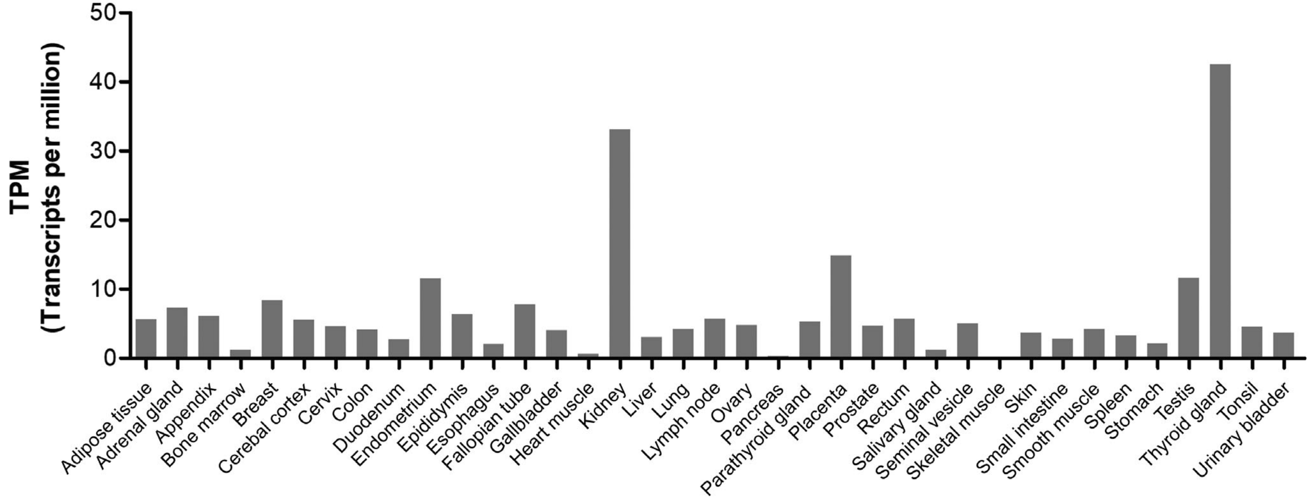


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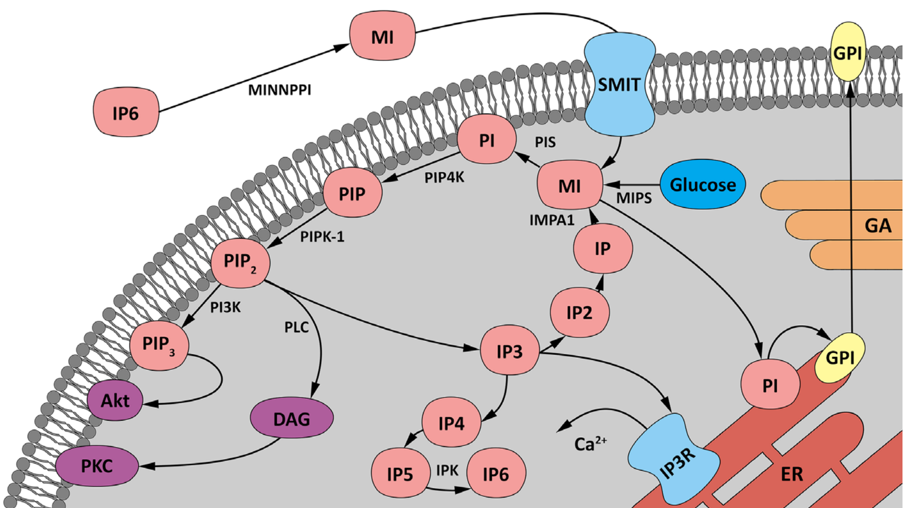
Figure 4 Inositol metabolism. Inositol is acquired by dietary ingestion of phytic acid, also known as inositol hexaphosphate (IP6). IP6 is metabolized into MI by the multiple inositol-polyphosphate phosphatase 1 (MINPP1) and then transported into the cytosol through the sodium–myo-inositol cotransporter (SMIT). Cytosolic glucose can also be used to synthesize MI through myo-inositol-phosphate synthase (MIPS). In the cytosol, MI can be metabolized into phosphatidylinositol (PI) by phosphatidylinositol synthetase (PIS). PI and its derivates are concentrated in membrane pools. PI can be further phosphorylated into phosphatidylinositol-(4)-monophosphate (PIP) by phosphatidylinositol-4-kinase (PIP4K), then into phosphatidylinositol (4,5)-biphosphate (PIP2) by phosphatidylinositol 4-phosphate 5-kinase 1 (PIPK1), and finally into phosphatidylinositol (3,4,5)-triphosphate (PIP3) by phosphoinositide 3-kinase (PI3K). Phospholipase C (PLC) metabolizes PIP2 into 1,2-diacylglycerol (DAG) and inositol (1,4,5)-triphosphate (IP3). In turn, DAG activates protein kinase C (PKC), recruiting it to the plasma membrane. Similarly, PIP3 recruits and activates Protein kinase B (Akt). On the other hand, IP3 is phosphorylated by inositol kinases (IPK) into inositol tetrakisphosphate (IP4), inositol pentakisphosphate (IP5), and inositol hexaphosphate (IP6) and dephosphorylated by inositol phosphatases into inositol biphosphate (IP2) and inositol monophosphate (IP). Furthermore, IP3 activates IP3 receptor (IP3R), inducing calcium release from the endoplasmic reticulum reservoir. IP can be used to synthesize glycosylphosphatidylinositol (GPI) anchors in the ER and transferred to the Golgi apparatus (GA) to end up fusing to the plasma membrane.



metabolized to Ins(1,4)P2 and sequentially dephosphorylated to render free MI. The number of inositol phosphates has grown since the identification of the IP3/Ca^{2+} signaling mechanism (Nishizuka, 1986).

Regarding GPI anchors, they were initially postulated in the mid-1970s, while complete GPI structures were first described in 1988. GPI anchors associate with membrane lipid rafts, make clusters, and transduce signals. Currently, numerous GPI-anchored proteins have been identified in eukaryotes, from protozoa to humans, with diverse functions, such as coating proteins, receptors, prions, adhesion molecules, and hydrolytic enzymes. In mammals, GPI-anchored proteins can arise from alternative splicing and have different functions (Albarran *et al.*, 2016). Differences in the GPI pathway of mammals and other eukaryotes are the current focus in drug development against specific pathogenic targets (Ferguson *et al.*, 2017).

PATHOPHYSIOLOGY OF INOSITOL AND ITS DERIVATIVES

In its free form, MI plays a highly relevant role as a cellular osmolyte, protecting cells from environmental stress, by osmotic compensation and consequent cell fluid and volume balance. With other osmolytes, all called ‘chemical chaperones’, MI also stabilizes and rescues misfolded proteins (Thurson *et al.*, 1989; Garcia-Perez & Burg, 1991; Gullans & Verbalis, 1993; Welch & Brown, 1996; Yancey, 2005). In addition to these functions, MI and its derivatives are involved in numerous biological processes, including protein tethering to the cell surface, cell signaling and vesicle trafficking, membrane excitability, regulation of ion channel opening, intracellular calcium signaling, cytoskeleton and chromatin dynamics and remodeling, gene expression, and epigenome regulation (Hammond *et al.*, 2004; Roest *et al.*, 2017; Bevilacqua & Bizzarri, 2018; Uličná *et al.*, 2018; Bilanges *et al.*, 2019). Consequently, alterations in MI transport and metabolism have been found associated with numerous pathological conditions, including metabolic syndrome, type 2 diabetes and gestational diabetes, thyroid dysfunctions, polycystic ovarian syndrome and other gynecological disorders, abnormalities in gamete production, fertilization and embryonic development, Down’s syndrome, psychiatric disorders (i.e., depression, panic disorders, obsessive compulsive disorder), Alzheimer’s disease, preterm broncho-pulmonary diseases (including respiratory distress syndrome and retinopathy of prematurity), liver and intestinal steatosis, and cancer. Several systematic analyses and meta-analyses have extensively reviewed the impact of MI and derivatives in health and disease (Berridge, 2016; Bizzarri *et al.*, 2016b; Chakraborty, 2018; Ramos *et al.*, 2019).

