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Review

Myo-inositol in assisted reproductive technology from bench to bedside

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Inositols are insulin-sensitizing compounds of promising efficacy in the management of polycystic ovary syndrome (PCOS). On the one hand, myo-inositol (myo-ins) plays a regulatory role in male and female reproductive function, influencing the development of oocytes, spermatozoa, and embryos. On the other hand, high concentrations of D-chiro-inositol (D-chiro-ins) in the ovary may adversely affect oocyte quality. This review analyses the available literature, which encourages the clinical use of myo-ins in assisted reproductive technologies (ARTs) due to its beneficial effects on female and male reproduction.

Highlights

Inositols are insulin-sensitizing compounds that have also regulatory functions in human reproduction.

In the ovary, myo-inositol (myo-ins) mediates the granulosa response to follicle-stimulating hormone stimuli and plays a crucial role in determining oocyte maturation. D-chiro-inositol, by contrast, is involved in the regulation of ovarian steroidogenesis and at high concentrations may be detrimental for oocyte quality due to its aromatase down-modulator activity.

Supplementation with myo-ins during assisted reproductive technologies reduces the total amount of gonadotropins used in polycystic ovary syndrome (PCOS) and non-PCOS women and leads to improvements in oocyte quality and maturation and embryo development and an increase in the rate of successful pregnancies.

Myo-ins also acts directly on spermatogenesis, improving sperm performance *in vitro* and *in vivo*.

An overview of the use of inositol in ART

Inositols are a class of organic **insulin** (see Glossary)-sensitizing compounds physiologically present in the human body and in several foods. They exist as various stereoisomers and exhibit a greater or lesser degree of phosphorylation. The most common naturally occurring form is myo-ins, but occasionally other configurational isomers, such as D-chiro-ins, are detected [1]. In human cells, part of myo-ins is physiologically converted to D-chiro-ins, and defined ratios of the two isomers are characteristic of specific organs and tissues [2]. An enzyme of the epimerase family regulates such transformation under the stimulus of insulin. As far as we know, the conversion of D-chiro-ins to myo-ins is negligible under physiological conditions [3].

Recently, myo-ins has attracted increasing attention in the field of reproductive medicine, particularly with regard to **ART** [4]. The use of oral supplementation with inositols in the treatment of patients with infertility deriving from ovulatory disorders has shown promise in cases of both **PCOS** [5] and non-PCOS [6]. Myo-ins may also be used in **controlled ovarian stimulation (COS)**, whereby it improves female fertility, the likelihood of successful ***in vitro*** **fertilization (IVF)**, and **intracytoplasmic sperm injection (ICSI)** [7].The use of oral supplementation with myo-ins in men with abnormal semen analysis improves seminal fluid characteristics, specifically sperm concentration, motility, and morphology, in addition to sperm capacitance and testosterone production [8,9]. Furthermore, treatment with myo-ins is able to increase post-thaw parameters verses control patients, with pre-freeze total motility (TM) reported to increase from 15% to 50%, progressive motility from 10% to 35% and cryosurvival rate from 30% to 40%. [10].

In this context, myo-ins could be considered a promising therapeutic option for the management of infertility and may be used in combination with other therapies to increase the likelihood of pregnancy in patients undergoing ART. The need for ART is growing year after year [11]; therefore, it is important to frequently address the current state of the field and what novelties may be gleaned. This review covers the use of inositols in both female and male fertility, starting from a mechanistic viewpoint and moving on to the existing clinical evidence for the use of these exciting molecules.

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Myo-ins and ovarian function

Both myo-ins and D-chiro-ins are involved in the physiology of female reproduction, with myo-ins being essential for proper development of the ovarian follicle and its metabolism. It plays a key role in glucose metabolism and mediates the response to gonadotropin stimuli, particularly those of **follicle-stimulating hormone (FSH)**, which regulates the proliferation and maturation of granulosa cells [12]. Myo-ins also modulates the FSH-mediated production of **anti-Müllerian hormone (AMH)**, thereby playing a crucial role in determining maturation and assuring good embryo quality [12].Since insulin stimulates **androgen** biosynthesis with the ovaries, D-chiro-ins is also involved in the regulation of steroidogenesis [2,13].

Thus, inositols impact ovarian physiology and act as regulators of human reproductive function. Assuming that ovarian functions physiologically depend on a correct ratio of inositol concentrations, it is likely that an imbalance between myo-ins and D-chiro-ins explains the hormonal alterations observed in certain pathological conditions such as PCOS [14,15].Therefore, their roles in the ovary differ depending on the follicular region under examination.

The follicular fluid provides support during the developmental stages of the maturing oocyte, with myo-ins levels in the follicular fluid being correlated with good oocyte and blastocyst quality [16]. The ovaries maintain insulin sensitivity even in insulin-resistant subjects and are therefore overstimulated by compensatory hyperinsulinemia. Insulin resistance, a typical feature of women with PCOS, elevates D-chiro-ins levels in the follicular fluid and consequently alters the myo-ins:D-chiro-ins ratio from 100:1 in healthy women to 0.2:1 in women with PCOS [17], with deleterious effects on oocyte quality as measured according to Gardner's grading system [18].

In granulosa cells, which surround the oocyte, myo-ins participates in gonadotropin intracellular signaling. This process leads to the production of **estrogens** from androgens, produced by theca cells, and to the final induction of ovarian follicle maturation. In healthy women, a high content of myo-ins in these cells is expected; however, since insulin and insulin-like growth factor-1 receptors are expressed at the level of the granulosa [19], it is plausible that hyperinsulinemic states are associated with an increase in D-chiro-ins content at the expense of myo-ins resulting in reduced ovarian sensitivity to FSH stimulation, as typically observed in women with PCOS [20] (Figure 1).

