Myoinositol: from gametes to embryonic development

Mariangela Palmieri¹
Claudia Crescenzo¹
Giuseppa Quaranta¹
Noemi Ciccarelli¹
Giovanni Russo¹
Sabatino Russo¹
Lodovico Parmegiani²

¹ Clinic Center Hera, Assisted Reproductive Technologies, Giugliano in Campania (NA), Italy
² GyneProMedicalCenters, Reproductive Medicine Unit, Bologna, Italy

Address for correspondence:
Mariangela Palmieri
Clinic Center Hera, Assisted Reproductive Technologies
Via Santa Caterina da Siena 36
80014 Giugliano in Campania, (NA), Italy
Phone: 081 5067919
E-mail: m.palmieri@clinicahera.it

Summary

Myoinositol (MI) is the main component of inositol family, and is widely distributed in cells and eukaryotic tissues. Physiologically, is involved in cell growth, lipid synthesis, cell cytogenesis, and morphogenesis. Growing evidence shows that MI, through the intracellular Ca²⁺ release, regulates a variety of signaling pathways including those relating to gametes development, like oocyte maturation, fertilization and embryonic development. It is studied for the treatment of several disorders like polycystic ovary syndrome and metabolic syndrome, as well as the improvement of gametes quality, the clinical outcome of IVF, from gametes to embryonic development, until the end of pregnancy.

KEY WORDS: myoinositol, inositol, oxidative stress, antioxidant, fertility.

Introduction

Myoinositol (MI) is the main component of inositol family, composed of nine stereoisomers. Inositol phosphates are carbocyclic polyols with chemical formula C₆H₁₂O₆ or (-CHOH-)₆, versatile molecules that perform important cellular functions (1). Their synthesis occurs mainly in liver and in kidneys and once produced, they enter in cells where subsequently are largely transformed by catalytic activity of various kinases. In humans inositol are synthesized from endogenous glucose, but since this metabolic reaction has a limited efficiency in quantitative terms, the necessary daily intake comes primarily through food. For this reason, inositol may be considered to belong to the group of essential B vitamins (2). The most abundant exogenous sources of inositol are represented by: whole grains, wheat germ, citrus fruits, meat in general and in particular the liver (3). They are present in animal tissues as constituents of phospholipids and in some plants, in form of phytic acid. The intestinal absorption of free inositol depends on a temperature and pH sensitive active transport, which takes place on stereospecific basis and inhibited by phlorizin. Numerous diseases are caused by inositol deficiency and, given the variety of processes in which is involved, inositol is used in many medical fields because, like to B vitamins, it is water soluble, well tolerated and devoid of toxicity. Inositol phosphates (IPs) are probably the key molecules belonging to the signal transduction system known to be involved in the regulation of several cellular functions (4). On the other hand, Inositol Phosphates (IPs) are probably the
most studied second messengers: indeed, the role of inositol 1, 4, 5-trisphosphate (IP₃) in calcium signaling through its receptors (IP₃Rs) is well known (6). IP₃ is produced via hydrolysis of a specific PIPs by phospholipase C; once the reaction has taken place; two different signal transduction molecules are produced: IP₃ and diacylglycerol that are involved in intracellular calcium ion increase and therefore, in the regulation of different signaling pathway. MI represents 99% of all inositols stereoisomers presents in human body and is widely distributed in cells and eukaryotic tissues (7). It is a precursor of D-chiro-inositol, and is synthesized from glucose: D-glucose 6-phosphate (G-6-P) is converted into 1-D-myo-inositol-3-phosphate (MIP), which is subsequently dephosphorylated to MI. This mechanism is characterized by a three-phase reaction catalyzed by 1-D-myo-inositol-3-phosphate synthase: an oxidation step with NAD that acts as acceptor of hydrogen, a step in which the intramolecular aldol cyclization occurs, and finally a step of reduction with NADH as a hydrogen donor which regenerates NAD (2).

Growing evidence shows that MI, through the intracellular Ca²⁺ release, regulates a variety of signaling mechanisms including those relating to gametes development, like oocyte maturation, fertilization and embryo development (8).