Considering MI and its derivatives participation in numerous biological processes and the association between abnormal levels of these molecules and numerous pathologies, it has already been established that an extra intake of MI as a dietary supplement may be beneficial for several clinical conditions. MI and inositol-P6 have been found to exert positive effects toward several conditions, such as polycystic ovarian syndrome, gestational and type 2 diabetes mellitus, metabolic syndrome and non-alcoholic fatty liver disease, pathologies related to excess adiposity and hyperglycemia, insulin resistance, infant respiratory diseases, and cancer-related events (Vucenik & Shamsuddin, 2006; Howlett *et al.*, 2015; Bizzarri *et al.*, 2016a; Laganà *et al.*, 2018; MacFarlane & Di Fiore, 2018; Michell, 2018; Owczarczyk-Saczonek *et al.*, 2018; Showell *et al.*, 2018; Tabrizi *et al.*, 2018; Facchinetti *et al.*, 2019; Vitagliano *et al.*, 2019). Specifically

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regarding polycystic ovarian syndrome, accumulating evidence suggests that one of the most important mechanisms of its pathogenesis is the insulin resistance; for this reason, the use of insulin sensitizers, such as inositol isoforms, gained increasing attention due to their effectiveness and safety profile (Monastra *et al.*, 2017; Laganà *et al.*, 2017; Reyes-Muñoz *et al.*, 2018).

MI supplementation can be considered totally safe for humans and, in particular, if 4 g dose/day is not exceeded. In this regard, the effects of a MI overdose (4–30 g dose/day) have been evaluated from 1 to 12 months in several trials, and only some mild side effects in a small percentage of patients were reported (Bizzarri *et al.*, 2016b). For all these reasons, several food supplements containing MI in combination with several compounds, including folic acid, melatonin, alpha-lactalbumin, glucomannan, sorbitol, sucrose, maltodextrin, and vitamin D, were developed. However, it is important to pay attention to the possible presence of mono-, di-, or polysaccharide excipients (i.e., maltodextrin, sucrose, and galactose) in these products, because they may affect MI absorption due to competition for the same transporters (Unfer, 2018). Other important aspects to consider in a food supplement 'therapy' are the pharmaceutical form (i.e., powder, tablets, soft gels), the chemical characteristics of the molecules used (i.e., pro-drug, salt form), the number and time of administration, and the interference of other substances on supplements absorption (i.e., caffeine, drugs). In this context, a recent review work has gathered and analyzed information from several scientific publications search engines (PubMed, Google Scholar, and ResearchGate) on MI bioavailability, identifying two studies that investigated the pharmacokinetic profile of MI administration. This analysis revealed an advantageous protocol of splitting a therapeutic dosage of 4 g MI into 2 g administrations, twice a day, to be the best approach for one day coverage (Orrù *et al.*, 2017).

MYO-INOSITOL AND DERIVATES IN THE MALE REPRODUCTIVE TRACT

The detection of MI in male reproductive tract secretions was first reported in the 1950s (Mann, 1951; Mann, 1954; Hartree, 1957). Specifically, Mann identified the boar seminal vesicles as an exceptionally rich source of MI, with amounts around 2.4 g/100 g fresh weight, much higher than those reported for most plant and animal tissues (10–100 mg/100 g) (Mann, 1954). Using a microbiological assay, Hartree determined MI levels in human semen from 2 pools of at least 10 samples each, finding 50–57.3 mg/100 g free MI and 53.6–62.8 mg/100 g total MI (Hartree, 1957). Results from Hartree's study also highlighted the difference between MI concentrations in human seminal plasma and blood, being ~100 times higher in the former (seminal plasma free MI: 50 mg/100 mL; serum-free MI: 0.6 mg/100 mL). In addition to free and total MI quantification in human seminal plasma, Hartree's study reported MI concentration in boar, bull, rabbit, ram, and stallion seminal plasma, showing highest values in boar [free MI (n = 3): 382-607 mg/100 g, total MI: (n = 2): 602, 725] and lowest in ram [free MI (4 pools of at least 10 samples each): 9.7–16.1, total MI (4 pools of 10 or more samples each): 37.3–45.4] (Hartree, 1957). Later, other studies identified free MI as a component of bull, guinea pig, monkey, rabbit, and ram seminal fluid (Mann, 1964). Moreover, Nixon reported MI in human semen from 20 patients classified as fertile, borderline, and subfertile, finding an average of 64 mg/mL free MI in the whole group (Nixon, 1964). In this report, no differences were found between MI levels and patient fertility status [subfertile (n = 4): 50–78 mg/100 mL; borderline (n = 4): 26–150; fertile (n = 12): 32–104], although a small number of individuals were evaluated in each group (Nixon, 1964).

In 1964, Eisenberg and Bolden described MI levels in rat testis (range: 39–66 mg/100mg; n = 3), epididymis (206–222), seminal vesicles (611–666), and seminal fluid (943, 1060; n = 2; Eisenberg & Bolden, 1964). The seminal vesicles were pointed as the principal MI storage site in the male tract. The authors suggested a possible role of MI in sperm maturation, based on the increased levels found from testis to the epididymis, although no experiments were reported to address the proposed role.

Other studies described high levels of MI in the male reproductive tract (Hartree, 1957; Lewin & Beer, 1973; Voglmayr & Amann, 1973; Ghafoorunissa, 1976; Pruneda *et al.*, 2007). Lewin & Beer's study (Lewin & Beer, 1973) identified prostate secretions as a major source of MI in human seminal fluid; in their report, high MI levels were found in the early portion of split ejaculates with elevated prostatic secretions, as judged by parallel measurement of acid phosphatase (prostate biomarker) and fructose (seminal vesicles biomarker). An extensive study by Hinton and collaborators later described free MI concentration in luminal fluid from rat, hamster, rabbit, rhesus monkey, baboon, ram, and boar testis (seminiferous tubules and rete testis), epididymis (fractioned in proximal and distal *caput* and *cauda*, mid, and distal *corpus*), and deferens duct (Hinton *et al.*, 1980). These authors reported the hamster as the species depicting the highest MI concentration (distal *cauda* epididymis: 90 mM), followed by rat (deferens duct: 49.17 mM) and rhesus monkey (proximal *cauda*: 17.40 mM). Taking into account the fluid absorption process reported in the epididymis, mainly in the testis proximal regions, the authors also estimated MI concentration in rat epididymis in the absence of absorption, showing values up to 10 times higher when compared with observed values (i.e., mid-*corpus* observed: 5.58 mM ± 0.46 versus estimated: 40.31 mM). The authors proposed a possible role of MI in sperm maturation, specifically on changes in the sperm membrane and development of progressive motility and gamete storage in the *cauda* epididymis. In line with these potential roles, a low concentration of MI in the epididymis has been associated with reduced fertility in a transgenic mouse model (Yeung *et al.*, 2004).

The presence of MI in the male gonad and other reproductive organs could result from its uptake from blood. There are reports showing a lower MI uptake by the testis or the epididymis, most likely as result of the blood–testis and blood–epididymal barriers blockage, suggesting active MI biosynthesis in the male gonad and accessory organs (Middleton & Setchell, 1972; Lewin *et al.*, 1976; Waites & Gladwell, 1982). In particular, the report by Lewin and collaborators described a study in which rats injected with radiolabeled MI showed lower radioactivity incorporated to the male reproductive tract organs than liver (% administered dose testis: 0.47% ± 0.07; epididymis: 1.66% ± 0.23; vas deferens: 0.26% ± 0.02; seminal vesicle: 2.04% ± 0.48; prostate: 0.65% ± 0.38; liver: 14.90% ± 2.06; mean ± SD), suggesting MI production in the male gonad (Lewin *et al.*, 1976). This report also described a 12-fold blood serum concentration of MI in the epididymis. MI transport in male reproductive tissues was later confirmed by Hinton & Howards (Hinton & Howards, 1982),

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who described the highest transporting activity in the initial segment and early proximal *caput* rat epididymis and the lowest in the testis and *cauda* epididymis. A low *cauda* transport activity was also reported by Cooper (Cooper, 1982).

In addition to MI uptake, Eisenberg described an active system to produce MI from glucose in rat testis, and identified the seminiferous tubules as the most active component for MI biosynthesis in the male gonad (Eisenberg, 1967). Later studies showed ram testicular MI synthesized from blood glucose (Voglmayr & White, 1971; Middleton & Setchell, 1972). In addition, other authors further characterized the presence and enzymatic activity of testis IPS and IMPA1 in rat (Robinson & Fritz, 1979) and bovine (Mauck *et al.*, 1980) models. These enzymes are subjected to a differential hormonal control, as revealed in a study in which IPS activity decreased in the reproductive organs and liver after hypophysectomy; on the other hand, animal thyroidectomy caused a similar decrease in the liver but not in the reproductive organs (Hasegawa & Eisenberg, 1981). While FSH and LH restored the activity to normal levels in the testis, prostate, and seminal vesicles, it did not in the liver of these animals.

