The Journal of Obstetrics and Gynaecology

Taylor & Francis
Taylor & Francis Group

Journal of Obstetrics and Gynaecology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijog20

An innovative approach to polycystic ovary syndrome

Vittorio unfer and his pioneering research on inositols

Mariano Bizzarri, Patrizia Logoteta, Giovanni Monastra & Antonio Simone Laganà

To cite this article: Mariano Bizzarri, Patrizia Logoteta, Giovanni Monastra & Antonio Simone Laganà (2021): An innovative approach to polycystic ovary syndrome, Journal of Obstetrics and Gynaecology, DOI: 10.1080/01443615.2021.1920006

To link to this article: https://doi.org/10.1080/01443615.2021.1920006

Published online: 25 Jun 2021.

JOURNAL OF OBSTETRICS AND GYNAECOLOGY
https://doi.org/10.1080/01443615.2021.1920006

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

An innovative approach to polycystic ovary syndrome
Vittorio unfer and his pioneering research on inositols

Mariano Bizzarri[a], Patrizia Logoteta[b], Giovanni Monastra[c] and Antonio Simone Laganà[d]

[a]Department of Experimental Medicine, Systems Biology Group Lab, Sapienza University of Rome, Rome, Italy; [b]Chief Commercial Officer, Lo.Li.Pharma Srl, Rome, Italy; [c]Systems Biology Group Lab, Rome, Italy; [d]Department of Obstetrics and Gynecology, "Filippo Del Ponte" Hospital, University of Insubria, Varese, Italy

ABSTRACT

Myo-inositol and D-chiro-inositol are insulin sensitising agents. In the ovary, myo-inositol acts as second messenger of Follicle Stimulating Hormone (FSH). Both molecules were administered to Polycystic Ovary Syndrome (PCOS) women. The gynaecologist Vittorio Unfer was the first to give specific value to myo-inositol for the treatment of PCOS: this important innovation opened new ways of research to identify efficient therapies based on myo-inositol alone or with low doses of D-chiro-inositol. Significant successes were also gained using myo-inositol in treating male and female infertility. Unfer's researches allowed to identify "the D-Chiro-Inositol Paradox in the Ovary" and the best myo-inositol/D-chiro-inositol ratio (40:1) for the treatment of PCOS. Furthermore, his studies allowed to improve the inositol's efficacy using alpha-lactalbumin. As shown in this review, the main stages of Unfer's scientific career have been closely intertwined with important phases of the recent pharmacological research about the topic.

KEYWORDS

Alpha-lactalbumin; fertilisation; inositol; insulin; insulin resistance; ovary; PCOS; Unfer; vitamin D

Introduction

Infertility and subfertility are a growing worldwide common problem, affecting a significant proportion of the population. As far Europe is concerned, there is also an increasing drop in the birth rate, and couple infertility is one of its causes. About 40% of infertility cases are related to women, 40% to men, 10% to both of them, and in 10% the reason is not clear (Kumar and Singh 2015).

On the one hand, many couples are frustrated in their desire for parenthood and ask the medical world to solve their problem; on the other hand, gynaecologists and andrologists have to face these difficult challenges and offer solutions, as well as concrete results.

In the last decade of the previous century, some researchers increasingly began to focus their attention on the study of some natural molecules, namely inositols, since they were found to be involved in the insulin intracellular signalling process. In addition, new data were demonstrating a robust link between these compounds and male and female fertility. However, some results were interesting, other disappointing. As we will highlight in the next sections, the intuition of very few researchers allowed that specific mistakes were later overcome.

Unfer's researches and studies

Among them, we mention Vittorio Unfer, born in Rome (Italy) in 1963, graduated with honours in Medicine and Surgery at the Sapienza University of Rome where then he specialised in gynaecology and obstetrics. He is an established researcher on nutrition and dietary supplements. Unfer is the founder of The Experts Group on Inositol in Basic and Clinical Research (EGOI), that brings together several international experts, researchers and opinion leaders, skilled in the field of inositol physiology and therapy. Unfer published many scientific books and papers in international journals in the field of Obstetrics, Gynaecology. Endocrinology, Drug safety, Genetics (about ninety of Unfer's papers are on PubMed). His scientific researches allowed him to achieve twenty patents which attest his commitment in these fields. In addition, Unfer has been lecturer in a hundred international conferences about specific topics in gynaecology.