**Myoinositol and oocytes**

MI is an important component of follicular microenvironment and plays a major role both in the oocyte nuclear development and cytoplasmic maturation. In fact, IP₃ modulates the intracellular Ca²⁺ release. It has been demonstrated that fully grown mammalian germinal vesicles (GV), that exhibit spontaneous intracellular calcium oscillations, are associated with an higher incidence of GV breakdown. MI supplementation was suggested to promote meiotic progression of these GV. On the contrary, depletion of MI will desensitize PI (Phosphatidylinositol) signaling and lower IP₃ release, thus leading to intracellular calcium transient dynamics disruption and oocyte maturation interruption (9).

Indeed, high concentration of MI in human follicles fluids is strongly associates with good-quality oocytes (10, 11).

**Myoinositol and PCOS**

Polycystic ovary syndrome (PCOS) is a female hormonal disorder and a major cause of menstrual disorders, ovarian dysfunction, and infertility. Observational studies suggest that up to 15% of women suffer from this condition during their reproductive life. The most common features of PCOS are hyperandrogenism, chronic anovulation, enlarged ovaries, and skin problems such as acne, hirsutism, and seborrhea (12). The etiopathology is not yet entirely clear, but recently it was demonstrated that the insulin resistance, not linked to the BMI (13, 14), and the resulting compensatory hyperinsulinemia (increase of insulin release to compensate for its low effect) plays a key role in the clinical development of this condition in almost all women. In particular, the hyperinsulinemia could directly stimulate ovaries to produce high levels of androgen and cause a reduction of sex hormone binding globulin (SHBG) serum levels (15).

For these reasons for many years, insulin sensitizers such as metformin, pioglitazone, or troglitazone were considered as possible treatment options in management of these problems. Metformin recently has been used on patients with a hyperinsulinemic state for the improvement of ovaries with anovulation, irregular menstrual cycles and infertility problems (16, 17). However, at therapeutic doses, it has been shown to have several side effects such as bloating, diarrhea, and nausea (18, 19).

Therefore, in recent years other therapeutic alternatives for PCOS treatment were investigated.

MI is one of the most interesting molecules studied for the treatment of PCOS. Several studies have demonstrated its effectiveness in improving fertility in patients with this syndrome (11, 20).

It acts as a direct messenger of insulin signaling and improves the level of glucose absorbed by tissues. This determines an improvement of insulin-resistance condition of women with PCOS, by restoring their hormonal status and consequently ovulation process (15, 21).

MI decreases the plasma insulin levels, glucose/insulin, HOMA index as well as other hormonal parameters such as luteinizing hormone (LH), LH/FSH, testosterone and prolactin (PRL) (10).
PCOS-induced insulin resistance determines a higher risk for development of type 2 diabetes, hypertension and dyslipidemia, all elements of metabolic syndrome (22). Because of its insulin sensitizing action and its high safety profile, MI is an effective approach for metabolic syndrome treatment (MS). In particular, available data have shown that in women with MS the treatment for 1 year with MI significantly reduces the parameters related to insulin resistance and dyslipidemia than diet alone. Furthermore, after one year of treatment, 20% of women did not fall more in the diagnostic criteria of MS (23, 24).

Recent evidences have demonstrated how, in ovarian level, PCOS women are characterized by a deficiency of MI and an excess of D-chiroinositol (DCI). These data further explain two features of the syndrome. In fact, women with PCOS have poor egg quality, due to a reduced energy intake (MI regulates glucose uptake) and overproduction of androgens (DCI in the ovary is responsible for the production of androgen-mediated insulin). Analyzing other functions exerted by MI in the ovary, it has highlighted its FSH sensitizing action, making it a crucial compound for follicular growth and probably for recruitment of dominant follicle (20, 25, 26).

Women with PCOS have an increased risk of ovarian hyperstimulation syndrome (27). In fact, high levels of serum ovarian androgens are implicated in high serum E2 levels production after ovarian stimulation with gonadotropins. PCOS patients treated with gonadotropins + MI showed a significant E2 levels reduction after administration of hGC. This was reflected in a lower number of cycles of in vitro fertilization (IVF) canceled due to high levels of E2 [sign of hyperstimulation syndrome (28)].