Chauvin & Griswold (2004) were the first reporting transcript expression of both enzymes involved in MI biosynthesis in mouse testis. In addition, they evaluated the expression of SLC5A3 cellular transporter. Expression of the three enzymes was found in several tissues, including testis and epididymis. Moreover, mRNA expression of the three enzymes was also detected in Sertoli cells, while *ISYNA1* and *IMPA1* expression was confirmed in round spermatids, and ISYNA1 mRNA in pachytene spermatocytes. In addition, Sertoli cells subjected to hypertonic conditions showed a significant increase in SLC5A3 and ISYNA1 mRNA levels, and an increased MI uptake. The authors also confirmed previous observations from Robinson & Fritz on the increase of MI production in Sertoli cells treated with dibutyryl cAMP, revealing a regulation of *SLC5A3* expression by FSH and dibutyryl cAMP (Robinson & Fritz, 1979). Considering MI osmoregulatory role in the kidney, and presence of high MI levels in luminal fluid, the authors hypothesized on the high production and secretion of the polyol by Sertoli cells, pachytene spermatocytes, and round spermatids that are in contact with the lumen. These cells also depict high expression of *ISYNA1* and *IMPA1* genes; in this regard, the induction of their expression under hypertonic conditions increased MI production, as response to osmolarity changes. Also, *SLC5A3* expression in Sertoli cells and MI increased expression and uptake in hypertonic conditions suggests MI as a key regulator of osmolarity in the male gonad. Recent studies described regulation of *ISYNA1* expression by p53 (Koguchi *et al.*, 2016); furthermore, p53 was reported to have a role in regulating mammalian spermatogenesis necrosis (Napoletano *et al.*, 2017). The impact of these findings on MI roles in spermatogenesis and sperm physiology has yet not been described. Testis expression of the three enzymes has been described in the Human Protein Atlas registries (HPA; https://www.proteinatlas.org; large-scale analysis of the human transcriptome; HG-U133A); the testis was the tissue with highest expression (Fig. 5). Prostate and seminal vesicles' mRNA expression of these enzymes was also reported in the HPA. These records confirmed findings previously described in animals.

MI and its derivatives may be involved in endoplasmic reticulum stress control in the testis (Gunes *et al.*, 2015) and other

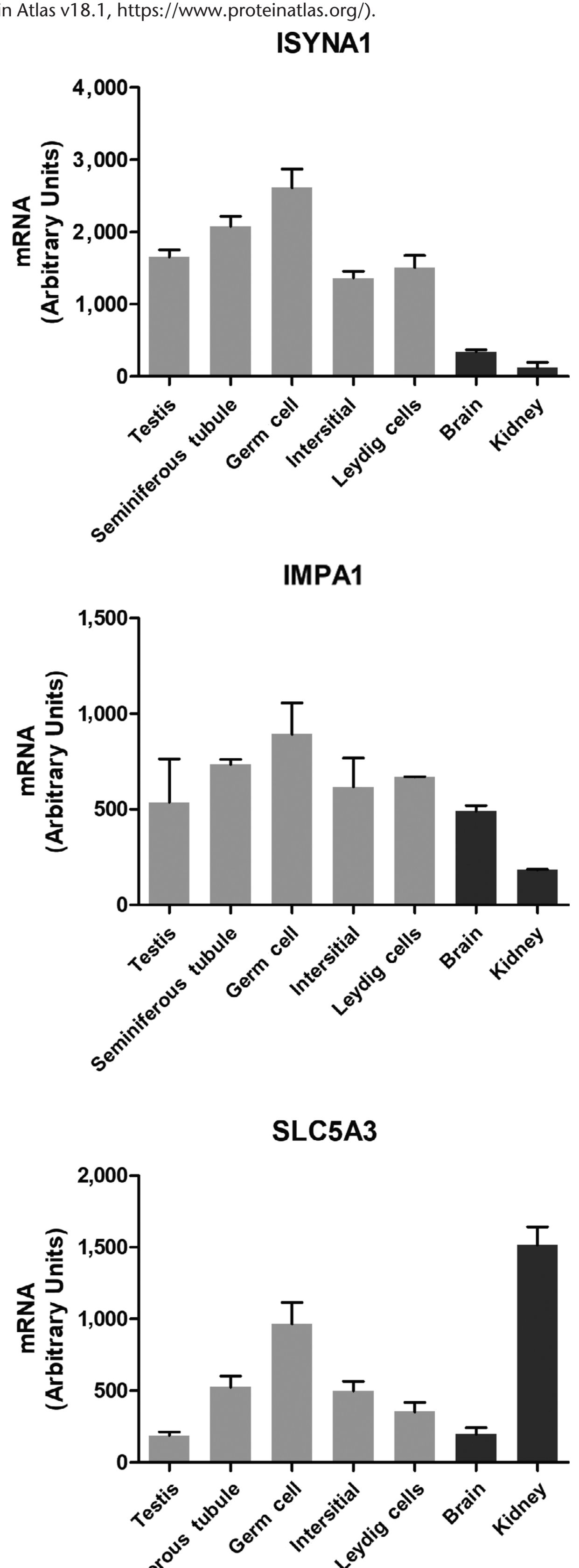

Figure 5 IMPA1, ISYNA1, and SLC5A3 expression in human testis RNA expression analysis of IMPA1 (top), ISYNA1 (middle), and SLC5A3 (bottom panel) in human testis, brain and kidney (Data retrieved from Human Protein Atlas v18.1, https://www.proteinatlas.org/).

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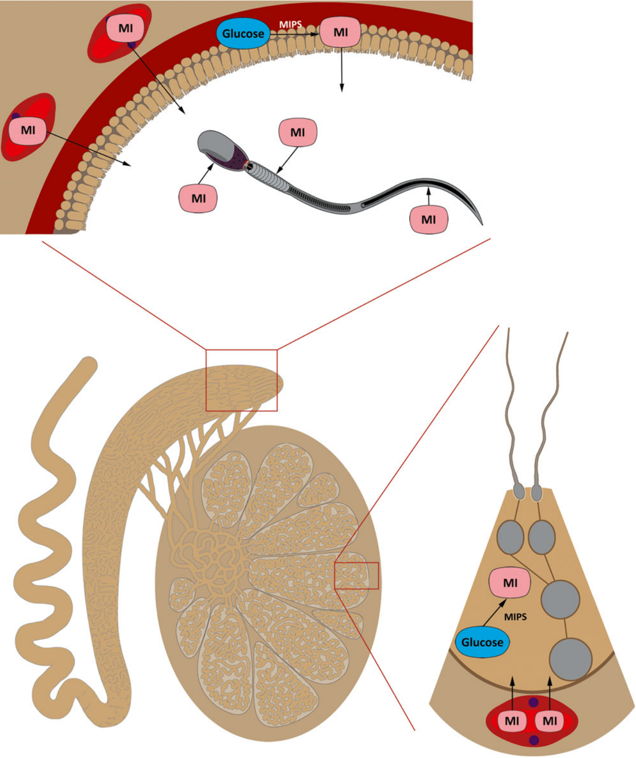

Figure 6 MI in the male genital tract and the spermatozoon. MI is produced from glucose in Sertoli cells inside the testis seminiferous tubules. Additionally, MI is acquired through blood supply in low levels due to the blood–testis barrier. Sperm cells can also acquire MI during epididymal transit.

male reproductive tissues, that is, epididymis and prostate, as reported in other tissues (Michell, 2008). In this regard, spermatogenesis is a highly complex proliferation and differentiation process leading to sperm morphogenesis that involves extensive protein synthesis, posttranslational processing, folding, and trafficking to an appropriate cellular localization (Fawcett, 1975; Neto *et al.*, 2016). Moreover, it requires the identification and destruction of both unfolded and misfolded proteins, by activating endoplasmic reticulum-associated proteasome-mediated degradation, in which MI and derivatives play a key role (Guzel *et al.*, 2017). Several studies have focused on Sertoli cells, since they have a key role in spermatogenesis by their close interaction with germ cells and the active protein synthesis (Crisóstomo *et al.*, 2018). Sertoli cells' endoplasmic reticulum has been extensively evaluated; a review work recently published by Vogl *et al.* (2018) extensively describes the distribution of the endoplasmic reticulum in testicular cells, the communication with the plasma membrane and mitochondria, and its function. Once spermatozoa are released from the testis, they undergo maturation while they transit the epididymis, acquiring progressive motility and ability to recognize the oocyte (Sullivan & Mieusset, 2016). Figure 6 summarizes the MI site of production in the testis and epididymis, as well as the sperm regions where MI and its derivatives may act, impacting male gamete function(s).