Thecal cells contain a myo-ins:D-chiro-ins ratio of 20:1 in healthy women, are surrounded by granulosa cells in concentric layers, and are the main site of ovarian androgen biosynthesis [21]. In women with PCOS, the ratio of myo-ins to D-chiro-ins in thecal cells is approximately four times lower, likely due to increased epimerase activity under the stimulus of excess insulin, resulting in increased androgen production and the hyperandrogenism classically observed in these patients [22]. In addition, D-chiro-ins reduces cytochrome P450 family 19 subfamily A member 1 (CYP19A1) or aromatase, the enzyme that converts androgens into estrogens, and cytochrome P450 side-chain cleavage (P450scc), two key steroidogenic enzymes, in primary cultures of human granulosa cells [13]. Based on recent evidence, D-chiro-ins may have aromatase downregulator activity and play a detrimental role if administered in high amounts to women with PCOS (specifically 2400 mg) as it reduces the production of estradiol (E_2) from testosterone leading to higher concentrations of androgens, which can further amplify the symptoms and signs of the syndrome [15,23].

Inositols, ART, and female fertility

Inositols, being natural insulin sensitizers, may have a positive role on ovulation induction and the success of IVF or ICSI in women with infertility due to ovulatory disorders. PCOS is the most

common cause of ovulatory dysfunction typified by ovulation disorders. In 80% of cases, it is associated with obesity, and up to 40% of patients also display metabolic syndrome with insulin resistance, consequently aggravating PCOS [24–26] (Figure 2); therefore, management of insulin resistance is essential in the treatment of women with PCOS in need of ART [26]. Diet and exercise represent the first method of treatment; however, in certain cases treatment with insulin-sensitizing molecules, including inositols, may be necessary.

PCOS patients undergoing ART represent a particularly interesting challenge to gynecologists experienced in reproductive medicine. Although they respond to COS protocols, the oocytes obtained are often of poor quality [27,28]. The quality of oocytes is defined by various factors including their ability to undergo meiotic maturation, fertilization, and proper embryonic development and to arrive at a successful pregnancy [28]. The role of myo-ins supplementation in women with PCOS undergoing ART has been investigated, specifically in improving oocyte quality, embryo quality, and the chance of achieving pregnancy [29–31]. However, further larger studies are still required; the 2023 International Evidenced-Based Guideline on the assessment and management of PCOS still considers inositol an experimental therapy [32]. To expand on this need, 25 women of childbearing age with PCOS and oligo- or amenorrhea were enrolled in a clinical study [33], where ovulatory disorder due to PCOS was apparently the only cause of infertility. These patients were continuously administered myo-ins combined with folic acid twice daily; 22/25 patients (88%) were restored at least one spontaneous menstrual cycle during treatment, of whom 18 (72%) maintained normal ovulatory activity during the follow-up period of 6 months. A total of ten single pregnancies were achieved (40% of patients). In a randomized controlled trial [34], two groups of women with PCOS received either folic acid or 4 g myo-ins + 400 mg folic acid daily, 1 month prior to the initiation of a protocol with gonadotropin-releasing hormone (GnRH) + FSH antagonist until the morning of ovulation trigger. The percentage of metaphase II oocytes (58% vs 78%, respectively), the percentage of good-quality embryos, and the fertilization rate (46% vs 65%) were significantly higher in the group that received myo-ins. Although no effect was observed on the cumulative pregnancy rate (35% vs 40%), [35] a similar study found higher pregnancy rates in women who underwent oral supplementation with myo-ins compared with controls (60% vs 32%). Furthermore, myo-ins supplementation increases the sensitivity of polycystic ovaries to gonadotropins, as measured by it leading to a reduction of up to 500 IU in the dose of FSH needed [7]. Another randomized controlled trial, in which the enrolled patients were divided into two groups and for 3 months received uninterrupted, twice daily, either 2 g of myo-ins + 200 µg of folic acid or 200 µg of folic acid alone, aimed to determine the effects of myo-ins on oocyte quality in a sample of women with PCOS [36]. At the end of treatment, in the group treated with myo-ins, the number of follicles >15 mm in diameter visible on ultrasound during stimulation (data not reported) and the number of oocytes retrieved at the time of pick-up transferred (myo-ins + folic acid median 12 vs folic acid median 8.05, $P < 0.05$), as well as the average number of embryos transferred (myo-ins + folic acid n =30, 68.1% vs folic acid control n = 9, 29%, P 0.02) were significantly higher. The average number of immature oocytes was also significantly lower in the group treated with oral supplementation with myo-ins (degenerated oocytes myo-ins + folic acid n = 2, 0.93% vs folic acid only n = 23, 14.37%, P 0.02; germinal vesicles myo-ins + folic acid n = 3, 1.4% vs folic acid only n = 15, 9.37%, P 0.02). To determine the effects of myo-ins on oocyte quality in PCOS patients undergoing ICSI, a prospective, controlled, and randomized study enrolled 60 infertile patients with PCOS undergoing ovulation induction followed by ICSI [37]. All participants were administered a standard long protocol. From the day of GnRH administration, 30 participants received oral myo-ins (2 g) combined with folic acid, twice daily, and 30 women received folic acid alone. In patients with PCOS, treatment with myo-ins and folic acid reduced germinal and degenerated oocytes at the time of oocyte retrieval vesicles (myo-ins 1.0 ± 0.9 vs control 1.6 ± 1.0, P 0.1) without significantly affecting the

Glossary

Androgens: steroid hormones (e.g., testosterone, dehydroepiandrosterone) produced by the adrenal glands, ovaries, and testes.

Anti-Müllerian hormone (AMH): a glycoprotein synthesized by the granulosa cells of the preantral follicle and the small antral follicles; regulates their sensitivity to FSH and subsequent recruitment into the ovarian cycle.

Assisted reproductive technology (ART): encompasses all noncoital conception methods used to treat infertility with donor or nondonor gametes.

Controlled ovarian stimulation (COS): an ART practice that, through the administration of hormones, induces the ovulation of more ovarian follicles.

Cryopreservation: a procedure in which gametes and/or embryos are maintained in a viable state for a period at cryogenic temperatures and then brought back to room temperature to restore their activity. It is a procedure commonly used in ART, 'social freezing', and fertility preservation programs.

Estrogens: the main female sex hormones. Their action induces the development and maintenance of female sexual characteristics. They also regulate the menstrual cycle and reproductive function, promote fertilization and implantation, and participate in the maintenance of pregnancy.

Follicle-stimulating hormone (FSH): a gonadotropin that in women stimulates the growth and maturation of follicles in the ovaries during the follicular phase of the ovarian cycle. In men, FSH promotes spermatogenesis by stimulating the testes to produce mature sperm and induces the production of androgen-binding proteins.