In line with these data, a recent clinical study with the aim of comparing the effect of supplementation MI or DCI on oocyte quality of PCOS patients showed that only MI instead DCI is able to improve the quality of oocytes (29). Costantino et al. (30) showed in a double-blind placebo-controlled trial, that MI significantly improved blood pressure, triglycerides, cholesterol, glucose, and insulin values. These improvements were achieved after a 16-week treatment period. Evaluating the hormone values showed a significant decrease in serum levels of total and free testosterone and at the same time an increase in progesterone levels, as marker of ovulation, in the group that received MI. This could demonstrate that MI has led not only positive changes in metabolic parameters, but also to a reduction of high values of androgens and subsequently to an improvement of skin problems such as acne or hirsutism.

In an observational study by Regidor et al. about 3602 infertile women treated for 2-3 months with $2 \times 2000$ mg myoinositol + $2 \times 200$ micrograms of folic acid per day, 70% of patients has restored ovulation after treatment. Already after 12 weeks the patients showed an increase in progesterone values from 2.1 ng/ml to 12.3 ng/mL, and at the same time a reduction in testosterone levels (96.6 ng/ml to 43.3 ng/dl) and free testosterone (1.2 ng/mL to 0.35 ng/mL), without relevant side effects. Pregnancy rates were equivalent or higher than those reported by the use of insulin sensitizer metformin in a comparison group (12).

Several studies have shown improvement in ovulation rates and regularization of menstrual cycles achieved by the combined use of 4 g of myoinositol with 400 mg of folic acid daily. Gerli et al. (31) demonstrated in a prospective study that 82% of patients ovulated after treatment with MI + folic acid, compared to 63% in placebo group. Similarly, the 70% of patients of the group treated with myoinositol has shown regular menstrual cycles after 16 weeks of treatment, whereas only 13% of women in the placebo group.

In a study of Raffone et al. (32), comparing the administration of myoinositol (2 $\times$ 2000 mg + 200 $\mu$g a day) with that of metformin (1500 mg daily) in women with a PCOS, it could be shown that the number of pregnancies was much higher in the group treated with myoinositol compared to the metformin group.

In a retrospective study evaluating the prevalence of gestational diabetes mellitus among PCOS patients, it was demonstrated that it was significantly lower (17 versus 54%) in the group continuously treated with MI (n=54) compared to the nontreated group (n=37). Such data suggest the possibility of MI use in the primary prevention of gestational diabetes mellitus in PCOS women (33, 34).

Kamenov et al. showed an improvement of ovulation induction with myoinositol alone and in combination with clomiphene citrate in polycystic ovary syndrome in patients with insulin resistance (35).
Another important factor is also linked to the difference of myoinositol and metformin in terms of safety profile and compliance for patients. In patients treated with metformin, side effects were commonly reported, particularly from mild to severe gastrointestinal side effects such as abdominal pain, nausea and diarrhea. Only in rare cases, serious side effects such as lactic acidosis have been found.

On the other hand, myoinositol seems to be a safe and well tolerated approach, in any case able to give results similar to those of metformin in terms of clinical efficacy.

A meta-analysis of Unfer et al. (10) has further validated these data. This study also demonstrated that the dosage of 4000 mg myoinositol + 400 µg folic acid has not in side effects-correlated, in comparison to those caused by other insulin sensitizers such as metformin (1500 mg daily).

This confirms that myoinositol is not only an effective alternative in the treatment of PCOS patients but also safe about side effects.

MI and sperm cells

It has been shown that oxidative stress plays a crucial role in the pathogenesis of male infertility because of its involvement in damage to sperm DNA (36-39). Spermatozoa appear to be very sensitive to negative action of reactive oxygen species (ROS), resulting in impairment of motility, morphology and integrity of sperm and, consequently, reducing its reproductive potential. White blood cells and sperm cells, prematurely released from the seminiferous tubules, seem to be the two main sources of ROS (40, 41). However, small amounts of hydrogen peroxide or other free radicals such as nitric oxide and superoxide anion, can promote sperm capacitation, the binding to zona pellucida and the acrosome reaction (40-46), suggesting that these ROS, at low concentration, can play a key role in sperm capabilities (43, 47). The functionality of sperm is therefore also dependent on maintaining a delicate balance between free radicals and antioxidant barrier: ROS at low levels are essential for survival and maintenance of normal cellular activities but, at the same time, they may damage sperm function and survival (48, 49).