At ejaculation, spermatozoa stored in the distal portion of the epididymis mix with the accessory glands secretions and are deposited in the female tract, where they undergo a set of complex and coordinated changes to accomplish fertilization (Satouh & Ikawa, 2018). Spermatozoa undergo capacitation, a process by which they develop full fertilizing potential, as evidenced by their ability to undergo acrosome reaction and to interact with the oocyte vestments (De Jonge, 2017; Puga Molina *et al.*, 2018). Several studies have identified MI phosphoderivatives in sperm mechanisms related to these processes, including progressive and hyperactive motility, sperm guidance, sperm capacitation, acrosome reaction, and sperm–oocyte interactions. With regard to sperm motility, it is well established that in the oviduct, spermatozoa change the low-amplitude symmetrical progressive tail movement for a more active high-amplitude and asymmetric one, defined as hyperactive motility, to detach from the oviductal epithelium and to penetrate the oocyte vestments (Suarez, 2008). While signaling pathways responsible for hyperactive motility have not been fully elucidated, evidence has been shown on the involvement of a signal pathway in which PIP2 is converted to PIP3 (Freitas *et al.*, 2017); moreover, the involvement of the PI3K–AKT pathway in sperm hyperactivated motility was previously reported (Sagare-Patil et al., 2013). With regard to sperm guidance, a thermotaxis mechanism has been described, by which temperature stimulation activates PLC causing hydrolysis of PIP2 to IP3 and DAG, and a consequent binding of IP3 to its receptor in an internal Ca^{2+} store, triggering Ca^{2+} release, altering sperm swimming directions (Bahat & Eisenbach, 2010). In addition, spermatozoa that undergo capacitation depict changes such as plasma membrane lipid rafts redistribution, and actin cytoskeleton reorganization (Watanabe *et al.*, 2017; Breitbart & Finkelstein, 2018), processes in which MI derivatives exert a relevant role. Regarding the former, the release of GPI-anchored proteins from the lipid rafts has been proposed as a crucial step for sperm fertilizing ability development (Watanabe *et al.*, 2017). It has been shown that a decrease in PIP2 synthesis inhibits actin polymerization and sperm motility, whereas increasing PIP2 synthesis enhances both activities; moreover, the increase in filamentous actin during capacitation has been related to the inactivation of the actin severing protein gelsolin, by its binding to PIP2 (Breitbart & Finkelstein, 2018). Once spermatozoa are capacitated, they are capable to undergo the acrosome reaction; in this regard, it has been proposed that PIP2, DAG, and PA modulate IP3 production, which is crucial for this process (Lopez *et al.*, 2012).

IN VIVO MYO-INOSITOL SUPPLEMENTATION AND IMPACT ON MALE FERTILITY

Based on current knowledge of MI presence and functions in the male reproductive tract, the effect of *in vivo* MI supplementation in patients with seminal abnormalities, idiopathic infertility and metabolic syndrome, and in normozoospermic (N) men has been evaluated in several studies. A summary of the publications in this topic is presented in Table 1: information on the study type and design and number of subjects in the study, diagnostics, type of treatment, evaluated variables, results and statistical differences, and citation.

Among them, a report of Calogero and co-workers described the impact of MI oral treatment in a placebo-controlled study done in men diagnosed with idiopathic infertility (Calogero *et al.*, 2015). The authors stated that patients in both groups depicted similar values for all parameters evaluated, although a statistical analysis between groups is not shown. In the MI-treated patients, lower levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were found, while increased

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serum inhibin B levels and no changes in Prolactin and Testosterone (T) levels were reported. Regarding semen parameters, sperm concentration, count, and progressive motility showed a significant increase, although discrete, in MI-treated patients. Results suggested a positive impact of MI treatment on men suffering infertility. In this regard, inositol and its phosphoderivatives are known to modulate FSH signaling in women (Laganá *et al.*, 2017), and in Sertoli cells as well (Casarini & Crepieux, 2019). This may represent an additional MI mechanism of action which, by sensitizing Sertoli cells to FSH, might potentially improve sperm parameters. As part of the study, the authors reported an increase in spontaneous acrosome reaction in MI-treated patients; while the authors speculated about the lack of functional relevance of this parameter, they did not discuss these unexpected high scores found in all groups, and the undesired increase found in the MI-treated patients. The values reported in samples from both groups assessed prior to treatment, as well as after treatment in placebo groups are similar (34%, 35%, and 36%, respectively) and much higher than those reported in the literature (i.e., Byrd & Wolf, 1986). Since the method used to assess the acrosome status is a standard procedure, it would not cause these high acrosome reaction values; whether the significant increase in the acrosome reacted spermatozoa in the MI-treated patients is the result of changes in phospholipids concentrations of the sperm membrane is not evaluated or discussed. These findings should warrant further investigations to assess the impact of MI on sperm membrane stability.

In the same year, Gulino and collaborators published a study designed to assess the effect of MI treatment in individuals distributed in three groups: one composed of healthy fertile (HF) men, another composed of oligoasthenozoospermic (OA) men, and a control group of healthy patients treated because of a female factor (Gulino *et al.*, 2016). This last group was not always included in the analysis, leaving open the question of their inclusion in the study design. The authors described the use of two well-established procedures to select the motile sperm fraction, swim-up and density–gradient centrifugation used in the IVF-ICSI treatment procedures. The swim-up method was done from the sperm pellet, a procedure that is widely discouraged because of reactive oxygen species (ROS) release from dead and damaged spermatozoa; however, results presented only referred to the density-centrifugation method. One very important point in this study is the lack of a placebo group, considering that MI formulation provided to the patients also contains folic acid. Although the authors described the results of each group, data are presented in a peculiar way for each reported parameter (semen volume, sperm count before and after sperm selection, and progressive motility). The authors refer to count, while reporting sperm concentration, as judged by the units (million/mL). An increase in sperm concentration but not in sperm motility is reported in both groups after treatment, and results are presented in a very confusing manner. Moreover, the authors report an increase in sperm concentration after sperm selection in both groups, although again data analysis is rather unconvincing.

In 2016, Montanino Oliva and co-workers studied a group of asthenozoospermic (A) patients diagnosed with metabolic syndrome, and evaluated sperm parameters before and after treatment with a commercial supplement containing MI (Montanino Oliva *et al.*, 2016a). Several parameters established by the National Cholesterol Education program were used to assess whether patients were affected with metabolic syndrome. Although no changes were found in body mass index (BMI), waist circumference (WC) and levels of Triglycerides, the homeostatic model assessment for insulin resistance (HOMA) index was decreased after MI supplementation. These changes were accompanied by a decrease in Estradiol (E2) and sex hormone-binding globulin (SHBG) levels; moreover, LH and free/total T levels were found increased in MI-treated men (Michalakis *et al.*, 2013). After treatment, sperm concentration, motility and morphology depicted a significant discrete increase, estimated in approximately 20%. No information was shown on the proportion of samples that reached normal values, or any sperm functional tests in treated men. While results appeared promising, they may not be totally attributed to the MI, since the commercial formulation also contains L-carnitine, L-arginine, vitamin E, selenium, and folic acid, and a placebo control group was not included in the study design, essential to confirm the relevance of the reported findings.

One year later, Dinkova and collaborators described the impact of MI treatment on semen parameters in a prospective longitudinal study done on A patients (Dinkova *et al.*, 2017). The authors indicated that semen parameters were evaluated in all patients prior and after treatment, but results of average sperm motility were only presented, and no indication of whether it was total or progressive motility was given. In response to MI treatment, the authors reported a significant increase in the percentage of motile spermatozoa, reaching normozoospermia in 34.9% of the cases, and no response in 12.8% of the cases. However, no information was provided on the total motile count, to determine the impact of MI treatment on sperm production and quality, information that was available since the authors indicate they evaluated all semen parameters. In the conclusion, the authors highlighted the safety of MI, and propose its use to manage idiopathic infertility, but the study does not address the impact of this compound in patients with unexplained infertility, but in men with alterations in sperm motility.

In the same year, a report by Capece and collaborators described a study done to assess the impact of MI patient treatment on routine semen parameters and DNA fragmentation (Capece *et al.*, 2017). MI was administered as a complex with other components to a group of patients with semen abnormalities. Although patient group was defined as oligoasthenoteratozoospermic (OAT), they were A, oligoteratozoospermic (OT), asthenoteratozoospermic (AT), and OAT. In addition to a hormone panel, semen analysis (WHO manual 2010) and DNA fragmentation (TUNEL) were evaluated (no cut off values of normality for TUNEL were indicated); after randomization both groups had comparable baseline values for all parameters. Results were presented as the difference between baseline and follow-up, displaying data in a complex way, making difficult the assessment of changes caused by the treatment. The authors claimed a significant overall increase in sperm concentration and progressive motility, and a decrease in DNA fragmentation, while no changes were reported in sperm vitality, total motility, normal morphology, as well as LH, FSH and T levels between treated and control patients. No other data was presented; in particular, results on A men (largest subgroup) in whom the impact of MI treatment on all parameters studies would have been important to report if contrasted with a specific placebo group.