Furthermore, he was the founder of Lo.Li.Pharma (2002), a pharmaceutical company strongly oriented towards research and development with the aim of providing innovative products, Medical Devices and Food Supplements, for obstetrics and gynaecology. Later this company has entered also other specialist fields (endocrinology, urology, neurology). Nowadays it sells products worldwide, in more than 50 countries.

Unfer has managed to unite the expertise in science with his great attention to solve some relevant therapeutic gaps, found during his direct experience in medical practice.

Almost twenty years ago, Unfer was the first promoter of the Myo-inositol (MI) use in the obstetric and gynaecological clinical practice. Later he tested this molecule also in andrology for the male fertility problems. From the beginning, he

CONTACT Giovanni Monastra g.monastra@gmail.com Systems Biology Group Lab, Rome 00185, Italy

focussed his attention on Polycystic Ovary Syndrome (PCOS), a complex endocrine disorder that was the bench test to evaluate in depth the use of inositols, going beyond some wrong ideas of the nineties. Unfer gained a relevant scientific role in identifying and developing an effective treatment for the various aspects of PCOS (in the first place, the recovery of fertility). As a researcher, he believes that the progress in science is primarily based on the open and continuous communication among experts. Discussions, exchange of ideas, also heated debates, fertilise the ground of science. Without this background of comparison and testing of hypotheses among scientists, the level of medicine, pharmacology, biology, would be much less advanced. The researches, aimed at clarifying the pathophysiologic mechanisms underlying PCOS and at identifying its best treatment, constitute a convincing example of this process. Unfer's achievements were recognised by well-known scientists, such as Josef Larner (Heimark et al. 2014) and John Nestler, who had an intense exchange of ideas with him (Nestler and Unfer 2015). By retracing his path in research, we have the opportunity to critically reread some significant phases of the development of the therapeutic approach to PCOS. To carry out this review, we systematically consulted multiple databases (MEDLINE, Embase and Google Scholar) and analysed all the scientific literature on inositols and Polycystic Ovary Syndrome over the past 40 years, highlighting the focal points of advancement in pathophysiology and therapy studies.

PCOS features and therapy: a metaphorical battleground

PCOS represents a relevant health problem that gynaecologists, but also endocrinologists, have to face. This syndrome consists of four different phenotypes and affects up to 10–15% of women of reproductive age in western countries, where it is one the main causes of infertility (Monastra et al. 2017). Currently, its definition requires the presence of two of the three following criteria: (1) chronic oligo-ovulation or anovulation, (2) hyperandrogenism (either clinically established or confirmed by laboratory testing); and (3) the presence of $\geq$12 follicles measuring 2–9 mm in diameter in each ovary and/or increased ovarian volume ($\geq$10 mL), detected by ultrasound examination (Rotterdam 2004). These criteria do not take in consideration other pathological characteristics, such as insulin resistance and related compensatory hyperinsulinemia, that play both a pivotal role in the pathogenesis of PCOS (hyperinsulinemia is present in approximately 80% of obese women with PCOS and 30–40% of lean women with PCOS) (Paul et al. 2016). In the early eighties, Burghen et al. were the first to demonstrate that hyperandrogenism correlates with hyperinsulinism in obese patients with PCOS (Burghen et al. 1980). In those years, insulin resistance was proved to be a feature of PCOS patients, even in the absence of obesity (Cotrozzi et al. 1983; Lagana and Pizzo 2015).