Insulin: a hypoglycemic hormone produced by beta pancreatic cells that facilitates the passage of glucose from the blood to cells. It promotes glycogen synthesis by encouraging the accumulation of glucose in the form of glycogen in the liver and inhibits hepatic glycogenolysis.

Intracytoplasmic sperm injection (ICSI): an *in vitro* procedure used in ART that involves the insemination of an oocyte by microinjecting a single spermatozoon directly into it.

***In vitro* fertilization (IVF):** an *in vitro* procedure used in ART comprising the union of ova and the sperm of the couple's male partner or a donor.

total number of oocytes retrieved. This approach, by reducing E_2 levels at the time of human choriogonadotropin (hCG) administration, should be considered an option to reduce the risk of **ovarian hyperstimulation syndrome (OHSS)** in PCOS patients. Similarly, a randomized controlled trial compared the effects of myo-ins and D-chiro-ins on oocyte quality in euglycemic PCOS patients, where it was demonstrated that, in PCOS patients with a normal insulin response, treatment with myo-ins rather than D-chiro-ins significantly improved oocyte and embryo quality (embryo grade 1 myo-ins 1.64 ± 0.88 vs D-chiro-ins 0.76 ± 0.43) during ovarian stimulation protocols [38].

A randomized, double-blind controlled trial of 102 patients studied the effects of myo-ins versus metformin in reducing the risk of OHSS and improving ART outcome in women with PCOS undergoing IVF [39]. They were divided into two groups and received oral supplementation with myo-ins 2 g twice daily or metformin 850 mg twice daily. Myo-ins and metformin were equally beneficial in reducing the risk of OHSS but myo-ins had a better ART outcome in women with PCOS undergoing antagonist cycles, demonstrating a significantly higher clinical and cumulative pregnancy rate [clinical pregnancy rate: myo-ins 18/50 (36.0%) vs metformin 9/50 (18.0%, *P* 0.04); cumulative pregnancy rate including frozen embryo transfer: myo-ins 16/37 (43.2%) vs metformin 10/44 (22.7%, *P* 0.05)].

Fewer studies on the use of inositols in non-PCOS patients undergoing ART are available. A systematic review and meta-analysis [7] of 812 patients investigated whether oral myo-ins supplementation was able to reduce the amount of gonadotropins and the duration of COS in women with and without PCOS undergoing IVF. During IVF, myo-ins was effective in both study groups in reducing gonadotropin use, but it effectively reduced the duration of COS only in the PCOS group. However, the meta-analysis included only a single trial with non-PCOS subjects and further investigations are warranted to draw accurate conclusions on the effects of myo-ins supplementation in these cases.

Another systematic review and meta-analysis [6] evaluated the efficacy of myo-ins supplementation in infertile women without PCOS undergoing ovulation induction for ICSI or IVF embryo transfer. Seven studies with 935 women were included. Myo-ins supplementation was associated with a significant improvement in the rates of clinical pregnancy (myo-ins 33.3% vs control 27.62%) and abortion rates (myo-ins 5.62% vs control 17.61%). Improved quality of retrieved oocytes was also observed, with a reduction of unsuitable oocytes retrieved and a significant reduction in total gonadotropin units used for COS in these patients (control −581.58 vs myo-ins −219.39, 95% CI, *P* 0.001). Oocyte quality is determined by myriad morphological parameters including cumulus cells, polar body, and cytoplasm [40]. However, no significant differences were found in total oocytes retrieved, MII-stage oocytes retrieved, days of stimulation, or E_2 peak level.

Inositols and testicular function

The male gonads are rich in myo-ins, with levels 30–40-fold higher than in blood [4]; this has led to investigations into the role of inositol in male testicular function and fertility [41]. The epididymis, in particular, has a higher concentration of inositols than other regions of the testis, as evidenced by historical studies across six mammalian species [42]. This higher concentration in the fluid of the epididymal tubules is generated by the metabolism of glucose in epididymal epithelial cells by enzymes that are present in the rat epididymis [43]. *In vitro*, spermatozoa have been shown to be able to synthesize myo-ins [44]. Furthermore, Sertoli cells, when cultured and maintained in a medium without inositol, release myo-ins into the medium [45]. When bound to phospholipids, myo-ins has important calcium mobilization functions by activating nuclear vesicle fusion and promoting the acrosomal reaction of the spermatozoa, which is essential for fertilization [43,45,46]. Calcium mobilization is also essential for sperm motility [43,47]; in this perspective, calcium is

Luteinizing hormone (LH): a gonadotropin that in women stimulates both the production of estrogen and progesterone and the production of small amounts of testosterone. In men it stimulates the production of testosterone by the Leydig cells of the testis.
Oligoasthenoteratozoospermia (OAT): the most common cause of male subfertility; comprises the simultaneous presence of a low concentration of spermatozoa, poor sperm motility, and poor sperm morphology.
Ovarian hyperstimulation syndrome (OHSS): an iatrogenic condition that can affect women undergoing COS for ART. In its most severe forms, it causes significant ovarian enlargement, abdominal and/or pleural effusions, abdominal pain, increased blood concentration and coagulability, and oliguria.
Polycystic ovary syndrome (PCOS): a complex functional alteration of the female reproductive system characterized by the presence of multiple cystic follicles detectable by ultrasound and responsible for chronic anovulation often accompanied by hyperandrogenism and metabolic syndrome.