Exogenous and endogenous in vitro ROS become crucial during IVF procedures, because the processing of semen sample removes seminal plasma but also its antioxidant component and not takes care of ROS production by immature sperm cells in the sample (50). Many studies have reported the benefits of antioxidants in vitro to protect sperm from exogenous ROS produced during sample preparation. Among these, Myoinositol (MI), cell mediator involved in Ca²⁺ release (3), has been and currently is the subject of many studies.

The presence of ISYNA1 and IMPA1 was confirmed even in the testes, epididymis, seminal vesicles and some parts of the brain. The elevated expression of these enzymes in epididymal and Sertoli cells (51, 52) is most likely due to serum MI inability to get through testicular tight junctions. Once produced, MI is transported into cells by a sodium/myoinositol cotransporter (SLC5A3) whose expression is sensitive to osmolar changes. MI is an osmolyte so, when the environment becomes hypertonic, Sertoli cells increase the expression of cotransporter protein (51).

Regarding to male reproductive function, MI appears to regulate: osmolarity and volume of seminal plasma; expression of proteins essential for embryonic development and sperm chemotaxis; sperm motility; capacitation; acrosome reaction. Recent studies also suggest a role of MI in maturation and migration of epididymal sperm (3). Furthermore, MI concentrations in deferens vessels, epididymis, seminal vesicles and prostate are 28 times higher than those in plasma where free MI has a concentration of about 29 µmol (53).

Tests on transgenic mice correlated a reduced male fertility to low concentrations of MI in epididymis, due to changes in osmolarity and volume of seminal fluid; while high levels of IMPA1 have been linked with decreased motility in asthenozoospermic patients (52).

All these findings have led to test MI as a possible oral or in vitro antioxidant agent in male infertility. According to Gulino et al., a systemic treatment of male infertility with MI improves the semen sample quality. After three months of intake of 4000 mg/die of MI, it was found a clear increase of spermatozoa number and motility in OAT and normospermic patients (54).

In a study of Calogero et al., a total of 194 patients was random selected to assume MI (4g/die, n = 98) or placebo (n = 96) for 3
months. The two groups did not differ significantly in age, body mass index, serum concentrations of hormones, sperm parameters and percentage of acrosome-reacted sperm. Treatment with MI or placebo did not affect the concentrations of serum PRL and testosterone nor the volume of seminal fluid compared to baseline. However, patients treated with MI showed a significant increase of serum inhibin B, concentration and total sperm count, progressive motility and percentage of acrosome-reacted spermatozoa compared to placebo. Furthermore, MI significantly reduces the FSH and LH levels compared to placebo (55).

Regarding the effect of MI in vitro, in the study of Colone et al., samples from normospermic and OAT subjects were incubated with 2 mg/ml of inositol for 30 minutes, 1 hour and 2 hours at 37°C and controlled CO2 and then evaluated with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The qualitative analysis of the sperm surface showed that OAT sperm, unlike normospermic sperm, was covered with an amorphous fibrous material which could be responsible for reduced or absent motility. After treatment, OAT samples showed no more amorphous material and also a marked increase in the thickness of intermediate section was seen. These data apparently suggest that inositol is capable of dissolving the amorphous fibrous material, probably inducing changes in pH of seminal fluid. Observations by TEM of ultrafine sections showed sperm cells with a lot of intact mitochondria surrounding axoneme in normospermic, unlike damaged mitochondria in OAT samples. After 2h incubation with inositol, these mitochondria appeared morphologically more similar to those of the control group (3).

In our previous study, we analyzed a total of 100 fresh and 25 thawed semen samples prior to and after addition of MI. Treatment of samples with MI showed an increase in the sperm total and progressive motility in both fresh and thawed samples. Furthermore, MI proved to be well tolerated by spermatozoa in vitro, demonstrating that it can be efficiently and safely used as an antioxidant in the laboratory practices and preparation of semen samples for ART (49).