Overall, PCOS is a heterogeneous disorder, favoured by the interaction of environmental and genetic factors (Monastra et al. 2017), and shows several reproductive, metabolic and cardiovascular anomalies, that can give rise to long-term health problems during the life span. Obviously, not all PCOS women wish to get pregnant, however for many of them this is an important purpose and PCOS constitutes a potential impairment. However, all of them desire to solve the abnormalities of the menstrual cycle as well as cure hyperandrogenism.

Until the early 2000s, PCOS women were treated with some insulin-sensitising, or insulin-mimetic, agents to significantly reduce hyperinsulinemia, such as metformin, thiazolidinediones (troglitazone, rosiglitazone and pioglitazone), and D-chiro-inositol (DCI) (Seli and Duleba 2004; Lagana et al. 2015). Physicians still followed this scientific approach of the end of twentieth century. Unfortunately, metformin and thiazolidinediones have very unpleasant (in few cases, serious) adverse effects that reduce patients' compliance and can severely limit their use. Indeed, metformin administration causes gastrointestinal symptoms (nausea, vomiting, diarrhoea and abdominal swelling) as well as metabolic disorders. Thiazolidinediones can induce fluid retention, body weight increase, fractures, coronary artery disease, myocardial infarction, and bladder cancer.

The treatment with DCI in PCOS: successes and failures

The other administered compound, DCI, is naturally present in the human body. It is an inositol stereoisomer such as MI. Towards the end of the nineties the renowned American pharmacologist Josef Larner, one of the most important researchers on insulin activity, was publishing his results on DCI as hypoglycaemic agent, useful for diabetes (Larner et al. 1998). DCI was tested also in PCOS. This molecule is produced in the tissues from MI with the intervention of a specific enzyme, a NAD–NADH-dependent epimerase, which is stimulated by insulin, with a non-reversible reaction. Of note, different tissues, based on their specific physiological roles, may have different MI/DCI ratio, however DCI levels are always much less than MI. Therapeutically, this feature should always be kept in mind, as Unfer did. DCI gave interesting but conflicting results in a few clinical trials with PCOS patients. Some studies carried out by John Nestler and his team (Cheang et al. 2008) had demonstrated that the impairment of insulin activity in PCOS could derive from a defect in the insulin second messenger pathway. Indeed, some low-molecular-weight inositol phosphoglycan mediators (IPGs) are

necessary for the physiological activity of insulin. The binding of insulin to its receptor gives rise to these IPGs, produced by hydrolysing the glycosylphosphatidylinositol lipids of the outer leaflet of the cell membrane. After their internalisation, the released mediators activate essential enzymes that control the oxidative and nonoxidative metabolism of glucose in cells. At that time, it was speculated that a deficit of IPGs (specifically DCI-IPG) in tissues can have a role in inducing insulin resistance (Nestler et al. 1999). However, from a therapeutic perspective we take into account that the DCI-IPG second messenger was found to directly stimulate (in dose–response manner) testosterone biosynthesis by human theca cells from healthy and PCOS patients (Nestler et al. 1998). Going back where it all began, in 1999 during the first controlled clinical study with DCI, 44 obese PCOS patients received 1200 mg DCI or placebo, daily by oral route for 6–8 weeks. The authors found an improvement of insulin sensitivity and ovulation, and reduced levels of free testosterone, whereas no effects were detected in the placebo group (Nestler et al. 1999). Afterwards in 2002, the same group of research gave 600 mg DCI or placebo, daily by oral route for 6–8 weeks, to lean PCOS women (Iuorno et al. 2002). In agreement with the first clinical trial, the DCI administration improved insulin sensitivity and ovulation, and decreased the circulating free testosterone. Then Insmed Pharmaceuticals made a large clinical study with DCI in PCOS women, administering 2400 mg DCI, doubling the dose used in the first trial. The study failed in confirming the effectiveness of DCI and the results were never published. The disappointing results were surely related to the high dose of DCI given. In September 2002 Insmed Pharmaceuticals gave up proceeding with DCI administration to PCOS patients and abandoned the project aimed to identify a drug based on DCI alone, considering that the expectations had been contradicted by the facts. Although the insulin-sensitising agents such as inositols were suitable to treat PCOS, something had to be corrected in the basic assumptions on the therapeutic effects exerted by these molecules.