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Table 1 *In vivo* supplementation with MI and impact on male fertility

Type	N	Diagnostics	Treatment	Results	Refs
Double-blind, randomized, placebo-controlled study	194	Men with Idiopathic infertility. No pregnancy after 2 years	Group 1: 2 g MI + 200 µg folic acid (n = 98) Group 2 (placebo): 200 µg folic acid (n = 96) Twice a day for 3 months	(mean ± SD; $p < 0.05$) (Comparison post-MI vs post-placebo) FSH (IU/L): Post = 10.7 ± 4.1, Pre = 16.7 ± 4.1 LH (IU/L): Post = 8.8 ± 2.6, Pre = 12.1 ± 2.6 Serum inhibin B (ng/L): Post = 105.0 ± 28.0, Pre = 86.0 ± 24.0 Sperm concentration (million/mL): Post = 26.4 ± 4.4, Pre = 20.2 ± 4.6 Sperm count (million): Post = 57.6 ± 14.4, Pre = 46.6 ± 12.6 Progressive motility (%): Post = 27.6 ± 1.8, Pre = 22.2 ± 2.1 Acrosome reaction (%): Post = 41 ± 11, Pre = 34 ± 8 Observations: No changes reported in placebo group.	Calogero *et al.* (2015)
Prospective study	62	13 OA 29 HF (healthy fertile) (N) 20 Control (female factor)	4 g MI + 400 µg folic acid Once a day for 2 months.	OA Sperm concentration (million spermatozoa/mL): Post = 10.06, Pre = 6.38; Change: +126.89% ($p < 0.01$). HF (N) Sperm concentration (million spermatozoa/mL): Post = 54.84, Pre: 51.93; Change: +10.50 (ns). Density-gradient centrifugation sperm selection Selected fraction–sperm concentration: OA men: Post = 4.15 million spermatozoa/mL, Pre = 1.63; Change: +306.56 ($p < 0.001$) HF: Post = 44.31 million spermatozoa/mL, Pre = 21.59, Change: +132.02 ($p < 0.01$) Sperm motility not improved in either group. Observations: -No placebo group was included for the OA and HF patients. -Data presented did not include standard deviation of the mean despite describing results of a patient group. Significant differences were not clear.	Gulino *et al.* (2016)
Prospective longitudinal	45	A men with metabolic syndrome	Andrositol (Lo.Li. Pharma, SRL, Rome, Italy) 1g MI + 30mg L-carnitine, L-arginine, vitamin E + 55 µg selenium + 200 µg folic acid Twice a day for 3-months	(mean ± SD) BMI (kg/cm2) Post = 27.0 ± 3.1, Pre = 28.1 ± 3.5 (NS) WC (cm) Post = 105.3 ± 3.3, Pre = 107.1 ± 4.2 (NS) Triglycerides (mg/dL) Post = 173.2 ± 13.4, Pre = 175.4 ± 12.5 (NS) HOMA (index): Post = 1.6 ± 0.7, Pre = 2.8 ± 1.2 ($p < 0.001$) E2 (pg/mL): Post = 20.9 ± 3.3, Pre = 32.4 ± 5.2 ($p < 0.01$) SHBG (nmol/mL):	Montanino Oliva *et al.* (2016b)

(continued)

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Table 1 (continued)

Type	N	Diagnostics	Treatment	Results	Refs
				Post = 35.8 ± 3.5, Pre = 55.0 ± 4.9 ($p < 0.001$) LH (mIU/mL): Post = 3.3 ± 1.2, Pre = 2.5 ± 1.3 ($p < 0.01$) Free T (pg/mL) Post = 47.2 ± 13.0, Pre = 33.0 ± 11.1 ($p < 0.001$) Total T (ng/mL): Post = 3.7 ± 1.4, Pre = 2.8 ± 1.2 ($p < 0.02$) Sperm concentration (million/mL): Post = 20 ± 4.2, Pre = 16.2 ± 3.4 ($p < 0.001$) Sperm motility (%): Post = 51.4 ± 7.2, Pre = 39.6 ± 6.1 ($p < 0.001$) Normal sperm morphology (%): Post = 30.1 ± 2.3, Pre = 24.9 ± 2.0 ($p < 0.001$). Observations: Study design lacked a placebo control group.	
Prospective longitudinal study	109	A	Andrositol (MLD Trading, Bulgaria) 1 g MI, 30 mg of L-carnitine, L-arginine, and vitamin E, 55 µg of selenium, and 200 µg of folic acid Twice a day for 3-months	(mean ± SD) Sperm motility: Post = 27.98% ± 9.69, Pre = 20.31 ± 8.5 ($p < 0.05$) Restauration to normozoospermia: 34.9% (38/109) No changes: 12.8% (14/109) Worsening: 1.84% (2/109) Observations: It is not indicated whether changes correspond to total or progressive motility.	Dinkova *et al.* (2017)
Single-center, randomized, single-blind, placebo-controlled study	60 (finally 56; 4 dropped out)	OAT A: 34 OT: 15 AT: 2 OAT: 5	Tradafertil (Tradapharma Sagl, Switzerland) 1 g MI, 300 mg Tribulus Terrestris, 200 mg Ecklonia Bicyclis Alga and Bovis Once a day for 3 months	Sperm concentration (million spermatozoa/mL) Average increase: MI-treated = 3.82, placebo-treated = 1.71 ($p = 0.048$) Progressive sperm motility Average increase: MI-treated = 4.86% placebo-treated = 1% ($p = 0.019$) DNA fragmentation Average (baseline–follow-up, %): MI-treated= −1.64, placebo-treated: −0.39 ($p = 0.001$) Observations: Results were presented as a difference between baseline and follow-up. No actual values of variables in MI- and placebo-treated patients were shown.	Capece *et al.* (2017)
Multicentric study	100	Men depicting one or more altered semen parameter O: 16 A: 43 OA: 41	Sinopol® (Laborest, S.R.L., Nerviano, Milan, Italy) 1 g MI, 800 mg alpha-lipoic acid, 400 mg folic acid, 100 mg betaine, 1.7 mg vitamin B2, 1.9 mg vitamin B6, 2.62 mg vitamin B12. Twice a day for 3 months	(mean ± SD) Sperm concentration: Post = 24.4 million/mL ± 23.4, Pre = 16.6 ± 13.1, Change=+41.2% ($p = 0.0009$) Sperm count: Post = 69.1 million spermatozoa ± 59.0, Pre = 46.5 ± 38.7, change = +50% ($p = 0.0017$) Progressive motility: Post = 24.8% ± 16.5, Pre = 19.5 ± 15.6, Change = +31.6% ($p = 0.0047$) Total motile sperm count:	Canepa *et al.* (2018)

(continued)

Table 1 (continued)

Type	N	Diagnostics	Treatment	Results	Refs
				Post = 22.3 million spermatozoa ± 30.8, Pre = 9.8 ± 11.5, Change=+120% (p = 0.0010) Normal sperm forms: Post = 7.9 % ± 4.1, Pre = 4.9 ± 3.1, Change=+60% ($p < 0.0001$) Follow-up 6 months: 40% pregnancies spontaneously (6%) or after ART procedures (4 IUI, and 30 FIV-ET or ICSI). Observations: No placebo group was included.	
Case–control open-label study	50	Women	MI vaginal suppositories (2 mg MI; Xyminal, Lo.Li Pharma, SRL, Rome, Italy) A two-phase strategy, lasting 2 menstrual cycles. Couples had intercourse, after which the post-coital test was done. In the second cycle, patients received 1 MI vaginal suppositories for 3 consecutive days, starting when leading follicle > 16 mm. The post-coital test was repeated after treatment.	No differences found in semen samples from Pre- and post-partner treatment, evaluated on day 9 of the menstrual cycle. Improvement in post-coital test semen parameters (mean ± SD) Total sperm motility: Post = 50.8 % ± 11.1, Pre = 40.8 ± 20.4 ($p < 0.05$) Progressive sperm motility: Post = 29.0 % ± 7.5, Pre = 15.8 ± 10.6 ($p < 0.001$) % Immotile spermatozoa: Post = 49.2 % ± 10.5, Pre = 59.2 ± 20.4 ($p < 0.05$) Observations: No placebo group is included.	Montanino Oliva *et al.* (2016b)

A, asthenozoospermic men; O, oligozoospermic men; OA, oligoasthenozoospermic men; OT, oligoteratozoospermic men; OAT, oligoasthenoteratozoospermic men; N, healthy fertile men. Text in light blue highlights outcome for fertility treatment.

Regarding the impact of MI on DNA fragmentation, phosphoinositide lipids (PPI) are found 10 times more enriched in the nucleus, where they regulate transcription, RNA splicing and DNA damage response. Wang *et al.* (2017) found an increase in intranuclear PPI levels within 1 sec of laser microirradiation. Moreover, PPI presence is essential for Ataxia telangiectasia and Rad3-related protein (ATR)-mediated DNA damage response. This is highly relevant, since double-strand breaks damage response is primarily led by ATR/ATM kinases (Larsen & Stucki, 2016), being this a ROS-mediated injury in sperm germ cells that may lead to translocations and cell death (Gunes *et al.*, 2015).