In vitro supplementation of MI can also significantly reduce the DNA fragmentation process. A cross-sectional study on semen samples from 10 patients was performed; baseline group was tested for DNA fragmentation after liquefaction and re-tested after 4 hours, divided into 4 subgroups: seminal plasma (SP), SP combined with MI (SP + MI), SP diluted 1:1 sperm with culture medium (CM), SP diluted 1:1 with CM and MI (CM + MI). Fragmentation rate of sperm DNA after 4 hours incubation was significantly increased in all groups, except in CM + MI group (56).

In a recent clinical study of Montanino Oliva et al., Authors demonstrated that treating fertile women with MI vaginal suppositories enhanced their partners’ sperm motility and also positively affected their conceiving capacity, without changes in cervical mucus structural and biochemical characteristics. Indeed, by means of the postcoital test on female cervical mucus, a significant improvement especially in progressive sperm motility was recorded after MI suppository use. Concomitantly, after MI treatment, a reduction of immotile spermatozoa percentage was observed (57).

In two works of Condorelli et al. (52, 58), seminal samples from 12 subjects (5 normospermic and 7 OAT), in the first work, and 40 samples (20 normospermic and 20 OAT), in second work, were incubated in vitro with 2mg/ml of MI or placebo for 2h. Data showed an increased percentage of spermatozoa with high membrane potential (MMP) in OAT patients, almost comparable with tested normospermic population. These results suggest an ameliorative effect of MI for mitochondrial functions in OAT patients and a protective effect in normospermic patients.

In a recent study, Carlomagno et al. showed that incubation of OAT samples with MI significantly improves their motility (9). The release of mitochondrial Ca2+, due to exposure to MI, probably results in a superior percentage of sperm cells with high mitochondrial membrane potential (MMP), a marker of apoptosis. When there is an apoptotic stimulus, the MMP is reduced (52).

Within this context, Rubino et al. investigated on outcome of ICSI cycles where spermatozoa were incubated with MI showing that, in OAT patients, MI improves motility and fertilization rate was higher in MI treated group (59). Marchetti et al. also showed that MMP, increased by MI, is positively correlated with fertilization rates (60). These studies therefore suggest a MI use for assisted reproduction techniques in vitro (52, 59). Moreover, with an improved quality of semen, the timing of the ICSI technique, in
terms of sperm selection, can be shortened to benefit the whole procedure. Our goal in an IVF laboratory is simulate in vivo conditions, if possible without mechanical stress and/or exposure to biochemical agents. Since MMP alterations are biochemically invasive for spermatozoa, further investigations are required on the physiology of sperm cell after exposure to MI before promoting this approach in standard clinical practice (61).

**Myoinositol and embryonic development**

We have already explained how MI is involved in the mammalian cell metabolism. The Ca\(_{2+}\) oscillations in mammalian oocytes play an important role in the acquisition of meiotic competence and guide them towards the final stages of maturation (62). The same oscillations, however, were also detected in zygotes. In fact, they express IP\(_3\)-receptors, suggesting that inositol is involved in mediating Ca\(_{2+}\) release even in the initial stage of development. Ca\(_{2+}\) fluctuations that occur in mammalian embryo cleavage stage could affect preimplantation embryonic development (63). A pre-treatment of women with MI before hormonal stimulation during IVF cycles might therefore increase the quality of oocytes (28, 29, 64) and embryos (65, 66), and possibly the implantation rate (67).

Experiments on farming species have supported the theory of a positive role of MI in mammalian pre-implantation development, as one of its supplementation to culture media has improved in rabbits and cattle the blastocyst formation, expansion and hatching, and gave birth to healthy animals (68, 69).

These data have prompted the hypothesis that the inclusion of this molecule in culture media of human embryos would have produced an increase in high quality embryos number in IVF cycles. To test this hypothesis, Colazingari et al. analyzed the MI effects on mouse preimplantation embryo, obtained by ICSI and cultivated in a standard medium usually used in IVF, in the same conditions that might apply to our species. The effects were evaluated by development rate analysis (65, 70, 71).