Myo-inositol in PCOS: a different paradigm and a new therapeutic approach

Few years later, Unfer decided to follow a new approach, based on a different paradigm. For the first time MI, the precursor of DCI was given to PCOS patients. MI and some of its derivatives are extensively distributed in the tissues of animals (vertebrates and invertebrates), plants, fungi and bacteria (Bizzarri et al. 2016). These molecules play important roles in vital processes and in the structures of the living beings, such as osmoregulation, signal transduction, cell regulation, ion channel physiology, cell membrane composition (Bizzarri et al. 2016; Chatree et al. 2020; Maffucci and Falasca 2020). MI is synthesised from glucose-6-phosphate by myo-inositol-3-phosphate synthase to give rise to myo-inositol-3-phosphate (Ins3P) (Lagana et al. 2016), then dephosphorylated to MI (Lackey et al. 2003). There was a well-defined therapeutic rationale in the MI use, based on its physiological role. Indeed, also MI exerts an insulin sensitising effect (Muscogiuri et al. 2016a), being one of the second messengers of this hormone, in the form of MI–IPG (Muscogiuri et al. 2016b). Therefore, the MI presence is just as essential as that of DCI for allowing insulin to play its role. However, for years several researchers did not agree on this (Baillargeon et al. 2006). Of note, cellular glucose uptake is mainly under MI control, and, in keeping with this physiological role, MI content is significant in tissues with high-glucose utilisation, such as the brain, the heart, and the ovary (Larner et al. 1988; Huang et al. 1993; Bevilacqua and Bizzarri 2018). Moreover, MI decreases free fatty acids release from adipose tissues by means of the adenylate cyclase inhibition (Croze and Soulage 2013). Instead, DCI concentrations are high in tissues that store glycogen (e.g. liver and muscle) and low in those where glucose is actively used (Pak et al. 1992). In addition to the above-cited effects, MI (as InsP3) in the ovary is one of the second messengers of Follicle Stimulating Hormone (FSH) (Milewska et al. 2016; Lagana et al. 2017), without any involvement of DCI. The concentration of MI in the mammalian female reproductive tract is substantially higher than in blood serum, suggesting that MI plays specific roles at the ovarian level, ensuring correct oocyte maturation (Lewin et al. 1982).

In the past, some similarities of action between MI and DCI had led somebody to believe these two stereoisomers almost interchangeable, instead their therapeutic rationale is very different, mainly at the ovarian level (Pizzo et al. 2014). Thus, a paradigm shift was necessary, and the new paradigm would prove correct.

The early clinical studies with myo-inositol in PCOS

In 2003, the first clinical study (double-blind RCT) on PCOS patients treated with MI was published (Gerli et al. 2003). The trial was suggested by Unfer as scientific director of Lo.Li.Pharma, his young pharmaceutical company founded just the previous year, which provided MI. This trial shows his original interest, as researcher and manager, specifically focussed on MI, in contrast with the treatments based on DCI. The authors administered 200 mg MI daily for 14 weeks to 136 women and placebo to 147 women. Their study achieved promising results, shedding light on a previously disregarded molecule for PCOS therapy. The ovulation frequency significantly increased ($p < .01$) in the study group (23%) compared with the placebo (13%) and the first ovulation occurred in a shorter time ($p < .05$). A significantly smaller number of patients receiving placebo has ovulated ($p < .05$). In the first week of treatment, Oestradiol (E2) serum levels were found higher only among MI treated women. A significant ($p < .01$) weight decrease was noted in the MI group, whereas patients in the placebo group had a weight increase ($p < .05$). The concentrations of HDL were found higher only in the MI treated group. Supported by these interesting results, some years later Unfer conducted another clinical trial, in collaboration with Papaleo and Baillargeon (Papaleo et al. 2007): also this study provided very encouraging results. Twenty-five PCOS women (28–38 years old) with oligo- or amenorrhoea (six or fewer menstrual cycles during

