Breakthroughs in the Use of Inositols for Assisted Reproductive Treatment (ART)

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Review

Breakthroughs in the Use of Inositols for Assisted Reproductive Treatment (ART)

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It is well known that myo-inositol (MI) and D-chiro-inositol (DCI) are insulin-sensitizing agents, and MI is of proven utility in polycystic ovary syndrome (PCOS). In addition, MI plays a pivotal role in the physiology of reproduction, and has beneficial effects on the development of oocytes, spermatozoa, and embryos. By contrast, DCI has little effect on spermatozoa, but high concentrations in the ovary can negatively affect the quality of oocytes and the blastocyst. Overall, the evidence in the literature supports the beneficial effects of MI in both female and male reproduction, warranting clinical use of MI in assisted reproductive treatment (ART).

Highlights

MI and DCI are insulin second messengers, but have different functions.

MI is involved primarily in cellular glucose uptake (e.g., the ovary, testis, and epididymis contain high concentrations of MI). By contrast, DCI levels are high in tissues where glycogen is stored (e.g., liver and muscle).

In the ovary, MI acts also as a second messenger downstream of follicle-stimulating hormone.

MI is involved in cell proliferation and improves oocyte and embryo quality.

Unlike MI, there are no data on the effects of DCI on sperm quality and on the first phases of fertilization and development, whereas high concentrations of DCI in the ovary are detrimental to oocyte and blastocyst quality.

MI also plays a pivotal role in male fertility at both the physiological and therapeutic levels.

MI oral supplementation during controlled ovarian stimulation and ART reduces the total amount of gonadotropins used and the length of controlled ovarian hyperstimulation in both PCOS and non-PCOS women.

Inositols: A Brief Presentation

This review describes the physiology of inositols, mainly in relation to reproductive processes, and, based on this evidence, provides therapeutic proposals for MI use in **assisted reproductive treatment** (**ART**; see Glossary), either as a dietary supplement or as an additive to the culture medium, with the aim of providing an effective method to combat the increasingly widespread problem of male and female infertility.

Inositols are natural molecules with nine stereoisomeric forms, of which MI and D-DCI are the most important. They function as **insulin** second messengers but have different activities [1–3]. MI is primarily involved in cellular glucose uptake, and its content is elevated in tissues with high glucose utilization including the brain, heart, and ovary [1,4]. On the other hand, DCI levels are high in tissues where glycogen is stored (e.g., liver and muscle) and low in tissues with high glucose utilization [1]. In addition, MI plays essential roles in both female and male reproduction. This stereoisomer is very safe, as stated by the FDA [5] and several studies [6].

Inositols in the Physiology of Reproduction

Several studies have demonstrated that MI plays pivotal roles in the physiology of both the female and male reproductive systems.

MI and DCI in the Ovary

MI and DCI, mainly when they are in the optimal ratio (40:1), are effective for treating **polycystic ovary syndrome (PCOS)**, the primary cause of infertility, in addition to menstrual dysfunction and metabolic disorders [7,8]. MI acts on cell proliferation and improves oocyte and embryo quality [9]. Furthermore, it plays a key role in the maturation of oocytes, which have IP3 receptors and MI transporters, as do maturing embryos [9]. In mammals, MI concentrations are considerably higher in female reproductive organs than in blood, in line with its different functions in the ovary. MI is a second messenger for **follicle-stimulating hormone (FSH)** in the ovary, in the form of myo-inositol 1,4,5-trisphosphate (MI-InsP3) [9]. In agreement with this physiological role, MI significantly enhances the serum levels of **anti-Müllerian hormone** (**AMH**; produced by granulosa cells under FSH stimulation) in women with diminished ovarian reserve (DOR), thus increasing the likelihood of pregnancy [10]. In mouse models, MI induces the meiotic progression of oocytes into fertilization-competent eggs, whereas its reduction within the ovary impairs physiological oocyte maturation [11]. MI also accelerates oocyte transport in the oviduct [12]. It is noteworthy that each organ can regulate the intracellular balance of inositol levels to determine the tissue-specific MI/DCI ratios that control metabolic processes [13]. The ratio is determined by the activity of an insulin-dependent epimerase which converts MI to DCI. Under normal conditions the ratio in the ovary is 100:1 [13], and in any case >70:1, as detailed below. Unlike MI, high concentrations of DCI in the ovary can negatively affect the quality of oocytes and the blastocyst. A recent study involved a homogeneous group of eight egg donors and 11 couples, comprising men with similar sperm quality [14]. The MI:DCI ratio was calculated in the follicular fluid and, after oocyte fertilization, this was found to be associated with different blastocyst grades. Blastocyst quality was found to progressively worsen with decreasing MI:DCI ratio in follicular fluid, in other words with increased DCI levels. MI and DCI proved to be, respectively, markers of 'high quality' and 'low quality' oocytes and blastocysts. A threshold of 70:1 for the MI:DCI ratio in follicular fluid was suggested as an indicator of blastocyst 'well-being'. Above this value blastocysts are of good quality and are suitable for successful ***in vitro* fertilization (IVF)**. It is noteworthy that DCI decreases, in a dose–response manner, the expression of the **aromatase** gene (*CYP19A1*) [15]. Aromatase, an enzyme expressed in granulosa cells (Figure 1), mediates the transformation of **androgens** to **estrogens**, and inhibition of its activity causes an increase in androgen levels, explaining the lower oocyte and blastocyst quality associated with high DCI levels.