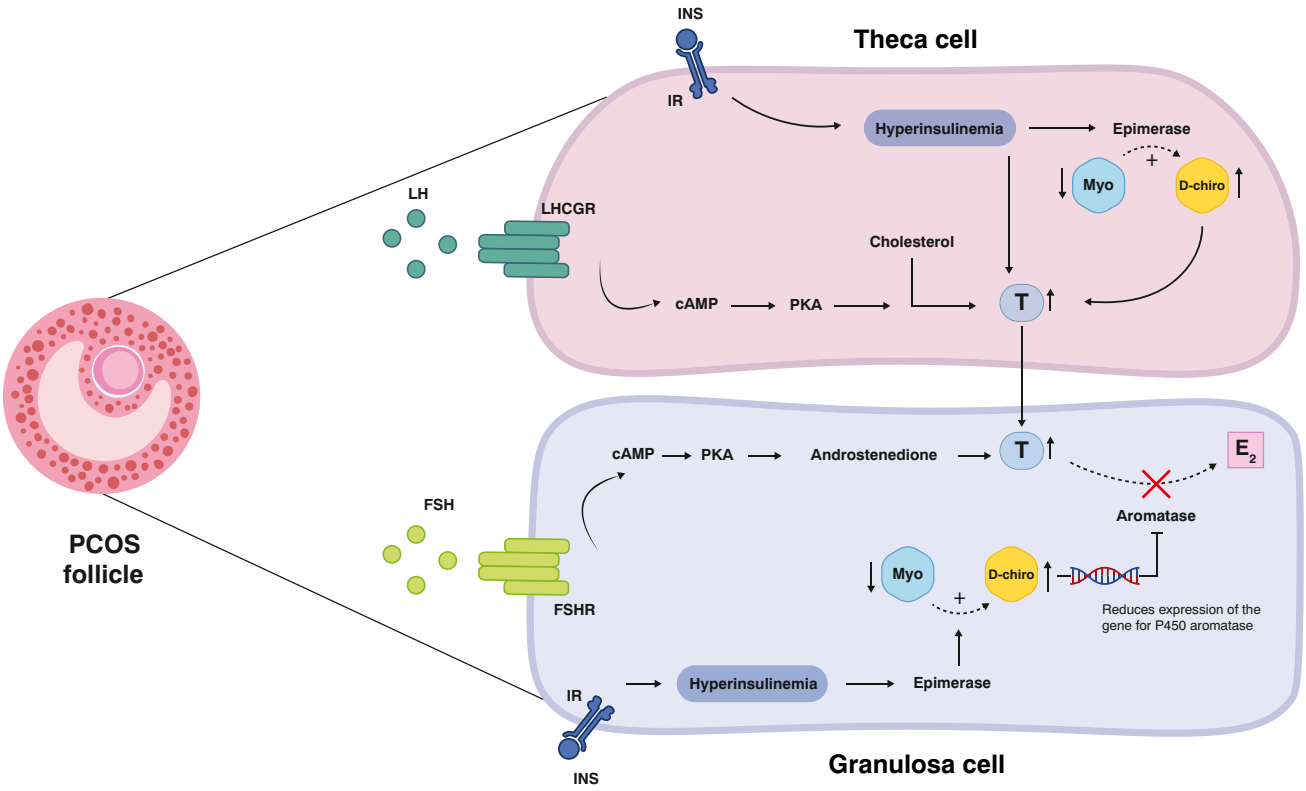

Figure 1. Effects of hyperinsulinemia on polycystic ovary syndrome (PCOS) follicles. In the theca cells of a PCOS follicle, hyperinsulinemia acts by enhancing ovarian epimerase activity, resulting in increased conversion of myo-inositol (myo-ins) to D-chiro-inositol (D-chiro-ins). Hyperinsulinemia in theca cells also has a direct effect in stimulating testosterone biosynthesis. Increased D-chiro-ins, on the one hand, in theca cells give a further boost to testosterone biosynthesis. On the other hand, excess D-chiro-ins in ovarian granulosa cells reduces the expression of the gene for P450 aromatase, hindering the conversion of testosterone to estradiol (E_2). Abbreviations: FSH, follicle-stimulating hormone; FSHR, follicle-stimulating hormone receptor; INS, insulin, IR, insulin receptor; LH, luteinizing hormone; LHCGR, luteinizing hormone/choriogonadotropin receptor; PKA, protein kinase A; T, testosterone.

necessary for the initiation and maintenance of this motility because it regulates the asymmetrical beating of the flagella, which is essential in ascending the female internal genitalia and reaching and attempting to fertilize the released egg. Finally, treatment with myo-ins is effective in counteracting the overproduction of reactive oxygen species (ROS), thus ensuring the molecular balance necessary for proper sperm maturation. In addition, myo-ins strengthens sperm protection against oxidative damage to DNA and proteins, increases sperm motility, and optimizes mitochondrial ATP production [48].

Inositols, ART, and male fertility

Male infertility is a multifactorial disorder that affects 1/20 men [49] and like female infertility is a common cause of recourse to ART. Levels of ROS negatively correlate with sperm quality [50]; for this reason, supplementation with multiple antioxidants has long been considered effective against male infertility. In recent years, however, inositols have been demonstrated to have a role in improving sperm quality [9,51], and they are used to treat men with fertility problems, principally **oligoasthenoteratozoospermia (OAT)** (Figure 3).

A double-blind, randomized, placebo-controlled study [52] evaluating the efficacy and safety of treatment with myo-ins in men with idiopathic infertility concluded that myo-ins significantly