a period of 1 year) were enrolled. Patients were affected by hyperandrogenism (hirsutism, acne or alopecia) or hyperandogenemia (elevated levels of total or free testosterone) and typical ovarian features on ultrasound scan. The subjects were administered daily with 2 g MI plus 200 μg folic acid until the end of the study (after six months) or the positive pregnancy test. After 34.6 ± 5.5 days of MI treatment, 22 patients (88% of the entire group) presented a first menstrual cycle and 18 of them had monthly menstruations in the follow-up period. They maintained spontaneous ovulation, with follicular growth and increase of serum progesterone levels during the luteal phase (mean 10.5 ± 1.8 ng/mL). Moreover, MI administration significantly reduced the concentrations of total testosterone and free testosterone in serum and improved the length of the successive cycles to 31.7 ± 3.2 days. Multiple pregnancies were not recorded.

In the same year, a double-blind RCT was also published (Gerli et al. 2007) which confirmed the previous positive results. After the randomisation of 92 patients, 45 of them received MI plus folic acid (4 g MI plus 400 μg folic acid), whereas 47 were administered with 400 μg folic acid as placebo. The ovulation frequency was found significantly higher ($p < .01$) in the study group (25%) in comparison with the placebo (15%), and the time to the first ovulation significantly decreased ($p < .05$). The placebo group had significantly less patients who ovulated ($p < .05$) compared to those treated. The E2 levels in blood increased during the first week of treatment only in women administered with MI. HDL in circulation were significantly higher in MI group. No changes were recorded after 14 weeks of MI or placebo therapy in fasting glucose concentrations, fasting insulin, or insulin responses to glucose challenge test. The authors found an inverse relationship between body mass and treatment efficacy. A significant weight loss ($p < .01$) was detected in the study group, whereas the weight of placebo patients increased ($p < .05$). These results supported again the beneficial effects of MI in women with oligomenorrhea and PCOS in improving ovarian function.

In the following years, Unfer went ahead in his studies and researches, achieving new confirmations to the effectiveness of MI in PCOS. Of note, Unfer administered MI to 46 women with hyperandrogenism (Minozzi et al. 2008). Patients, affected by mild to moderate hirsutism, were treated for six months with 2 g MI twice a day. The therapy allowed to significantly reduce hirsutism ($p < .001$) and insulin resistance ($p < .01$), analysed by Homeostasis Model Assessment (HOMA) index. Likewise, total androgens, FSH and LH concentrations decreased. Instead, the E2 levels raised. There was a small non-significant reduction of total cholesterol and LDL cholesterol, and an increase in HDL cholesterol concentration. In addition, also other studies and researches, not driven by him, confirmed his results (Facchinetti and Neri 2020). Hence, it was confirmed Unfer's hypothesis that the deficiency of MI is deeply involved in the development and persistence of PCOS, and that MI supplementation can be an effective treatment (Unfer 2010).

MI human plasma concentration has a mean value of 32.5 ± 1.5 μM/L, with a range of 26.8–43.0 μM/L, according to Leung et al. (Leung et al. 2011). Monastra et al. (Monastra et al. 2018b) administered 6 g MI in a single oral administration to 18 healthy volunteers (men and women). They found that baseline levels in the study subjects were 32.17 ± 4.76 μM/L, using gas chromatography-mass spectrometry for this detection. After MI administration, its average peak plasma concentration (at 180 min) increased about threefold with respect to baseline, reaching 95.06 ± 7.31 μM/L. Of note, this MI dose was chosen to get a good detection of this molecule, however this dosage is not very different from that normally given to patients, i.e. 4 g per day per os for at least 3 months. In the study by Montanino et al. (Montanino Oliva et al. 2018), where the authors used gas chromatography-mass spectrometry, the basal MI value found in PCOS patients was less than in normal women, i.e. 17 ± 3.5 μmol/L, in agreement with Unfer's hypothesis that MI deficiency is a common feature in PCOS patients. After a treatment with 2 g MI plus 50 mg alpha-lactalbumin, twice a day by oral route for three months, this concentration raised by 106%, reaching 35 ± 3.8 μmol/L.