MI in Male Reproduction

It has been known for a long time that the male reproductive organs are particularly rich in free MI [16]. High concentrations are found in rat testis, epididymis, and prostatic fluid [17]. The seminal fluid of mammals is a rich source of free MI and contains higher levels than blood. Inositol concentrations in the testicular fluids of rat, hamster, and monkey were found to be ~30–40-fold higher than in blood plasma; in addition, increased MI levels were reported in caput luminal fluid [18]. Epididymis, in particular, has an inositol concentration higher than testis. The capacity of the male reproductive system to synthesize MI differs according to the site: in rete testis fluid it is ~50% higher than in cauda epididymal plasma and >fivefold higher than in seminal plasma [19]. It has long been hypothesized that MI may play an important role in spermatozoa maturation in the seminiferous tubules and in their migration to the epididymis and vas deferens. Because MI can be synthesized *in vitro* by spermatozoa, one can hypothesize that most testicular inositol is formed within the seminiferous tubules [20]. Further, primary cultures of Sertoli cells, maintained in a chemically defined medium without inositol addition, released MI into the medium during three successive 24 h periods. The amounts released were greater in cells stimulated by dibutyryl cAMP. These results were interpreted to indicate that MI in the fluid of seminiferous tubules most probably originates from Sertoli cells that synthesize inositol from glucose. In fact, additional inositol in epididymal tubule fluid could readily be generated by glucose metabolism in epididymal epithelial cells. Rat epididymis contains the enzymes necessary for such biosynthesis, and this

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may account for the high levels of free MI seen in the luminal fluid of the cauda epididymidis [21]. Some studies also indicate that inositol biosynthesis may be essential for normal metabolism of the germinal epithelium because *de novo* MI synthesis could be necessary to generate adequate intracellular levels of nucleotide precursors that are essential for maintaining the integrity of adult epithelial cells [22]. In addition to such intracellular effects, Chauvin and Griswold conjectured that MI may exert extracellular activity as an osmoprotective molecule to safeguard sperm cells – by analogy to the same role it plays in kidney [23]. In this regard, it is worth recalling that **osmolytic activity**, probably dating back to the prebiotic era, was useful from an evolutionary point of view because it provides vital protection to cells against environmental and metabolic stress [24]. As far as the phospholipid-bound MI is concerned, one of the important functions of IP3 in the male reproductive system is to mobilize calcium that activates the nuclear fusion of vesicles [25]. Release of calcium from the acrosome may also play an important role in promoting membrane fusion and therefore exocytosis [26]. Furthermore, hyperactivation of spermatozoa motility is crucial for fertilization because it promotes their migration in the oviduct to reach the egg. It is well known that Ca^{2+} is necessary for the initiation and maintenance of this motility because it regulates asymmetric flagellar beating. Therefore, Ca^{2+} mobilization from storage sites is a fundamental step, and IP3 is deeply involved in this physiological pathway as an important releasing factor for stored Ca^{2+} [27] (Figure 2).

Treatment with MI in Impaired Female Fertility (PCOS and Other Conditions)

The first study (1992) on the role of MI in IVF found a positive correlation between serum concentrations of MI and successful IVF pregnancy. Serum samples with high MI content showed clear trophic properties when added to *in vitro* embryo cultures, and supported better post-implantation development of mouse embryos [28]. Subsequent studies showed that high MI levels in human follicular fluid correlate positively with satisfactory oocyte quality [29]. Moreover, addition of MI to the culture medium stimulated meiotic progression by mouse germinal vesicle oocytes, a process that requires intracellular calcium mobilization [11]. On the other hand, Goud and coworkers demonstrated that inositol-1,4,5-trisphosphate plays an important physiological role during *in vitro* maturation, fertilization, and early cleavages of human oocytes and embryos [30].