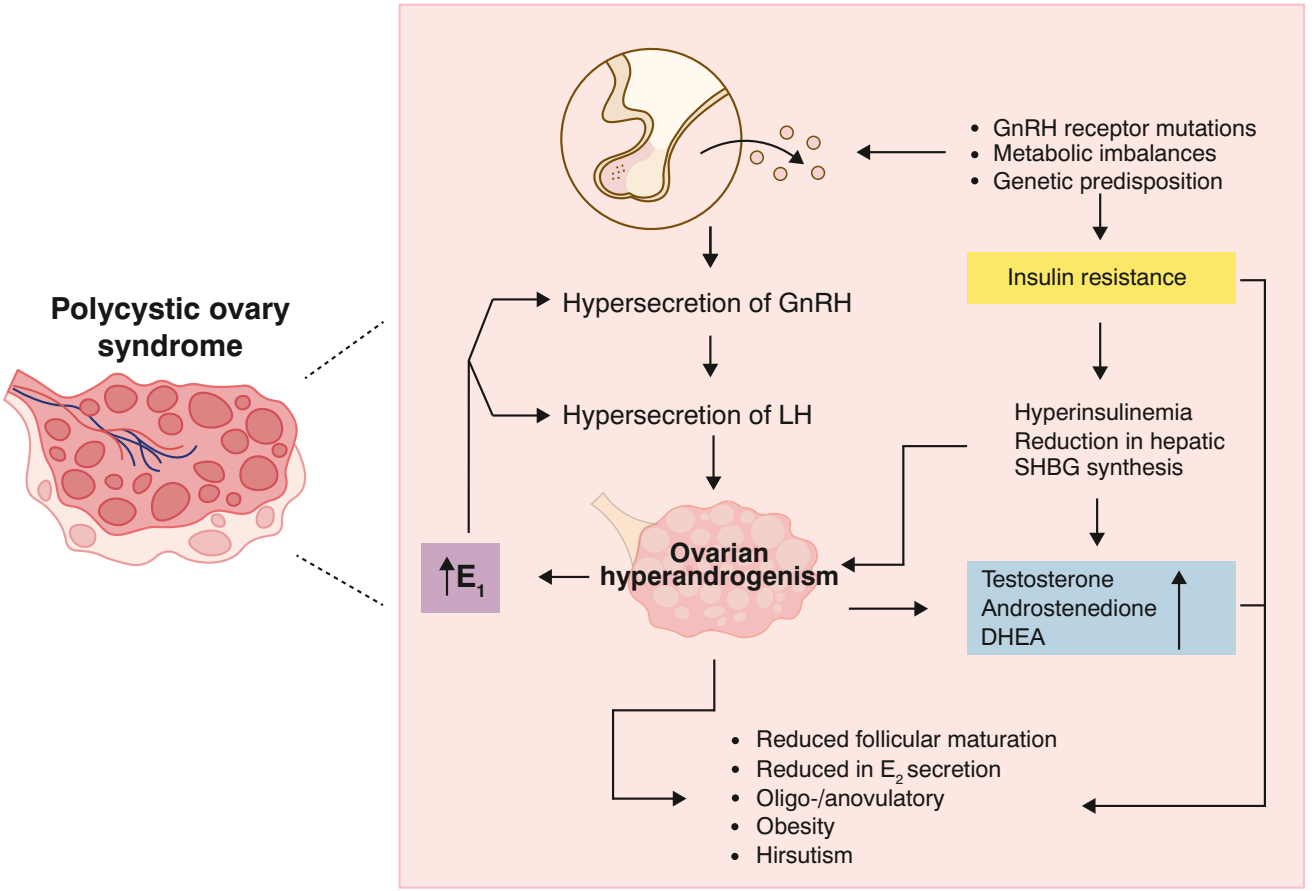

Figure 2. Pathophysiology of polycystic ovary syndrome (PCOS). A schematic representation of the pathophysiology of PCOS. Contributing factors such as gonadotropin-releasing hormone (GnRH) receptor mutation, metabolic imbalances, and genetic predisposition lead to insulin resistance and hypersecretion of GnRH by the pituitary gland. Subsequently, downstream pathways trigger ovarian hyperandrogenism characterized by elevated testosterone, androstenedione, and dehydroepiandrosterone (DHEA), leading to the clinical manifestation of PCOS as outlined by the Rotterdam criteria. Abbreviations: E_1, estrone; E_2, estradiol; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.

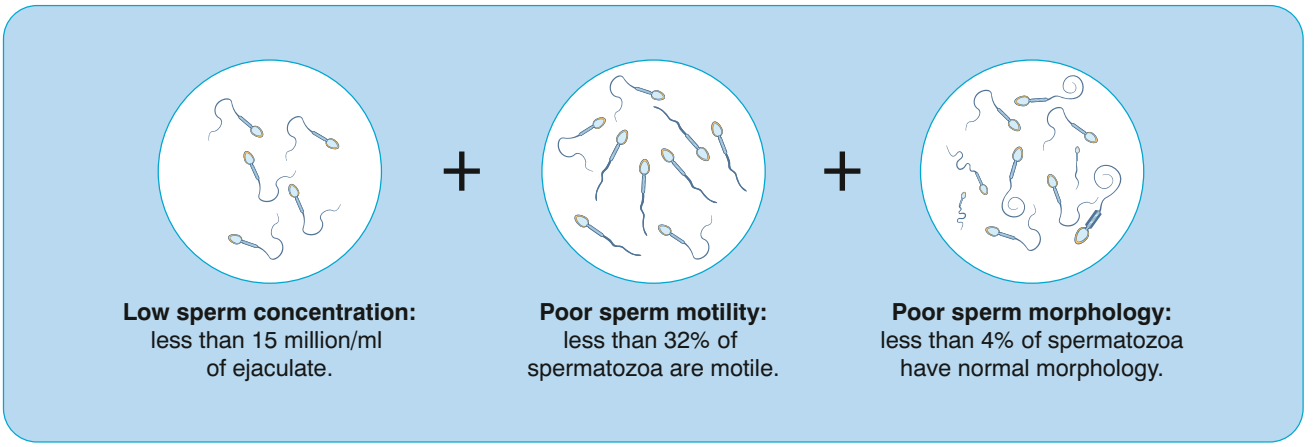

Figure 3. Characteristics of oligoasthenoteratozoospermia (OAT). OAT as identified by three key factors – low sperm concentration, poor sperm motility, and poor sperm morphology – according to the current diagnostic criteria [60].