In a recent report, Canepa and co-workers described a treatment with a commercial formulation containing MI, in individuals depicting one or more altered semen parameters (Canepa *et al.*, 2018). While patient treatment resulted in an increased sperm concentration and count, progressive motility, total motile sperm count, and normal sperm morphology, no placebo group was included in the study design. This was highly relevant, considering that treatment was done with a complex containing MI, alanine, betaine, folic acid and vitamins B2, B6, and B12. The authors also reported a reproductive follow-up of patients from the study group, indicating that 40% pregnancies were achieved, either spontaneously or after ART procedures up to 6 months after treatment; however, no control placebo group was reported to compare this outcome and confirmed its association with the treatment.

Another study that evaluated the reproductive outcome after MI patient treatment comes from a report by Montanino Oliva *et al.* (2016b) on sperm performance done in women treated with MI vaginal suppositories. Briefly, this case–control open-label study used two-phase strategies and lasted two menstrual cycles. Ovulation was monitored in both cycles after which a post-coital test was done. In the second cycle, patients used a MI vaginal suppository for three consecutive days, starting when the leading follicle was over 16 mm. As result, no differences were found when the semen samples from the pre- and post-partner treatment were evaluated on day 9 of the menstrual cycle. However, a significant improvement was observed in the post-coital test semen parameters from women treated with MI, finding an increase in total and progressive sperm motility, and a concomitant decrease in the percentage of immotile spermatozoa. Although promising, no placebo control was included in the study design and no other reports from this group or others replicated these findings.

IN VITRO SEMEN SUPPLEMENTATION WITH MYO-INOSITOL AND IMPACT ON SPERM FERTILIZING POTENTIAL

In addition to the effect of *in vivo* supplementation, the impact of MI was further evaluated in a set of *in vitro* studies

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using semen samples depicting normal and abnormal sperm parameters. A summary of the reports is shown in Table 2, following a structure similar to that described for Table 1.

Firstly, a report by Calogero's group described the impact of *in vitro* MI supplementation on sperm mitochondrial membrane potential in semen samples from N and OAT patients (Condorelli *et al.*, 2011). After liquefaction, semen samples were supplemented with MI or placebo, resulting in increased percentages of spermatozoa depicting high mitochondrial potential, and reduced percentages of spermatozoa with low mitochondrial membrane potential in OAT patient samples. Contrasting with these findings, no significant changes in mitochondrial potential were found in N semen samples after MI supplementation. Moreover, MI supplementation of semen samples from N and OAT men resulted in higher, although not significant, percentages of spermatozoa depicting Phosphatidyl Serine (PS) externalization as a signal of apoptosis. Moreover, a trend toward a decrease in abnormal chromatin compactness was also observed in spermatozoa from semen samples incubated with MI, although differences again did not reach statistical significance. Assessment of a larger number of subjects could have helped confirming or disregarding these findings that may impact in other sperm fertility-related functions. Following these findings, one year later the same group reported the results of semen supplementation with MI followed by swim-up sperm selection in a cohort of N and OA patients (Condorelli *et al.*, 2012). In this study, the authors reported enhanced progressive cell motility and mitochondrial membrane potential of spermatozoa from OA men after MI treatment prior to the sperm swim-up selection. Furthermore, post-swim-up sperm motility (total and progressive) and sperm count were reported significantly increased in both N and OA patient MI-treated samples. The authors include in the report a figure with results on total and motile sperm motility, although values differ from those listed in the table. Moreover, authors report no significant changes in PS externalization and chromatin compactness. In a review published in 2017, Condorelli and collaborators proposed some biological MI roles, as potential prokinetic, antioxidant and hormonal modulator. In addition, they summarized prior findings from their group and others about the impact of MI on sperm quality, and propose an algorithm to identify asthenozoospermic patients that may benefit from its supplementation, which includes the assessment of MMP (Condorelli *et al.*, 2017).

To further assess MI benefits, Scarselli *et al.* (2016) described its effect on semen samples depicting hyper viscosity. Specifically, this study enrolled patients without testicular pathology but abnormal high semen viscosity. As working protocol, seminal plasma was removed by centrifugation, spermatozoa were resuspended in a commercial MOPS-buffered medium (G-MOPS™ PLUS) and separated in two aliquots, one of which was supplemented with MI; this treatment resulted in increased sperm motility when compared to the control, suggesting a positive effect of MI on sperm motility in sperm suspensions devoid of seminal plasma proteins. When samples were classified according to the response in High (≥60–100%)-, Medium (≥30–<60%)-, Poor (≥1–<30%)-, and No (0%)-responders, hyperviscous semen samples were found to respond in over 90% of the cases, being 57.6% defined as High-responders and 24.2% as Medium-responders. They also evaluated the effect of the same short incubation with MI on samples from patients with grade II and III varicocele. In these cases, although the overall percentage of motile spermatozoa was similar in treated and control samples, a response was found in over 60% of the samples following criteria defined above.

In the same year, Palmieri *et al.* (2016) described the impact of *in vitro* sperm treatment with MI on total and progressive motility in fresh semen samples from N, O, A and OA subjects. Samples supplemented with MI and incubated for a short time at 37 °C showed an improvement in total and progressive sperm motility compared to controls. As indicated by the authors, a higher progressive than total motility was unexpected, since semen analysis was done following 2010 WHO guidelines. Semen samples were also subjected to swim-up or discontinuous density-gradient sperm selection procedures in the presence or absence of MI; in this section, the authors presented these findings as a sperm capacitation rather than a sperm selection procedure. A decrease in sperm total motility after selection with MI was found; in addition, similar values for sperm progressive motility were reported regardless of treatment. A similar evaluation done using pentoxifylline also did not improve sperm motility. In the same report, the authors evaluate the effect of MI supplementation on frozen-thawed semen samples. In their study, incubation with MI resulted in an overall significant although discrete improvement in progressive sperm motility, depicting a large variance among samples.

In 2017, Artini *et al.* (2017) reported results on MI *in vitro* supplementation in a cohort of N and OAT patients using a commercial product. A short treatment resulted in a discrete increase in progressive motility of both N and OA patient's semen samples, compared to control samples incubated in standard medium. Furthermore, total sperm motility was also improved in N patient samples treated with MI, an effect that was not found in the Condorelli's study.

The impact of *in vitro* semen supplementation with MI on DNA fragmentation was addressed in a cross-sectional study presented at the 2015 ESHRE meeting by Parmegiani *et al.* (2015). Although a preliminary report, a significant overall decrease in DNA fragmentation was determined in MI-treated spermatozoa.

Another 2015 ESHRE meeting report by Paternostro and collaborators evaluated MI impact on sperm cryopreservation (Paternostro *et al.*, 2015). Semen samples were assessed according 2010 WHO criteria and cryopreserved using slow freezing with medium supplemented with or without MI. Comparing with the control aliquot, an improvement of sperm motility was observed in 50% of the cases. An average reduction in DNA fragmentation after thawing samples supplemented with MI was found in a large proportion of the samples. This was a promising study, considering the well-known negative impact of sperm cryopreservation on sperm motility and DNA damage. No details on the response to cryopreservation with MI in relation to the initial semen parameters were presented.