PCOS women undergoing ART are a particularly challenging target group and have been the subject of several recent studies. Wdowiak [31] investigated the activity of oral MI in PCOS women enrolled for **intracytoplasmic sperm injection (ICSI)**. Over the 3 months before ICSI, 60 control PCOS patients received 200 µg of folic acid twice per day; the remaining 52 PCOS subjects were treated with 2 g of MI plus 200 µg folic acid, also twice per day. Further controls (no treatment) were 105 healthy women. A significant difference in the number of pregnancies was found: pregnancy was reported in 34.62% of the MI-treated group but in only 20% of the PCOS controls. **Superoxide dismutase (SOD)** concentration in follicular fluid increased significantly only in the MI group. **Catalase (CAT)** activity was not modified [31].

In another IVF clinical trial [32], 133 PCOS and 137 non-PCOS women with preserved ovarian reserve were treated daily by the oral route for 3 months during the preconception period and ovarian stimulation. The first group (PCOS) received 1 g MI plus 400 µg folic acid, whereas the second group (non-PCOS) received 400 µg folic acid plus 2 µg cyanocobalamin. The total number of mature oocytes in the MI-treated patients was significantly higher than in the second group. Pregnancy rates per embryo transfer, 'take home baby' index, and miscarriage rates were comparable. In conclusion, MI improves oocyte quality, thus potentially improving IVF outcome [32].

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Further support was provided by Emekçi Özay *et al.* [33] who administered 4 g MI plus 400 μg folic acid, before and during **controlled ovarian hyperstimulation (COH)** with recombinant (r)FSH and intrauterine insemination (IUI), to 98 infertile PCOS women undergoing controlled ovulation induction and IUI. Controls (n = 98) received rFSH and 400 μg folic acid. Of the treated subjects, nine accomplished spontaneous pregnancy. During COH + IUI treatment three cycles were canceled in the study group and eight in the control group. In the treated patients, a significant decrease in total rFSH dose and cycle duration was reported; in addition, clinical pregnancy rate was higher (18.6%) in patients receiving MI compared with controls (12.2%) [33].

Systematic review and meta-analysis, including eight randomized controlled trials (RCTs) with a total of 812 participants [34], confirmed that oral inositol supplementation during **controlled ovarian stimulation (COS)** and ART can reduce the total amount of gonadotropins used and the length of COS in both PCOS and non-PCOS women undergoing IVF. According to the analysis, MI was effective in reducing gonadotropin administration in both PCOS and non-PCOS women. However, MI supplementation decreased the length of COH only in PCOS women. Although the data from the current literature do not take pharmacoeconomic aspects into account, based on the above results the authors assert that MI therapy may significantly reduce the overall cost of IVF procedures, with direct benefits for the patients [34].

Zheng and colleagues [35] performed systematic literature review and meta-analysis concerning the efficacy of MI administration to infertile (non-PCOS) patients undergoing ovulation induction for ICSI or IVF and embryo transfer (IVF-ET). Seven trials, with a total of 935 women, were taken in consideration. MI treatment was associated with a significantly higher clinical pregnancy rate and proportion of grade 1 embryos. The abortion rate in the MI group was significantly lower than in controls. Furthermore, the study group required significantly fewer total units of gonadotropins such as rFSH compared with controls. MI therefore increases both the clinical pregnancy rate in infertile women undergoing assisted motherhood and the quality of embryos, as well as reducing the number of unsuitable oocytes and the amount of stimulation drugs required [35].

Further evaluation of MI supplementation, in a larger cohort of patients, will be necessary to assess its economic advantages in IVF treatments and its effect on long-term fertility outcomes (clinical pregnancy rate and live birth rate in ART and spontaneous ovarian cycles).