increased sperm concentration (myo-ins 26.4 ± 4.4 million/ml vs placebo 20.8 ± 4.3 million/ml, $P > 0.05$), total sperm count (myo-ins 57.6 ± 14.4 million spermatozoa/ejaculate vs placebo 47.8 ± 11.2 million spermatozoa/ejaculate, $P > 0.05$), and progressive motility (myo-ins 27.6 ± 1.8% vs placebo 23.3 ± 2.1%, $P > 0.05$), as well as the percentage of acrosome reactions compared with placebo (myo-ins 41 ± 11% vs placebo 36 ± 10%, $P > 0.05$). Moreover, supplementation with myo-ins was able restore **luteinizing hormone (LH)** (before treatment 12.1 ± 2.7 IU/l vs after treatment 8.8 ± 2.6 IU/l, $P < 0.05$), FSH (before treatment 16.7 ± 4.1 IU/l vs after treatment 10.7 ± 4.1 IU/l, $P < 0.05$), and inhibin B (before treatment 86.0 ± 24.0 ng/l vs after treatment 105.0 ± 28.0 ng/l, $P < 0.05$) in serum towards levels typically seen in healthy males. With the aim of evaluating the effect of myo-ins administration on semen parameters in male patients undergoing IVF cycles, sperm samples were collected from 62 patients divided into three different groups: healthy and fertile patients, patients with OAT, and a control group [8]. Before and after administration of 4000 mg/day of myo-ins and 400 µg of folic acid for 2 months, sperm samples were analyzed to assess sperm volume, count, and motility before and after the density gradient separation method. After treatment, there was a significant increase in sperm concentration before (126.89%) and after (302.56%) the density gradient separation method in the group of OAT patients and a significant increase in sperm count after the density gradient separation method (132.02%) in the group of healthy, fertile patients. Motility values were higher in healthy men than in OAT before-treatment patients (49.79% vs 30.26%), but there was no improvement in either group after treatment. Moreover, a prospective longitudinal study [53] examined the effects of administering myo-ins to asthenospermic males with metabolic syndrome. Hormonal and metabolic profiles and semen sample parameters were assessed at the start of the study and after 3 months of treatment. The administration of myo-ins normalized the metabolic profile of these patients and improved their insulin sensitivity. Moreover, significant increases were observed in levels of free testosterone (before 33.0 ± 11.1 pg/ml vs after 47.2 ± 13.0 pg/ml, $P < 0.001$) and total testosterone (before 2.8 ± 1.2 ng/ml vs after 3.7 ± 1.4 ng/ml, $P < 0.02$), sperm concentration (before 16.2 ± 3.4 × 10^6/ml vs after 20 ± 4.2 × 10^6/ml, $P < 0.001$), motility (before 39.6 ± 6.1% vs after 51.4 ± 7.2%, $P < 0.001$), and normal morphology (before 24.9 ± 2.0% vs after 30.1 ± 2.3%, $P < 0.001$).

In addition, the effects on sperm performance of a nutraceutical mixture containing mainly myo-ins were studied *in vivo* [9], analyzing sperm samples from 51 men: 21 healthy normozoospermic and 30 with OAT. In the latter group, 15 patients were treated orally with the nutraceutical mixture and in the remaining 15 patients only myo-ins was used directly on ejaculated sperm. Comparing pathological and normal samples, motility, viability, Bcl-2 phosphorylation, and cholesterol efflux increased after the *in vitro* and *in vivo* treatments. Treatment with myo-ins facilitated the transition from non-capacitated to capacitated spermatozoa, favoring the likelihood of fertilization.

Myo-ins has been used *in vitro* to test its effect on sperm quality in normal and OAT patients undergoing IVF compared with standard sperm medium [54], whereby *in vitro* incubation of seminal fluid with myo-ins at a concentration of 15 µl/ml of a solution of myo-ins (2 g/ml) improved progressive motility in both normospermic (after myo-ins treatment 36.6 ± 28.9 × 10^6/ml vs before treatment 32.7 ± 21.2 × 10^6/ml) and OAT (after myo-ins treatment 6.6 ± 6.2 × 10^6/ml vs before treatment 4.4 ± 2.3 × 10^6/ml). To evaluate whether *in vitro* incubation of spermatozoa with myo-ins can improve fertilization rates in ICSI cycles, a prospective, bicentric, randomized study was conducted on 500 oocytes injected in 78 ICSI cycles [55]. Notably, the fertilization rate (myo-ins 78.9 ± 28.6% vs placebo 63.2 ± 36.7%, $P = 0.002$) and the percentage of grade A embryos at day 3 (myo-ins 59.9 ± 35.6% vs placebo 43.5 ± 41.5%, $P = 0.019$) were significantly higher when sperm was treated *in vitro* with myo-ins compared with the control. An additional study analyzed the effects of myo-ins on sperm motility in ejaculates of patients with severe

varicocele (grade III) or semen hyperviscosity [56]. This investigation included normal-viscosity ejaculate from 30 patients with varicocele and high-viscosity ejaculate from 33 patients without any testicular pathology. In both groups, the pellet was divided into two parts and both incubated for 15 min at 37°C, one with myo-ins and the other, as a control, in phosphate-buffered saline only. Incubation with myo-ins improved progressive sperm motility in the high-viscosity samples compared with the control group (myo-ins 38.9% ± 3.0 vs control 24.35% ± 2.41). No statistically significant difference in total progressive sperm motility was observed in the varicocele samples compared with the control group, probably because the heavy structural and biochemical defects that typically affect varicocele patients are not restored by inositol.

Finally, some evidence indicates that pretreatment of seminal fluid with inositols improves the sperm parameters of thawed spermatozoa and preserves their viability, with cryopreserved sperm having reduced fertility compared with fresh sperm. The reduction results from lower viability after thawing or from sublethal dysfunction in a part of the surviving subpopulation [10,57]. The effects of *in vitro* supplementation with myo-ins on the **cryopreservation rate** of cryopreserved human sperm has been studied, with *in vitro* treatment with myo-ins in human sperm ejaculated from infertile men leading to a 10% increase in the cryopreservation rate in samples with abnormal sperm parameters before freezing. Comparable results have been obtained by other authors [48,58,59].

Outstanding questions

Is there an economic advantage in supplementing myo-ins to reduce the number of rFSH units used in ART?

Would prevention protocols involving myo-ins supplementation be appropriate for patients at high risk of developing OHSS?

How do myo-ins and D-chiro-ins affect long-term fertility?

For pregnancies achieved via ART in patients supplemented with myo-ins, up to what gestational age should myo-ins administration be maintained to reduce the risk of miscarriage and/or preterm delivery?

Can myo-ins enter the routine of cryopreservation programs to preserve gamete quality?

Concluding remarks

Administration of myo-ins has proved to be a simple treatment capable of restoring spontaneous ovarian activity and consequently fertility in most patients with PCOS, with possible applications in healthy populations due to its safety profile (see Outstanding questions). By mediating the FSH signal, myo-ins leads not only to a reduction in the number of intermediate-sized follicles and an increase in the number of large follicles, thus helping to reduce the risk of ovarian hyperstimulation, but also to improved oocyte quality and maturation and embryo development and increased rates of successfully completed pregnancies in women with PCOS, especially if myo-ins supplementation continues during gestation. Myo-ins also acts directly on sperm and spermatogenesis, improving the performance of OAT sperm *in vitro* and *in vivo*. The positive effects of these treatments could be of great help to couples who have difficulty conceiving a child naturally and/or via ART. The cryoprotective effect of myo-ins demonstrated by some studies could be important not only in routine ART but also in sperm cryopreservation protocols to increase the chances of fertility preservation.