Last, but not least, it is necessary to emphasise that MI is a totally safe molecule, as stated by Food and Drug Administration (FDA 2017) and demonstrated by some clinical studies (Carlomagno and Unfer 2011), with no side effects at the therapeutic dose (maximum 4 g/die).

Myo-inositol to improve female fertility

The positive effects due to MI administration in improving female fertility gave new opportunities to many women, regardless of whether they are suffering from PCOS or not (Papaleo et al. 2009a; Lagana et al. 2018a). This area of research was initially developed starting from pioneering Chiu's studies. His attention was focussed on the content of follicular fluid (FF), since it is a metabolically active microenvironment where oocytes are hosted and grow. FF plays a key role in the processes of fertilisation and embryogenesis. Chiu et al. demonstrated that the amount of MI contained in human follicles is related to the quality of oocytes. Higher MI concentrations represent an excellent marker of good quality oocytes and embryos (Chiu et al. 2002). Then, a research conducted in mice confirmed this effect due to MI (Chiu et al. 2003). This evidence is in line with a previous study that had demonstrated a directly proportional relationship between inositol content in human serum and reproductive outcome in IVF (Chiu and Tam 1992). On the other hand, the results achieved with MI in PCOS women, that wish getting pregnant, represent a further confirmation (Gerli et al. 2007; Genazzani et al. 2008; Costantino et al. 2009; Papaleo et al. 2009b). In particular, a study by Unfer et al. showed that the MI treatment in patients with PCOS undergoing IVF decreases germinal vesicles and degenerated oocytes at ovum pick-up, without compromising total number of retrieved oocytes. This procedure allows to have lower E2 levels at hGC administration and can reduce the risk of ovarian hyperstimulation in these patients. Of note, the administration of MI to PCOS women undergoing IVF permitted to decrease the quantity of recombinant FSH (rFSH) administered and the number of days of stimulation, as clearly shown in a systematic review

by Laganà et al. (2018b). This evidence proves that MI can increase FSH sensitivity providing further support to the beneficial effect of MI administration on ovarian function and oocyte development. Then, Unfer and co-workers highlighted that only MI plays a beneficial role for oocytes, differently from DCI (Unfer et al. 2011a). The authors enrolled 84 euglycemic PCOS patients, undergoing ovulation induction for ICSI; among them 43 were administered with 2 g MI and 41 patients with 0.6 g DCI, both twice a day for 8 weeks before rFSH administration. This comparison study demonstrated that the number of mature oocytes was significantly higher in the MI group than in the DCI group. Furthermore, the authors found that the number of immature oocytes decreased in the MI treated women as well as the mean number of top-quality embryos and the total number of pregnancies increased in the patients administered with MI respect to those treated with DCI. The results clearly demonstrated that in these PCOS women MI treatment works better than DCI in improving oocyte and embryo quality for ovarian stimulation.