MI and Embryo Development *In Vitro*

Preimplantation mouse embryos supplemented *in vitro* with MI exhibit (i) increased percentage of progression to the most advanced stage of development; (ii) overall increased percentage of development to the expanded blastocyst stage; (iii) increased average number of blastomeres forming the embryos at the blastocyst stage [36]. A plausible mechanism may include rapid incorporation of MI into phosphatidylinositides (PtdIns) and increased production of intracellular second messengers that enhance proliferation [37,38]. In particular, MI supplementation of the culture medium of late preimplantation mouse embryos induced Akt phosphorylation at serine 473 [39]. This demonstrates that, in the early stages of development, new phosphorylation of Akt occurs in the mid-to-late preimplantation stages, and this process depends on the availability of MI. Previous evidence showed that the development of preimplantation mouse embryos (8–16 cell stage) requires the activity of PI3K, an enzyme that produces PIP3 from PtdIns [40,41]. The increased synthesis of phosphoinositides [42], and the resulting increase in PIP3, may account for these observations. In conclusion, enhanced phosphorylation of Akt in the presence of MI may be responsible for the faster development rate of cultured embryos. Reasonably, MI supplementation to dividing blastomeres

Glossary

Androgens: steroid hormones (e.g., testosterone) that are produced by testis, ovary, and the adrenal gland.
Anti-Müllerian hormone (AMH): a glycoprotein hormone synthesized by follicle granulosa cells of the preantral and small antral follicles. It regulates their sensitivity to FSH and subsequent recruitment to the ovarian cycle.
Aromatase: an enzyme involved in transforming androgens into estrogens. It can be found in several tissues: ovary, placenta, testis, adrenal, adipose tissue, brain. In the ovary aromatase is predominantly in the granulosa cells (endoplasmic reticulum) and its expression is stimulated by FSH. D-chiro-inositol (DC) exerts an inhibitory effect on the aromatase.
Assisted reproductive treatment (ART): describes collectively all noncoital methods of conception that are used to treat infertility with donor or non-donor eggs and sperm, including procedures such as IVF and ICSI.
Controlled ovarian hyperstimulation (COH) and controlled ovarian stimulation (COS): a method of ART consisting of carefully monitored administration of agents designed to induce ovulation by a greater number of ovarian follicles and thus increase the probability of an oocyte being fertilized.
Estrogens: steroid hormones that induce the development and maintenance of female features in the human body. Estrogens regulate the menstrual cycle and reproductive system. During the menstrual cycle, estrogens favor the fertilization, implantation, and nutrition of the embryo in its early stages. Estrogens are produced from androgens in the ovary through the action of aromatase.
Follicle-stimulating hormone (FSH): a glycoprotein that controls the growth and development of ovarian follicles. It stimulates aromatase activity and thus induces estrogen production by ovarian granulosa cells.
Insulin: a peptide hormone produced by the β cells of pancreatic islets. It regulates blood glucose concentrations by stimulating glucose uptake from the blood by liver, muscle, and adipose tissue. Imported glucose can be used to provide energy or can be stored.
Intracytoplasmic sperm injection (ICSI): an IVF technique to overcome male infertility. In this procedure, a single sperm is injected into an egg removed

enhances the pathway leading to Akt phosphorylation and accelerates development. In conclusion, the direct effects of MI on oocyte health and subsequent development may improve ART outcome.

MI Treatment in Impaired Male Fertility

MI was used to treat men with fertility problems, principally **oligoasthenoteratozoospermia (OAT)** – a disorder that includes oligozoospermia (low sperm count), asthenozoospermia (reduced sperm movement), and teratozoospermia (malformed sperm cells). MI was used either *in vivo*, *in vitro* or, in both for the same patient.

In Vivo Treatment

Gulino *et al.* [43] enrolled 62 male patients undergoing IVF cycles and divided them into three groups: the first group (A) of 29 healthy fertile men (**normospermic**), a second group (B) of 13 OAT patients, and a third (control) group of 20 healthy patients undergoing ART for a female cause of infertility. Spermatozoa count, progressive motility, and spermatozoa count after density-gradient separation were significantly worse in group B (OAT patients) compared with both group A (normospermic patients) and the control group. No differences were found between group A and the control group. Patients from group A and B were orally administered 4 g of MI and 400 µg of folic acid for 2 months. This treatment significantly improved two parameters: spermatozoa count in OAT patients, and, most importantly for IVF, spermatozoa count after density gradient separation both in normospermic patients and OAT patients. No effect was observed on sperm motility [43]. Furthermore, a double-blind, randomized, placebo-controlled study demonstrated that oral treatment with MI (2 g with 400 µg of folic acid, twice per day for 3 months) in patients with idiopathic infertility significantly enhanced sperm concentration, total count, and progressive motility of spermatozoa, as well as the number of acrosome reaction-positive spermatozoa. At the same time, MI rebalanced the concentrations of **luteinizing hormone (LH)**, FSH, and inhibin-B [44].