Declaration of interests

V.U. is employed at Lo.Li. Pharma Srl, Rome, Italy. All other authors have nothing to declare.

References

1. Singh, S. *et al.* (2023) Polycystic ovary syndrome: etiology, current management, and future therapeutics. *J. Clin. Med.* 12, 1454
2. Bizzarri, M. *et al.* (2023) Myo-inositol and D-chiro-inositol as modulators of ovary steroidogenesis: a narrative review. *Nutrients* 15, 1875
3. Kiani, A.K. *et al.* (2021) From myo-inositol to D-chiro-inositol molecular pathways. *Eur. Rev. Med. Pharmacol. Sci.* 25, 2390–2402
4. Facchinetti, F. *et al.* (2020) Breakthroughs in the use of inositols for assisted reproductive treatment (ART). *Trends Endocrinol. Metab.* 31, 570–579
5. Facchinetti, F. *et al.* (2019) Short-term effects of metformin and myo-inositol in women with polycystic ovarian syndrome (PCOS): a meta-analysis of randomized clinical trials. *Gynecol. Endocrinol.* 35, 198–206
6. Zheng, X. *et al.* (2017) Inositol supplement improves clinical pregnancy rate in infertile women undergoing ovulation induction for ICSI or IVF-ET. *Medicine (Baltimore)* 96, e8842
7. Laganà, A.S. *et al.* (2018) Myo-inositol supplementation reduces the amount of gonadotropins and length of ovarian stimulation in women undergoing IVF: a systematic review and meta-analysis of randomized controlled trials. *Arch. Gynecol. Obstet.* 298, 675–684
8. Gulino, F.A. *et al.* (2016) Effect of treatment with myo-inositol on semen parameters of patients undergoing an IVF cycle: *in vivo* study. *Gynecol. Endocrinol.* 32, 65–68
9. Santoro, M. *et al.* (2021) Sperm performance in oligoasthenoteratozoospermic patients is induced by a nutraceuticals mix, containing mainly myo-inositol. *Syst Biol Reprod Med* 67, 50–63
10. Saleh, R. *et al.* (2018) Increased cryo-survival rate in ejaculated human sperm from infertile men following pre-freeze *in vitro*