Myo-inositol to improve male fertility

As previously mentioned, Unfer investigated the efficacy of MI, without DCI, also to solve male infertility and obtained satisfying results. Again, this intuition was derived by the analysis of the physiological data, which demonstrate that male reproductive organs are particularly rich in free MI. It was a clear hint that MI is central also in male reproduction (Eisenberg and Bolden 1964; Voglmayr and Amann 1973; Ghafoorunissa 1975; Hinton and Setchell 1981). Indeed, this stereoisomer plays a vital role for the maturation of spermatozoa in the seminiferous tubules and for their migration to the epididymis and then to the vas deferens. MI biosynthesis seems to be indispensable for the normal metabolism of the germinal epithelium. MI de novo synthesis could be required for maintaining satisfactory intracellular levels of nucleotide precursors, essential to keep the integrity of the mature epithelial cells (Morris and Collins 1971). In addition, MI may act as osmoprotective molecule to safeguard the sperm cells, exerting the same function that it does in the kidney to protect other kind of cells (Chauvin and Griswold 2004). As far as the phospholipid-bound MI is concerned, one of the essential roles that IP3 plays in the male reproductive system consists in the mobilisation of calcium ions which activate the nuclear fusion of vesicles (Sullivan et al. 1993). The release of calcium from the acrosome could be fundamental to promote membrane fusion and therefore exocytosis (Walensky and Snyder 1995). Moreover, the fertilisation process needs the spermatozoa hyperactivated motility which allows these cells swimming along the oviduct to reach the egg. As we know, Ca^{2+} is required for starting the sperm motility and to keep it, since Ca^{2+} controls the asymmetrical flagellar beating (Correia et al. 2015). The above evidence was essential for determining the best exploitation of inositols in ART. MI was given to men with fertility problems, mainly affected by oligoasthenoteratozoospermia (OAT), a disorder including oligozoospermia (low sperm count), asthenozoospermia (reduced sperm movement), and teratozoospermia (malformed sperm cells). This stereoisomer was used in vivo, *in vitro* and in both ways in the same patient (Colone et al. 2010; Condorelli et al. 2011; Colone et al. 2017). Also other researchers, following his strategy, reached convincing results (Condorelli et al. 2012; Calogero et al. 2015; Rubino et al. 2015; Gulino et al. 2016; Montanino Oliva et al. 2016; Palmieri et al. 2016; Artini et al. 2017; Condorelli et al. 2017; Dinkova et al. 2017).

Tissue targets of insulin-resistance and "the D-Chiro-Inositol paradox in the ovary"

Unfer focussed his attention on the tissue-specific nature of insulin resistance in PCOS women. Indeed, although the liver, fat and muscle become insulin-resistant in these patients, the ovaries maintain the physiological sensitivity to the hormone (Monastra et al. 2017). In 2011, Unfer elaborated the so-called "DCI paradox" in the ovary, a milestone that allowed to understand the intriguing enigma of the very different effects of MI and DCI in PCOS patients (Carlomagno et al. 2011b). As mentioned above, the epimerase enzyme, under insulin stimulation, converts MI to DCI in all the tissues where it is present; the ovary is one among them. Hence, in insulin-resistant tissues there is a decrease of DCI concentration; nevertheless, Unfer suggested that the situation should be different in the ovary. He suggested that in women with PCOS, hyperinsulinemia likely stimulates epimerase activity in the ovary, resulting in an overproduction of DCI and a concomitant depletion of MI. It was postulated that the consequential deficiency of MI could be responsible for the poor oocyte quality and the impairment of the FSH signalling. Clearly, DCI supplementation in PCOS patients is ineffective, if not harmful in such women as they already have high levels of this molecule in the ovary. DCI may exert some beneficial effects at systemic level by properly modulating insulin-based activity, however it may hamper ovarian function at high levels. Indeed, high release of DCI-phosphoglycans, under insulin stimulation, enhances de novo testosterone biosynthesis from ovarian theca cells, thus raising serum androgen levels (Nestler et al. 1998). In addition, DCI may impair the subtle equilibrium in between MI and DCI within ovary cells. Both DCI and MI are required to ensure a proper glucose metabolism in cooperating with insulin, although MI seems to play a more critical role in oocyte, as suggested by the fact that almost 99% of intracellular inositol pool is constituted by MI. In 2013, Larner and co-workers (Heimark et al. 2014) formally recognised that Unfer was the first to discover what occurs in the ovaries of PCOS women: «In a paper entitled "The D-Chiro-Inositol Paradox in the Ovary" (Carlomagno et al. 2011b), the authors speculate that PCOS patients with hyperinsulinemia likely present an enhanced MI to DCI epimerization in the ovary; this would result in an increased DCI/MI ratio (i.e. overproduction of DCI), which would in turn would lead to a MI deficiency in the ovary». The present data with theca cells from PCOS subjects and controls demonstrates that this is indeed the case". Shortly after Unfer published a similar paper on this topic (Unfer et al. 2014). This study was carried out on PCOS and