The embryo culture, in presence or absence of MI was monitored daily until reaching the expanded blastocyst stage. At each interval of time post-fertilization (p.f.), the embryos grown in medium supplemented with MI showed a faster division rate, with a higher percentage of embryos in more advanced stage.

The evolutionary difference between embryos cultured in presence or absence of MI was particularly evident 96h p.f., when most of the blastocyst belonging to the first group (+MI) were expanded. These embryos showed an higher number of blastomeres, suggesting that faster growth rate observed in all division phases reflects an higher proliferation activity (65).

The MI is transported into mammalian cells, including oocytes and early embryos, from a sodium co-transporter (72) and in part by an independent sodium transporter (73). During preimplantation mouse development, the activity of these transporters produces a strong MI absorption, progressively increasing from the initial stage to single cell up to the blastocyst (73, 74).

Previously, information about MI effects on mouse development were limited to oocytes meiotic maturation and early embryonic stages. In fact, Chiu et al. (11) had previously shown that the addition of MI to oocytes culture in the germinal vesicle stage increased their maturation rate in metaphase II (MII). After fertilization in vitro of these oocytes and the transfer of the resulting 2-cell embryos, a higher implantation rate and an higher post-implantation viability were observed, compared to control embryos.

About the nature of the biochemical pathways induced by mouse embryos blastomeres exposure to MI, existing information suggests that the possible path aroused by MI, responsible for a reduced length of cell cycles and more efficient proliferation activities, will be initiated by a rapid incorporation into phosphoinositides (74), namely, PIP\(_3\), enzymatically favored by the activity of PI\(_3\)K.

PIP\(_3\) is converted by phospholipase C (PLC) in diacylglycerol (DAG) and IP\(_3\) that, mobilizing Ca\(_{2+}\) exchange, has already been recognized accelerate various cellular processes, including proliferation (75-77). PI\(_3\)K is also responsible for the activation of Akt (78), which is known to promote the proliferation of blastomeres in mouse embryos (79). Since MI has been shown to increase the Akt phosphorylation as well as its activity in rat skeletal muscle cells (80), it
has been speculated that MI supplementation may be a critical step for the improvement of preimplantation embryo development (81). Our group is currently collecting data about outcome of Intracytoplasmic Morphologically-selected Sperm Injection (IMSI) cycles with “physiological” sperm selection (based on hyaluronic acid binding ability). We are injecting sibling oocytes with MI-sperm or untreated-sperm, comparing fertilization rates and the following preimplantation embryonic development.

A second open question in current research involves the safety of preimplant embryo after MI exposure, for post-implantation and post-natal development.

MI also seems particularly important for early post-implantation embryonic development (82): its serum concentration in fetuses and infants is much higher than in adults (79); its administration during pregnancy reduces the possibility of developing gestational diabetes (83, 84) and, in the fetuses of curly tail mutant mice (ct) (82), the natural occurrence of spina bifida.

Pregnancy is a physiological state in which there is a high metabolic demand for tissue oxygen. This oxygen demand increase leads to a higher production of reactive oxygen species (ROS), damaging the cell membranes for a lipid peroxidation process (9).

During pregnancy, the main source of the peroxidized lipids is the placenta, and their concentration increases in blood during the entire gestation (79). Furthermore, pregnancy has a negative effect on the activity of several antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in the liver and placenta (80). This evidence clearly shows that during pregnancy women need to confront an increased oxidative stress. It has been shown that some disorders related to pregnancy both depend on high levels of oxidative stress and imbalance of some micronutrients in maternal blood. Several studies have been conducted to investigate the role of MI, sometimes associated with melatonin, in restoring and maintaining an healthy pregnancy and a good fetal development.

The neural tube defects (NTDs) in humans are the most frequent malformations that occur during pregnancy. Characterized by a defective closure of the neural tube during the first 4 weeks after conception. Failure to neural tube closure could lead to serious illnesses and birth defects, and in certain conditions incompatible with life (spina bifida and anencephaly).