myo-inositol supplementation. *Clin. Exp. Reprod. Med.* 45, 177–182
11. Lazzari, E. *et al.* (2023) Projecting the contribution of assisted reproductive technology to completed cohort fertility. *Popul. Res. Policy Rev.* 42, 6
12. Milewska, E.M. *et al.* (2016) Inositol and human reproduction. From cellular metabolism to clinical use. *Gynecol. Endocrinol.* 32, 690–695
13. Sacchi, S. *et al.* (2016) Modulation of gonadotrophin induced steroidogenic enzymes in granulosa cells by D-chiroinositol. *Reprod. Biol. Endocrinol.* 14, 52
14. Pizzo, A. *et al.* (2014) Comparison between effects of myo-inositol and D-chiro-inositol on ovarian function and metabolic factors in women with PCOS. *Gynecol. Endocrinol.* 30, 205–208
15. Facchinetti, F. *et al.* (2020) Inositols in polycystic ovary syndrome: an overview on the advances. *Trends Endocrinol. Metab.* 31, 435–447
16. Gupta, D. *et al.* (2020) Myo-inositol's role in assisted reproductive technology: evidence for improving the quality of oocytes and embryos in patients with polycystic ovary syndrome. *Cureus* 12, e8079
17. Unfer, V. *et al.* (2014) Hyperinsulinemia alters myoinositol to D-chiroinositol ratio in the follicular fluid of patients with PCOS. *Reprod. Sci.* 21, 854–858
18. Ravanos, K. *et al.* (2017) Can high levels of D-chiro-inositol in follicular fluid exert detrimental effects on blastocyst quality? *Eur. Rev. Med. Pharmacol. Sci.* 21, 5491–5498
19. Mani, A.M. *et al.* (2010) IGF1 induces up-regulation of steroidogenic and apoptotic regulatory genes via activation of phosphatidylinositol-dependent kinase/AKT in bovine granulosa cells. *Reproduction* 139, 139–151
20. Unfer, V. *et al.* (2020) Altered ovarian inositol ratios may account for pathological steroidogenesis in PCOS. *Int. J. Mol. Sci.* 21, 7157
21. Heimark, D. *et al.* (2014) Decreased myo-inositol to chiro-inositol (M/C) ratios and increased M/C epimerase activity in PCOS theca cells demonstrate increased insulin sensitivity compared to controls. *Endocr. J.* 61, 111–117
22. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum. Reprod.* 19, 41–47
23. Cheang, K.I. *et al.* (2008) Insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator correlates with insulin sensitivity in women with polycystic ovary syndrome. *Metabolism* 57, 1390–1397
24. Chiu, T.T.Y. *et al.* (2002) Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. *Hum. Reprod.* 17, 1591–1596
25. Bayona, A. *et al.* (2022) Prevalence of PCOS and related hyperandrogenic traits in premenopausal women with type 1 diabetes: a systematic review and meta-analysis. *Hum. Reprod. Update* 28, 501–517
26. Dietz de Loos, A.L.P. *et al.* (2021) Improvements in PCOS characteristics and phenotype severity during a randomized controlled lifestyle intervention. *Reprod. BioMed. Online* 43, 298–309
27. Sayutti, N. *et al.* (2022) PCOS and role of cumulus gene expression in assessing oocytes quality. *Front. Endocrinol. (Lausanne)* 13, 843867
28. Nikbakht, R. *et al.* (2021) Evaluation of oocyte quality in polycystic ovary syndrome patients undergoing ART cycles. *Fertil. Res. Pract.* 7, 2
29. Dinicola, S. *et al.* (2021) Inositols: from established knowledge to novel approaches. *Int. J. Mol. Sci.* 22, 10575
30. Hernandez Marin, I. *et al.* (2021) A multicenter clinical study with myo-inositol and alpha-lactalbumin in Mexican and Italian PCOS patients. *Eur. Rev. Med. Pharmacol. Sci.* 25, 3316–3324
31. Bizzarri, M. *et al.* (2022) An innovative approach to polycystic ovary syndrome. *J. Obstet. Gynaecol.* 42, 546–556
32. Teede, H.J. *et al.* (2023) Recommendations from the 2023 International Evidence-based Guideline for the assessment and management of polycystic ovary syndrome. *Fertil. Steril.* 120, P767–P793
33. Papaleo, E. *et al.* (2007) Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. *Gynecol. Endocrinol.* 23, 700–703
34. Akbari Sene, A. *et al.* (2019) The myo-inositol effect on the oocyte quality and fertilization rate among women with polycystic ovary syndrome undergoing assisted reproductive technology cycles: a randomized clinical trial. *Arch. Gynecol. Obstet.* 299, 1701–1707
35. Artini, P.G. *et al.* (2013) Endocrine and clinical effects of myo-inositol administration in polycystic ovary syndrome. A randomized study. *Gynecol. Endocrinol.* 29, 375–379
36. L, C. *et al.* (2011) Effects of myo-inositol supplementation on oocyte's quality in PCOS patients: a double blind trial. *Eur. Rev. Med. Pharmacol. Sci.* 15, 509–514
37. Papaleo, E. *et al.* (2009) Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. *Fertil. Steril.* 91, 1750–1754
38. Unfer, V. *et al.* (2011) Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. *Eur. Rev. Med. Pharmacol. Sci.* 15, 452–457
39. Rajasekaran, K. *et al.* (2022) Myoinositol versus metformin pretreatment in GnRH-antagonist cycle for women with PCOS undergoing IVF: a double-blinded randomized controlled study. *Gynecol. Endocrinol.* 38, 140–147
40. Hoshino, Y. (2018) Updating the markers for oocyte quality evaluation: intracellular temperature as a new index. *Reprod. Med. Biol.* 17, 434–441
41. Ghasemi, A. *et al.* (2019) The effect of myo-inositol on sperm parameters and pregnancy rate in oligoasthenospermic men treated with IUI: a randomized clinical trial. *Int. J. Reprod. Biomed.* 17, 749–756
42. Voglmayr, J.K. and Amann, R.P. (1973) The distribution of free myo-inositol in fluids, spermatozoa, and tissues of the bull genital tract and observations on its uptake by the rabbit epididymis. *Biol. Reprod.* 8, 504–513
43. Vazquez-Levin, M.H. and Verón, G.L. (2020) Myo-inositol in health and disease: its impact on semen parameters and male fertility. *Andrology* 8, 277–298
44. Voglmayr, J.K. and White, I.G. (1971) Synthesis and metabolism of myoinositol in testicular and ejaculated spermatozoa of the ram. *J. Reprod. Fertil.* 24, 29–37
45. Sullivan, K.M. *et al.* (1993) Calcium mobilization is required for nuclear vesicle fusion *in vitro*: implications for membrane traffic and IP3 receptor function. *Cell* 73, 1411–1422
46. Walensky, L.D. and Snyder, S.H. (1995) Inositol 1,4,5-trisphosphate receptors selectively localized to the acrosomes of mammalian sperm. *J. Cell Biol.* 130, 857–869
47. Condorelli, R.A. *et al.* (2012) Myoinositol: does it improve sperm mitochondrial function and sperm motility? *Urology* 79, 1290–1295
48. Ponchia, R. *et al.* (2021) Oxidative stress measurement in frozen/thawed human sperm: the protective role of an *in vitro* treatment with myo-inositol. *Antioxidants (Basel)* 11, 10
49. Levine, H. *et al.* (2017) Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum. Reprod. Update* 23, 646–659
50. Agarwal, A. *et al.* (2006) Relationship between oxidative stress, varicocele and infertility: a meta-analysis. *Reprod. BioMed. Online* 12, 630–633
51. Azizi, M. *et al.* (2022) Effect of myo-inositol on sperm quality and biochemical factors in cryopreserved semen of patients with asthenospermia. *Andrologia* 54, e14528
52. Calogero, A.E. *et al.* (2015) Myoinositol improves sperm parameters and serum reproductive hormones in patients with idiopathic infertility: a prospective double-blind randomized placebo-controlled study. *Andrology* 3, 491–495
53. Montanino Oliva, M. *et al.* (2016) Effect of myoinositol and antioxidants on sperm quality in men with metabolic syndrome. *Int. J. Endocrinol.* 2016, 1674950
54. Artini, P.G. *et al.* (2017) *In vitro* effect of myo-inositol on sperm motility in normal and oligoasthenospermia patients undergoing *in vitro* fertilization. *Gynecol. Endocrinol.* 33, 109–112
55. Rubino, P. *et al.* (2015) Improving fertilization rate in ICSI cycles by adding myoinositol to the semen preparation procedures: a

prospective, bicentric, randomized trial on sibling oocytes. *J. Assist. Reprod. Genet.* 32, 387–394
56. Scarselli, F. *et al.* (2016) Analysis of myo-inositol effect on spermatozoa motility, in hyper viscous ejaculates and in patients with grades II and III varicocele. *Arch. Ital. Urol. Androl.* 88, 279–283
57. Watson, P.F. (2000) The causes of reduced fertility with cryopreserved semen. *Anim. Reprod. Sci.* 60–61, 481–492
58. Abdolsamadi, M. *et al.* (2020) Does myoinositol supplement improve sperm parameters and DNA integrity in patients with oligoasthenoteratozoospermia after the freezing–thawing process? *Cell Tissue Bank.* 21, 99–106
59. Mohammadi, F. *et al.* (2019) Supplementation of sperm freezing medium with myoinositol improve human sperm parameters and protects it against DNA fragmentation and apoptosis. *Cell Tissue Bank.* 20, 77–86
60. Jakubik-Uljasz, J. *et al.* (2020) Relationship between sperm morphology and sperm DNA dispersion. *Transl. Androl. Urol.* 9, 405–415