Finally, Montanino Oliva *et al.* carried out a prospective, longitudinal study of men with reduced sperm motility and metabolic syndrome. They demonstrated that 3 month treatment with 1 g of MI, 30 mg of L-carnitine, L-arginine, and vitamin E, 55 µg of selenium, and 200 µg of folic acid (twice per day) had beneficial effects on both metabolic and reproductive parameters. Indeed, MI normalized the metabolic profile of these patients by improving their insulin sensitivity, and also enhanced testosterone levels and greatly improved semen characteristics such as sperm concentration, motility, and morphology [45]. Similar results were also obtained in another trial [46].

In Vitro Treatment

Sperm cells in patients with OAT are characterized by low motility and high levels of inositol monophosphatase 1 (IMPA-1) that catalyzes the dephosphorylation of phosphatidylinositol (PI). This suggests that the related signal transduction pathways are crucial for the maintenance of male germ cell motility [47]. Indeed, an *in vitro* study demonstrated that the spermatozoa of OAT patients are covered by an amorphous fibrous material that increases seminal fluid viscosity and reduces sperm motility. Furthermore, the mitochondria in the intermediate tract of spermatozoa of these patients had damaged cristae. After incubation with MI, the amorphous fibrous material disappeared and cristae damage decreased [48]. In addition, MI can act directly on mitochondria to enhance the mitochondrial membrane potential (MMP). Because low MMP is considered to be a marker of apoptosis related to the functional features of sperm cells, MI might exert a beneficial effect on various parameters including motility, fertilization capacity, and embryo quality. *In vitro* studies on the sperm of patients undergoing IVF

from an ovary. The fertilized egg can then be returned to the uterus.
***In vitro* fertilization (IVF):** a technique that involves extracorporeal fertilization of gametes by coincubation of oocytes with sperm *in vitro*.
Luteinizing hormone (LH): a glycoprotein that stimulates ovulation and the subsequent development of the corpus luteum. In the ovarian follicle thecal cells, LH induces the synthesis of androgens that are precursors to estrogens.
Normospermia: production of spermatozoa that are normal in number and motility.
Oligoasthenoteratozoospermia (OAT): the most common cause of male subfertility. OAT includes oligozoospermia (low number of sperm), asthenozoospermia (poor sperm movement), and teratozoospermia (abnormal sperm shape).
Osmolytic activity: this influences the properties of biological fluids and preserves the integrity of cells by modulating the viscosity and ionic strength of aqueous solutions.
Physiological intracytoplasmic sperm injection (PICSI): an advanced form of ICSI that employs hyaluronan to select the best spermatozoa (those bound to hyaluronan).
Polycystic ovary syndrome (PCOS): a heterogeneous endocrine disorder affecting up to 10–15% of women of reproductive age. PCOS is a relevant cause of infertility. In addition to hyperandrogenism, insulin resistance plays a key role in PCOS.
Superoxide dismutase (SOD) and catalase (CAT): two essential antioxidant enzymes.