In 2018, Saleh and collaborators reported a randomized controlled cryopreservation trial in a group of infertile men, supplementing semen cryoprotectant with MI (Saleh *et al.*, 2018). After thawing, total and progressive motility and the progressive cryosurvival rate (CSR = post-thaw progressive motility/pre-freeze progressive motility x100) were higher in samples supplemented

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Table 2 *In vitro* supplementation with MI and impact in male fertility

Type	N	Diagnostics	Treatment	Outcome	Refs
Prospective study	17	5 N 12 OAT	Semen supplemented with 2 mg/mL MI and incubated for 2 h	MMP (flow cytometry following JC-1 staining) Showed improvement in MMP only in OAT patients ($p < 0.05$). MI supplementation did not induce changes neither in % spermatozoa with signs of apoptosis (PS externalization) nor in chromatin compactness. PS externalization (mean ± SEM) N With MI = 7.9 ± 4.1; Without MI = 2.7 ± 1.1 (NS) OAT With MI = 8 ± 2.5; Without MI = 4.6 ± 0.8 (NS) Abnormal chromatin compactness (mean ± SEM) N With MI = 17.5 ± 3.1; Without MI = 19. ± 3.6 (NS) OAT With MI = 23.7 ± 2.9; Without MI = 27.3 ± 2.9 (NS)	Condorelli *et al.* (2011)
Prospective study	40	20 N 20 OAT	Semen supplemented with 2 mg/mL MI and incubated for 2 h, followed by swim-up for 30 min.	OAT (mean ± SEM) Progressive sperm motility (%): Post = 42 ± 4.0; Pre = 28.0 ± 4.0 ($p < 0.05$). MMP % spermatozoa with high MMP: Post = 80.0% ± 2.0; Pre = 58.0 ± 4.0 ($p < 0.05$) % spermatozoa with low MMP: Post = 15% ± 3.0; Pre = 38.0 ± 4.0 ($p < 0.05$). Sperm vitality and chromatin compactness were similar in sperm suspensions from MI-treated and placebo control samples. Post-swim-up sperm motility (% total and progressive) and sperm count were increased in both N ($p < 0.05$) and OA ($p < 0.05$) MI-treated patient samples.	Condorelli *et al.* (2012)
Prospective study	33	Patients without testicular pathology but abnormal high semen viscosity (semen threads of >2 cm length)	Semen centrifuged at 1800 rpm/10 min, resuspended, and incubated with 2mg/mL MI (Andrositol® DGN, Lo.Li. Pharma, Rome, Italy: 133 mg/mL MI in 9 mg/mL sodium chloride) for 15 min at 37 °C	(mean ± SD) Sperm motility (%): MI-treated = 38.9% ± 3.0; control = 24.35 ± 2.41 ($p < 0.0001$) Samples classified according to Andrositol® DGN criteria: High (≥60–100%)-, Medium (≥30–<60%)-, Poor (≥1–<30%)-, and No (0%)-responders. Hyper viscous semen response> 90% of cases. High-responders: 57.6% Medium-responders: 24.2% Poor-responders: 12.1% No-responders: 6.1%	Scarselli *et al.* (2016)
Prospective study	30	Patients diagnosed with grade II and III varicocoele.	Semen centrifuged at 1800 rpm/10 min, resuspended, and incubated with 2 mg/mL MI (Andrositol® DGN, Lo.Li. Pharma, Rome, Italy: 133 mg/mL MI in 9 mg/mL sodium chloride) for 15 min at 37 °C	Varicocoele patients response in> 60% of the samples High-responders: 20% Medium-responders: 16.7% Poor-responders: 26.7% No-responders: 36.7% Overall % motile spermatozoa was comparable between MI-treated and control samples	Scarselli *et al.* (2016)
Prospective study	100	46 N 19 O 15 A	Semen supplemented with 15 µL/mL of MI (Andrositol®-LAB (Lo.Li.	(mean ± SD) Total motility (%):	Palmieri *et al.* (2016)

(continued)

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Table 2 (continued)

Type	N	Diagnostics	Treatment	Outcome	Refs
		20 OA	Pharma, Rome, Italy) and incubated 15 min at 37 °C	MI = 50.23 ± 18.92, control = 46.55% ± 18.62 ($p \leq 0.0001$) Progressive motility (%): MI = 56.91 ± 20.68, control = 47.76 ± 20.64 ($p \leq 0.05$) Swim-up or discontinuous density-gradient sperm selection procedures in the presence or absence of MI. Total sperm motility (%): MI = 70.87 ± 31.46, control = 73.99 ± 28.94 ($p \leq 0.05$) Progressive motility (%): MI = 70.67% ± 26.72, control = 69.97 ± 27.27). (ns). A similar evaluation with pentoxifylline did not improve sperm motility	
Prospective study	*25*	*3 N, 7 O, 6A 9 OA (Sample from biopsy or fresh ejaculate; volume of less than 1.5 mL)*	*Frozen-thawed sample supplemented with 3,2 mg/mL MI and incubated 15 min at 37 °C*	*Total sperm motility (%): MI = 14.88% ± 16.86, control = 11.4 ± 16.51 (ns) Progressive motility (%): MI = 16.4 ± 20.64, control = 9.8 ± 14.1 ($p < 0.05$)* Note: high variability in the values among samples	Palmieri *et al.* (2016)
Prospective study	63	31 N 32 OA	Semen supplemented with 2 mg/mL MI (Andrositol®-LAB, Lo.Li. Pharma, Rome, Italy) and incubated 30 min at 37 °C	(mean ± SD) Total motile sperm concentration: N MI-treated = 50.2 ± 20.9 million/mL, control = 44.6 ± 21.2 ($p < 0.05$) Progressive motile sperm concentration: N MI-treated = 36.6 ± 28.9 million/mL, control = 31.8 ± 28.5 ($p < 0.05$) OAT MI-treated = 6.6 ± 6.2 million/mL, control = 4.5 ± 2.1 ($p < 0.05$). Observations: No significant changes in Total motile sperm concentration in OAT patients	Artini *et al.* (2017)
Cross-sectional study	10	Men attending infertility clinic.	Sample divided into four aliquots, 1 left in seminal plasma (SP-4h), 1 in SP + 2 mg/mL MI (Andrositol-LAB; Lo.Li. Pharma; Rome, Italy) (SP-4h + MI), one in SP + 1:2 medium (SP-4h + culture medium), 1 diluted as before + MI SP-4h + culture medium + MI). All aliquots incubated for 4 h	DNA fragmentation (sperm chromatin dispersion method; %) (500 spermatozoa/sample). baseline: 24% (1200/5000 spermatozoa), SP-4h: 51.6% (2580/5000), SP-4h + MI: 40.9% (2045/5000), SP-4h + culture medium: 35.8% (1790/5000), ($p < 0.001$ all between aliquots and baseline) SP-4h + culture medium + MI=24.5% (1225/5000) ($p = 0.576$)	Parmegiani *et al.* (2015) (ESHRE Meeting abstract)
Prospective study	*90*	*Men subjected to basic semen analysis*	*Semen cryopreserved (slow freezing) with freezing medium supplemented with 2 mg/mL MI*	*Sperm motility Improvement = 45/90 Decrease = 28/90 DNA fragmentation Reduction (3.7%) = 66/90 Increase (2%) = 9/90 No variation (16%) = 15/90* Note: limited to samples with ≥5 million spermatozoa/mL	*Paternostro et al.* (2015) (ESHRE Meeting abstract)
Randomized controlled trial done	*50*	*Infertile men (9 excluded; final 41) Evaluated as total (n = 41) (only shown) or normal (n = 15) and abnormal (n = 26)*	*Semen samples supplemented with 1 mg MI to cryoprotectant, slow freezing cryo.*	*Median (25th–75th percentile) paired t-test analysis Total motility (%): Pre-freeze = 50 (30–50) Post-MI: 15 (10–35) Post-control = 10 (6–30) (p = 0.15, post-MI vs post-control)*	Saleh *et al.* (2018)

(continued)

Table 2 (continued)

Type	N	Diagnostics	Treatment	Outcome	Refs
Prospective study	*40*	*N*	*Semen divided into two aliquots ad cryopreserved: one with 2 mg/mL MI; one without MI (control), slow freezing cryo.*	*Progressive motility (%):* *Pre-freeze = 35 (20-35)* *Post-MI: 10 (5-20)* *Post-control = 5 (3-15)* *(p = 0.14, post-MI vs post-control)* *Cryo-Survival Rate (%):* *Post-MI = 40 (25–70)* *Post-control = 30 (13–58)* *(p = 0.041, post-MI vs post-control)* (mean ± SE) *Total sperm motility (%) (CASA):* *MI = 19.62 ± 2.68, control = 16.32 ± 2.75 (p = 0.045).* *Progressive sperm motility (%) (CASA):* *MI = 16.97% ± 1.86, control: 12.77 ± 1.78). (p = 0.042).* *Normal sperm morphology (%) (CASA):* *MI = 11.9 ± 0.97, control = 9.57 ± 0.9 (p = 0.019).* *ROS (reactive oxygen species)* *(ns; MI vs control)* *TAC (total antioxidant assay)* *(p < 0.001 MI increased compared to control).* *MDA (malondialdehyde)* *(p < 0.05 MI decreased compared to control)* *DNA fragmentation (TUNEL)* *(p < 0.05 MI increased compared to control).*	Mohammadi *et al.* (2019)
Prospective study	30		Semen preparation media with 2 mg/mL Androsito®-LAB (Lo.Li Pharma, Rome, Italy). IUI	Clinical pregnancy: MI = 33.3%, control = 20.4% (*p*: 0.0492). Note: MI-treated couples compared to a control group (retrospective) of 28 cases matched for clinical fertility history and women age	Poverini *et al.* (2014)
Prospective study	376	Group MI: (*n* = 144) Group control (*n* = 232) Samples grouped in N, O, and OA cases	Preparation medium supplemented with MI. Density-gradient centrifugation selection method for spermatozoa washed twice in the MI presence or absence	Pregnancy rate: A + MI=28.8%, A-MI = 18.8; OA + MI=33.3%, A-MI = 25.0% (ns) Note: Sperm parameter values were similar in cell suspensions incubated with or without MI	Park *et al.* (2016)
Prospective bicentric randomized study	78	Group MI (*n* = 262 oocytes) Group placebo (*n* = 238 oocytes)	Semen supplemented with 2mg/mL Androsito®-LAB (Lo.Li Pharma, Rome, Italy) or placebo for 20 min. Washed and subjected to swim-up with MI or placebo-supplemented medium for 30-60 min Spermatozoa recovered used for ICSI	(mean ± SD) Motile spermatozoa in swim-up selected (%): MI = 72.9 ± 24.3, placebo = 67.8 ± 24.2 (*p* = 0.004) Fertilization rate (%): MI = 78.9 ± 28.6, placebo: 63.2 ± 36.7 (*p* = 0.002) Day 3, grade A embryos (%): MI = 59.8 ± 35.6, placebo = 43.5% ± 41.5 (*p* = 0.019) Expanded blastocysts (%): MI = 56.6 ± 31.2, placebo = 61.6 ± 61.5 (ns) Implantation/pregnancy rates Implantation rate (%): MI = 21.3, placebo: 13.6% (ns) Pregnancy rate (%): MI = 35.1, placebo = 20.0 (ns)	Rubino *et al.* (2015)