Nowadays, it is generally known that folic acid supplementation in the periconceptional period can prevent the majority of neural tube defects (NTDs) (85). Several studies of NTDs in mouse models have provided various tests on two different NTD subtypes that are classified as sensitive folate and folate-resistant. In humans about 30% of NTDs are folic acid resistant (86).

Recently, several studies have shown that a new therapy for folate-resistant DTN is the administration of a combination treatment with folic acid and MI. This new therapy has been shown to successfully prevent the majority of cases of neural tube defects, even those folate resistant (87).

Children who suffer from respiratory distress syndrome have an immature respiratory system that often leads to premature death. Over the past 30 years, the strategy for the prevention of RDS was addressed to the acceleration of fetal lung maturity in utero by drugs administered to the mother as glucocorticoids (GC) (88), and the development of surfactant substitutes for the treatment of surfactant deficiency to birth.

Possible serious side effects of GC have led to the development and testing of other drugs able to accelerate lung maturity. Studies aimed to investigate the pulmonary surfactant constituents have shown that during fetal development the surfactant system contains very high phosphatidylinositol levels (89). By collecting these data, further studies have shown that myo-inositol, administered to the mother has a positive effect on fetal lung mechanics, reducing the side effects of glucocorticoids such as decreased lung protein content (90).

Conclusions

The intracellular Ca²⁺ increase, MI-induced, plays an important role in various cellular processes like oocytes maturation, fertilization and embryonic development (pre- and post-implantation).

High concentration of MI in human follicles fluids is strongly associated with good-quality oocytes (10, 11) and several studies have demonstrated its effectiveness in improving fertility in patients with polycystic ovary syndrome (11, 20). It acts as a direct messenger of insulin
signaling and improves the level of glucose absorbed by tissues. This determines an improvement of insulin-resistance condition of women with PCOS, by restoring their hormonal status and consequently ovulation process (15, 21). Furthermore, comparing the administration of myo-inositol with that of metformin in women with a PCO syndrome, it could be shown that the number of pregnancies was much higher in the group treated with myo-inositol compared to the metformin group (32).

Regarding to male reproductive function, MI appears to regulate: osmolarity and volume of seminal plasma; expression of proteins essential for embryonic development and sperm chemotaxis; sperm motility; capacitation; acrosome reaction. Recent studies also suggest a role of MI in maturation and migration of epididymal sperm (3). Several research works have tested MI as a possible oral or in vitro antioxidant agent in male infertility, all with positive results: MI improves sperm quality (54), it can also reduce the DNA fragmentation process (56) and increases percentage of spermatozoa with high membrane potential (MMP) (52), which suggests an ameliorative effect of MI for mitochondrial functions in OAT patients and a protective effect in normospermic patients.

Regarding to MI involvement in embryonic development, it was demonstrated that zygotes express IP₃-receptors, suggesting that inositol is involved in mediating Ca²⁺ release even in the initial stage of development. Ca²⁺ fluctuations that occur in mammalian embryo cleavage stage could affect preimplantation embryonic development (63). A pre-treatment of women with MI before a hormonal stimulation during IVF cycles might therefore increase the quality of oocytes (28, 29, 64) and embryos (65, 66) and possibly the implantation rate (67). These observations have prompted the hypothesis that MI supplementation in culture media of human embryos would increase the high quality embryos number in IVF cycles. MI also seems particularly important for early post-implantation embryonic development (82): its serum concentration in fetuses and infants is several times higher than in adults (79). Several studies have been conducted to investigate the role of MI, sometimes associated with melatonin, in restoring and maintaining a healthy pregnancy and a good fetal development (87).

MI administration, both in vivo and in vitro, has not shown considerable side effects.

So, it could be potentially used in all phases of IVF cycle, starting from preparation to hormonal stimulation until the end of pregnancy. It would be appropriate to further investigate the possible consequences, even if not apparent, of MMP variations, induced by MI, on the physiology of gametes and embryonic cells.

**Competing interests**

The Authors declare that they have no conflict of interests regarding the publication of this paper.

**References**

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46. Grieuve JF, Dumont E, Renard P, Callegari JP, Le Lannou D. Reactive oxygen species, lipid peroxidation and enzy-