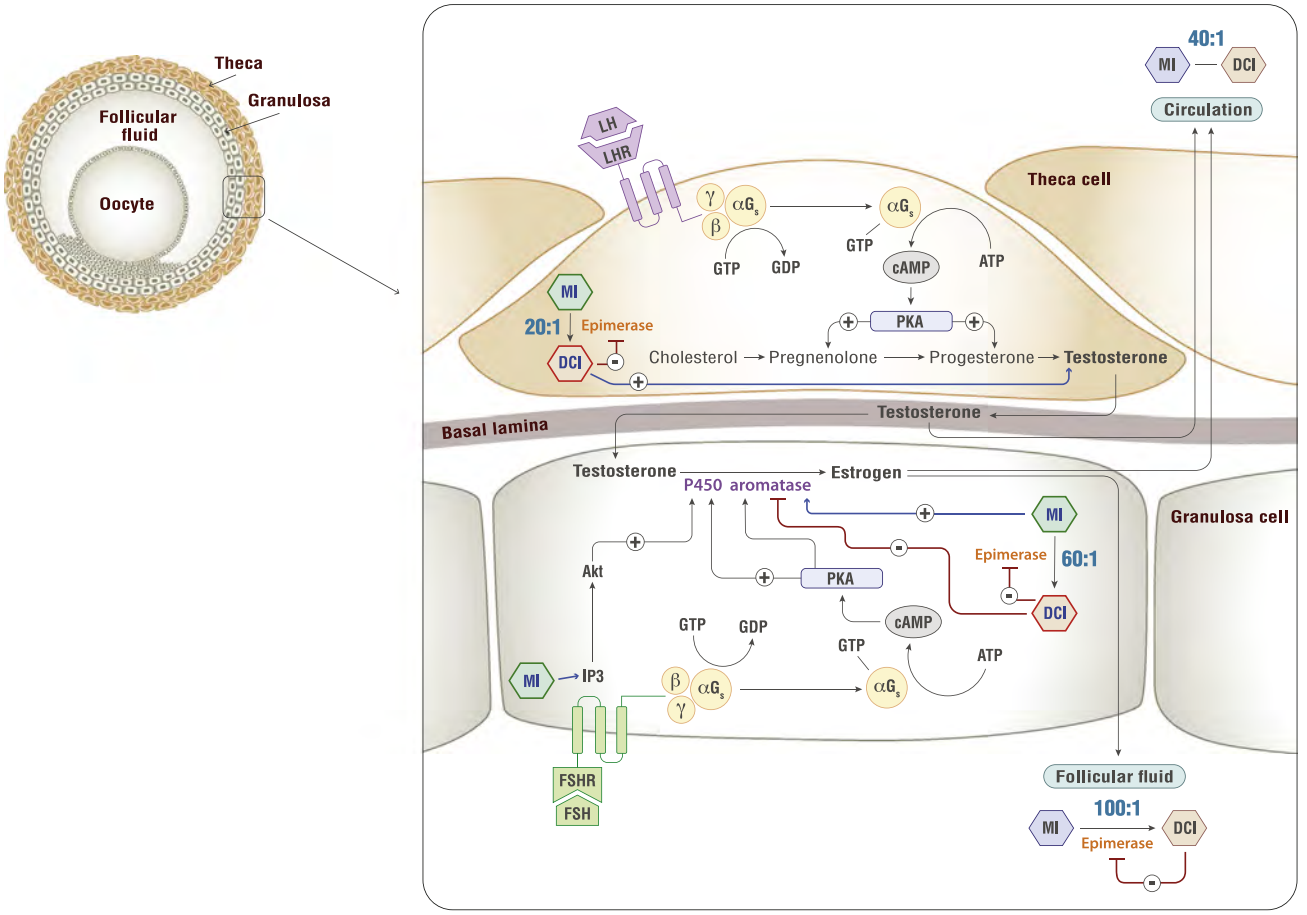

Figure 1. Physiological Actions of Myo-Inositol (MI) and D-Chiro Inositol (DCI) in the Theca and Granulosa Cells of the Ovary. Each ovarian follicle is surrounded by two sheets of cells, an outer layer (theca) and an inner layer (granulosa). A proportion of testosterone synthesized by theca cells is transferred to neighboring granulosa cells and the remainder is exported into the blood. Estrogens are synthesized from testosterone and other androgens in granulosa cells by aromatase enzyme (cytochrome P450 aromatase, CYP19A1) localized in the endoplasmic reticulum. Granulosa cell differentiation and aromatase induction are stimulated by follicle stimulating hormone (FSH), whose receptors are expressed exclusively by granulosa cells. MI and DCI are present in both theca and granulosa cells. Their ratio has so far been experimentally determined only in theca cells, where it is 20:1; no data are available for granulosa cells. We can speculate that, between the outer layer and the follicular fluid, where the physiological MI/DCI ratio is 100:1, there is an increasing gradient of MI that is determined by progressive DCI-mediated downregulation of isomerase activity; above certain levels, DCI is detrimental to the oocyte. If this hypothesis is correct, the concentration of MI in granulosa cells is likely to be higher, reaching an MI:DCI ratio close to 60:1. In theca cells MI (as IP3) acts as a second messenger in the FSH signaling pathway. Furthermore, it is noteworthy that MI and DCI have opposing effects on aromatase: MI stimulates aromatase activity whereas DCI inhibits. Abbreviations: Akt, serine/threonine kinase; FSHR, follicle-stimulating hormone receptor; Gs, G protein; IP3, inositol 1,4,5-trisphosphate; LH, luteinizing hormone; LHR, luteinizing hormone receptor; PKA, protein kinase A.

(both normal and OAT) demonstrated that incubation of seminal fluid with MI improves progressive motility in both groups [49]. Motility improvement in the OAT group was related to a substantial increase in the percentage of spermatozoa with high MMP, and this did not differ significantly from that of the normozoospermic men. After incubation with MI, the total number of spermatozoa recovered after swim-up was significantly enhanced in both normal and OAT patients. Other studies have supported these findings on the use of MI in both *in vivo* and *in vitro* ART [50].