A, asthenozoospermic men; O, oligozoospermic men; OA, oligoasthenozoospermic men; OT, oligoteratozoospermic men; OAT, oligoasthenoteratozoospermic men; N, healthy fertile men. Text in light blue highlights outcome for fertility treatment. Rows in italic highlight information on frozen-thawed semen samples. SD, standard deviation of the mean; SEM, standard error of the mean.

with MI prior to cryopreservation. A preliminary report had been presented at ASRM 2017 (Saleh *et al.*, 2017).

Recently, Mohammadi *et al.* (2019) reported the results on semen supplementation with MI prior to cryopreservation in semen samples from N young men without pathologies related to semen abnormalities (i.e., varicocoele, accessory gland infection, cigarette smoking, among others). Cryopreserved samples were thawed after one month and analyzed. As result, a significant improvement was observed in total and progressive motile spermatozoa, as well as normal sperm

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forms compared to controls. In addition, sperm freezing in the presence of MI resulted in a significant increase in antioxidant activity (ZB-TAC-96A commercial kit), as well as a decrease in lipid peroxidation (malondialdehyde production) and DNA fragmentation assessed by TUNEL assay. No significant changes were observed in ROS between both groups. The study is well designed, and the succesfull outcome encourages its use in abnormal semen samples.

In addition to the studies designed to assess the impact on semen parameters in fresh and frozen-thawed human spermatozoa, the effect of *in vitro* sperm incubation with MI on male fertility was addressed by some authors. Among them, Poverini *et al.* (2014) reported a prospective study done with couples counseled for IUI cycles using MI supplemented swim-up media, revealing an increase in clinical pregnancy rate after treatment. Another study presented in a 2016 meeting, described the impact of MI addition in the sperm preparation medium on sperm quality and pregnancy rates in IUI procedures (Park *et al.*, 2016). The prospective study comprised a cohort of patients divided into two groups, MI and control. Samples were subgrouped in N, O, and OA cases, and density-gradient centrifugation was used to select motile spermatozoa. The study revealed a trend toward higher pregnancy rates in A and OA patients with semen samples treated with MI, although similar sperm parameters were determined in cell suspensions regardless of treatment.

To address the impact of sperm treatment with MI on *in vitro* fertilization and embryo development in IVF-ICSI procedures, Rubino *et al.* (2015) published a report of a prospective bicentric randomized study to assess the impact of MI in fertilization outcomes of microinjected oocytes. Semen samples were supplemented with MI or placebo, washed and then subjected to swim-up with MI or placebo-supplemented medium; spermatozoa recovered after selection were used for ICSI. Fertilization rate and embryonic development were registered. MI-treated spermatozoa depicted a discrete significant increase in sperm motility after swim-up. Moreover, a significant increase in fertilization rate and the percentage of day 3 grade A embryos was found in the MI group when compared to the control. However, the percentage of expanded blastocysts was similar in both groups, and no differences were found in implantation or pregnancy rates. This was the first report describing the positive impact of MI supplementation in *in vitro* sperm selection on fertilization rate and early embryo development after ICSI procedures.

IN VIVO AND IN VITRO SEMEN SUPPLEMENTATION WITH MYO-INOSITOL AND IMPACT ON SPERM FERTILIZING POTENTIAL

In addition to the *in vivo* and *in vitro* protocols tested to assess the impact of MI treatment on male infertility, in a 2017 report, Korosi and collaborators described the effect of combining both approaches on sperm performance in IVF-ICSI procedures (Korosi *et al.*, 2017). The prospective randomized controlled trial of couples involved male partner treatment with MI. In addition, semen samples from these patients provided for the ART procedures were subjected to gradient centrifugation and swim-up procedures for motile sperm selection, followed by incubation with MI. Finally, the sperm suspension was subjected to a final sperm selection by means of hyaluronan hydrogel binding (PICSI dish). Couples with OAT male partners who did not receive any *in vivo* and *in vitro* MI treatment served as control. Patient treatment with MI was associated with a higher fertilization rate and a higher percentage of good-quality embryos. Pregnancies were obtained only in the MI-treated group, indicating a positive impact of the supplement. These findings are summarized in Table 3.

CONCLUDING REMARKS/ PERSPECTIVES

This review work has presented an overview of MI identification, natural dietary sources and absorption, biosynthesis and concentrations in human tissues and body fluids. Moreover, it has outlined information on MI functions in its free form as well as through its derivatives (PI, PIP, GPI, IPG) and their impact in health and disease, highlighting the relevance of this molecule in numerous functions. The following sections described MI presence, biosynthesis and functions in the male genital tract and spermatozoa, although the literature is scarce regarding the underlying changes in reproductive tissues and sperm structure and functions in association with MI deficiency. Based on the background information about the molecule and its relevance in several diseases, and the available formulations to treat patients or use it in *in vitro* assays, its supplementation was initially tested and reported in several clinical studies mainly from one country, and has been extended to other countries as reflected by recent reports. Studies evaluated the impact of *in vivo* MI treatment on some hormones, as well as *in vivo* and *in vitro* supplementation on sperm parameters from fresh and frozen-thawed semen. Other studies have assessed the impact of MI treatment on natural and assisted male fertility. In summary, several studies have reported the beneficial use of different

Table 3 *In vivo* and *in vitro* supplementation with MI and impact in male fertility

Type	*N*	Diagnostics	Treatment	Outcome	Refs
Prospective randomized controlled trial	35	MI-treated group: 22; control: 13 couples with OAT male partners who did not receive any *in vivo* and *in vitro* MI treatment	Male partner treatment with a MI based supplement (Folandrol, Exeltis, Hungary; a formula equivalent to Andrositol® described by Montanino Oliva et al in 2016) for two months ART semen sample subjected to gradient centrifugation and swim-up procedures for motile sperm selection, followed by incubation for 2 h with 2 mg/mL Ins (Andrositol®-LAB (Lo.Li. Pharma, Rome, Italy) The sperm suspension was subjected to a final sperm selection by means of hyaluronan hydrogel binding (PICSI dish)	(mean ± SD) Fertilization rate (%): MI = 84.8 (134/158), control = 60.5 (66/109) ($p < 0.001$) Day 3, good-quality embryos (%): MI = 54.7, control = 32 ($p = 0.001$). Number of pregnancies: MI-treated group = 11, control group = 0	Korosi *et al.* (2017)

OAT, oligoasthenoteratozoospermic men. Text in light blue with outcome from fertility treatment is highlighted.

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formulations of MI on sperm motility and DNA damage. In any case, several studies failed to include appropriate controls, and almost no investigation has reported the underlying mechanisms of action. The background information on its possible impact on male reproductive tract organs and sperm functions encourages further investigations to unravel MI roles in sperm physiology and the underlying molecular mechanisms. Moreover, larger prospective randomized controlled multicenter studies will contribute to confirm the positive effect of MI supplementation in male infertility management.

NOTESNotes

[1] MI: myo-inositol. Inositol has also been proposed as the accepted abbreviation for myo-inositol, both free and in chemical combination (http://www.sbcs.qmul.ac.uk/iupac/cyclitol/myo.html).

[2] Alternative names: myo-inositol 1-phosphate synthase, short name MI-1-P synthase; MIP synthase, short name hIPS; myo-inositol 1-phosphate synthase A1, short name hINO1.

[3] Alternative name: sodium/myo-inositol transporter 1, SMIT1.

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