In another study [51], a total of 125 semen samples (100 fresh and 25 thawed) from 100 men aged 22–60 years (46 normozoospermic, 19 oligozoospermic, 15 asthenozoospermic, and

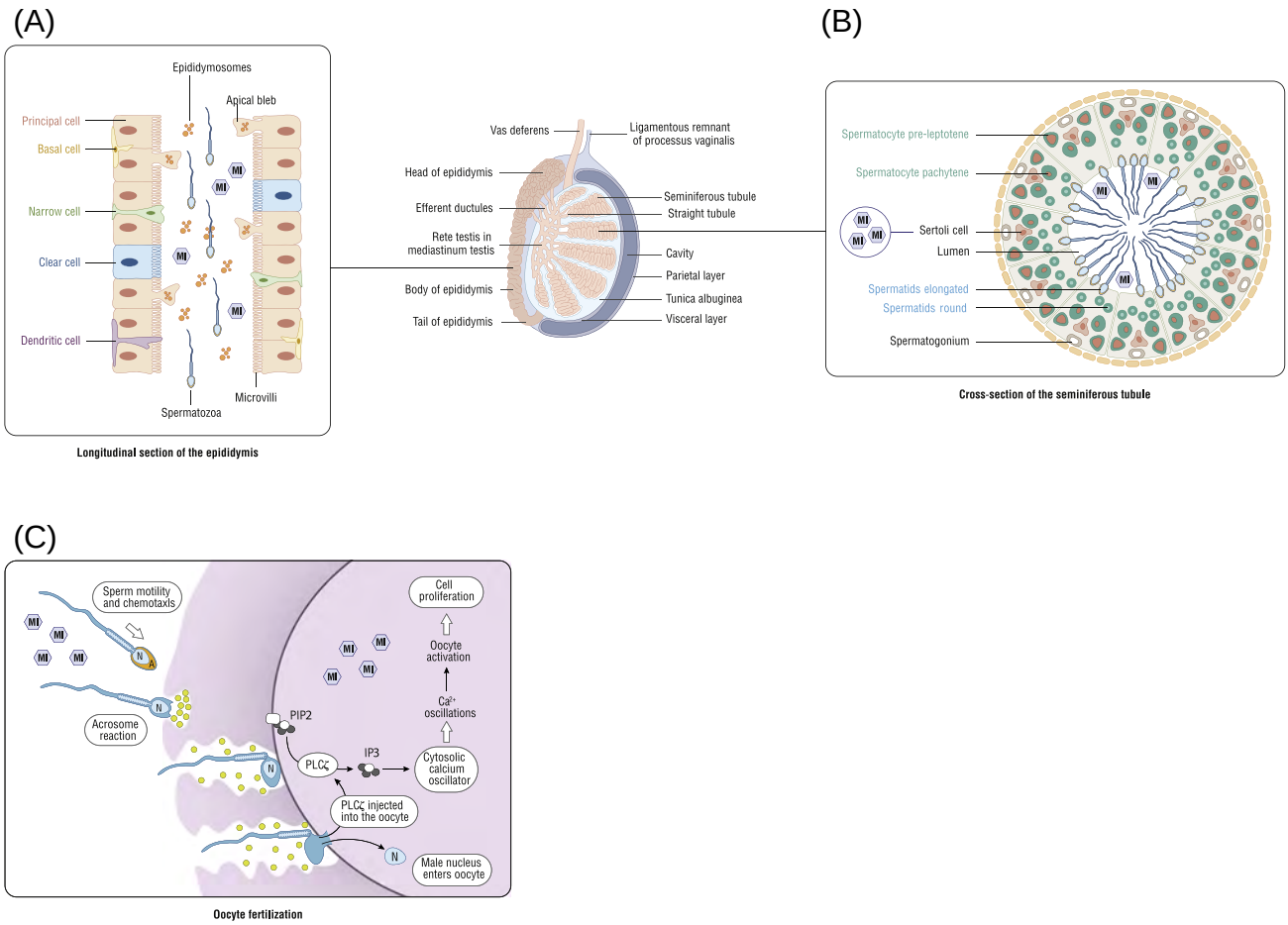

Figure 2. Myo-Inositol (MI) Physiological Actions in Testis, Epididymis, and Oocyte Fertilization. Significant concentrations of MI are found in the secretions of the male reproductive tract, where they are ~30–40-fold higher than in blood plasma. Because the blood–testis barrier inhibits MI entry, the majority of MI in the testis does not come from the blood, and MI is instead synthesized locally from glucose. Both spermatozoa and Sertoli cells can produce MI, and most testicular MI is probably generated by the seminiferous tubules. The epididymis also expresses MI biosynthesis enzymes, and this may account for the high levels of free MI in the luminal fluid of the cauda epididymidis. Inositol concentrations in epididymis are reported to be higher than in testis. MI levels differ according to the site: in rete testis fluid MI is ~50% higher than in cauda epididymal plasma, and >fivefold higher than in seminal plasma. MI is involved in spermatozoa maturation in the seminiferous tubules (panel B) as well as in their migration to the epididymis (panel A) and then to the vas deferens. In addition, MI may act as an osmoprotective molecule to safeguard sperm cells. Both MI *per se* and as IP3 are essential for the final steps of oocyte fertilization (including sperm motility). Then, following membrane fusion, PLCζ is introduced into the oocyte. This enzyme cleaves PIP2 to generate IP3 (oocyte activation). IP3 induces Ca^{2+} mobilization which in turn triggers the nuclear fusion of vesicles (panel C). Abbreviations: A, acrosome; IP3, inositol 1,4,5-trisphosphate; N, nucleus; PIP2, phosphatidylinositol 4,5-bisphosphate; PLCζ, phospholipase Cζ.

20 OAT) were evaluated *in vitro* before and after MI addition (2 mg/ml for 15 minutes). This treatment induced an increase in sperm total and progressive motility in all samples [51]. Concerning MI safety, previous *in vitro* studies demonstrated that 2 mg/ml of MI, added for 2 h to the medium, is well tolerated by spermatozoa [49,52].

In a study by Artini and colleagues [53], including 73 male subjects, the authors found that incubation of spermatozoa with MI (2 mg/ml) improved progressive motility in both normal and OAT patients relative to standard medium. Interesting results were also found by Papaleo *et al.* [54]. MI was also tested [55] for its ability to protect the sperm of infertile subjects undergoing ART against cryopreservation damage leading to reduced post-thaw sperm recovery (viability

≤50%) [56]. After thawing, MI supplementation led to a significant increase in the cryo-survival rate (CSR) in samples with abnormal pre-freeze sperm parameters [55].

A similar study [57] evaluated the effect of supplementing the cryopreservation medium with MI on post-thaw sperm quality from 40 normozoospermic men. MI significantly improved post-thaw progressive motility and normal morphology in treated samples relative to controls. Lipid peroxidation, assessed by measuring malondialdehyde levels, decreased in samples frozen with MI, and treatment was associated with increased total antioxidant capacity. In addition, MI in the cryopreservation medium significantly reduced DNA fragmentation compared with controls [57].

Combined *In Vivo* and *In Vitro* Treatment

Finally, in a prospective RCT [58], oral and *in vitro* treatments were combined before **physiological intracytoplasmic sperm injection (PICSI)**. The study group received 1 g MI, 30 mg L-carnitine, L-arginine, and vitamin E, 55 µg selenium, and 200 µg folic acid twice per day for 2 months, controls were untreated. The semen of the treated patients was incubated for 2 h with 2 mg/ml of MI before PICSI. The authors reported a significantly improved fertilization index, a higher rate of good quality embryos, and increased pregnancy rates relative to controls [58].

In conclusion, the above data convincingly support the besneficial effects of MI on sperm preparation for ART procedures. However, further studies will be necessary to confirm the general applicability of these findings.

Concluding Remarks

Studies over recent decades have significantly increased our knowledge on the use of inositol in ART. Growing evidence argues that MI plays a key role in the physiology of reproduction, and that MI supplementation is beneficial for several aspects of ART. MI and/or its derivatives are essential for the development of oocytes, spermatozoa, and embryos. By contrast, DCI levels above a certain threshold may adversely affect ovarian physiology. In particular, the MI:DCI ratio in follicular fluid may be a pivotal biomarker of reproductive health. MI is also involved in regulating sperm cell motility, capacitation, and acrosome reaction, and MI supplementation enhances sperm quality. Finally, MI supplementation of dividing blastomeres promotes their development by stimulating Akt phosphorylation. By contrast, there are no data on the effects of DCI on sperm quality or on the earliest phases of human development.

In conclusion, the literature highlights the beneficial effects of MI treatment for ART. However, further in-depth investigations will be necessary to evaluate the impact of MI on the ART live birth rate and to elucidate the role of MI/DCI in spontaneous ovarian cycles.

Outstanding Questions

What are the economic advantages of MI in reducing the number of rFSH units used in IVF?

What proportions of the economic benefit of this decrease pertain to patients and the public healthcare system, respectively?

Does MI affect long-term fertility outcomes such as clinical pregnancy rate and live birth rate in ART and in spontaneous ovarian cycles?

By what mechanism does MI improve sperm performance?

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