non-PCOS patients and provided the experimental evidence that PCOS women have an abnormal MI-DCI ratio in FF. The authors observed that this ratio changes from 100:1, in healthy women, to 0.2:1 in PCOS patients. Since MI is one of the second messengers of FSH (Milewska et al. 2016), PCOS can harm even the signalling pathway of this hormone.

From inositol levels in tissues to the correct therapy

Since many years Unfer was driven by the firm conviction that the respective physiological concentrations of two natural molecules, such as MI and DCI, in each district of the body should lead the researchers and physicians to establish the best therapy. All the available data in healthy subjects (Garzon et al. 2019) suggest that DCI should be administered, at least in most cases, at very low levels. Since his effort was ever to improve the already satisfactory effect obtained with MI alone, he addressed his attention to the mean concentrations of both inositol stereoisomers, i.e. their reciprocal ratio, in plasma of healthy women. The aim was defining, in the most reliable way, the correct dosage of MI and DCI to administer orally in PCOS (and not only), in harmony with the woman's physiology. In 2016, Facchinetti and co-workers, based on unpublished data obtained by some Unfer's collaborators, had established that the MI/DCI plasma ratio is around 40:1 (Facchinetti and Neri 2016), confirming some Unfer's ideas based on previous studies (Dinicola et al. 2014; Unfer and Porcaro 2014). Translated at therapeutic level, it means that PCOS women, under inositol treatment aimed at restoring a physiological condition, should take 4 g MI and 0.1 g DCI in powder, but the use of innovative technologies for the improvement of drug absorption have led to the administration of 550 mg of MI and 13.8 mg of DCI in a pharmaceutical form of soft gel capsule, patented by the company of Unfer, corresponding to the plasma ratio 40:1 of MI and DCI (Carlomagno et al. 2012; De Grazia et al. 2012).

On this line of researches and studies, a further confirmation and deepening of the results already obtained in FF was provided in 2017. A prospective observational study, performed in an IVF centre, allowed to correlate the concentrations of MI and DCI in the FF in healthy subjects with the oocyte and blastocyst quality (Ravanos et al. 2017). The trial involved 8 egg donors and eleven couples undergoing *in vitro* fertilisation. The donors, all selected to have a homogeneous condition, underwent the same standard stimulation protocol. To avoid any confounding factor, male partners were chosen from normospermic subjects. MI/DCI ratio, calculated in FF, was correlated with different blastocyst grades. The authors found that the MI/DCI ratio was significantly higher in the samples evaluated as good quality blastocysts, in comparison to those graded as poor-quality blastocysts. Almost all the transferred blastocysts, rated as good quality, were correlated to lower DCI levels in FF and gave satisfying results for the implantation rate and pregnancy rate. These data demonstrate that the reduction of the MI/DCI ratio plays a negative role in the development of the follicle, that is of the oocyte and blastocyst quality. It was possible to define a good quality threshold of the MI/DCI ratio in FF, fixed very close or higher than 70:1 (up to 100:1), whereas the blastocysts of low quality were under this value, which may represent a new biomarker for estimating the good features of blastocysts, and a prognostic factor of embryo implantation and pregnancy success. These results gave a further support to pre-treat women undergoing IVF with MI, in order to improve oocyte quality and ART outcome.

Alpha-lactalbumin story

In parallel with these studies, Unfer has opened up another line of research to solve the problem of inositol resistance, which refers to the therapeutic inefficacy of inositols in some patients (named 'resistant'), a condition found in several clinical studies (Iuorno et al. 2002; Gerli et al. 2007; Raffone et al. 2010; Kamenov et al. 2015). In 30–40% of PCOS patients, indeed, inositols failed to significantly improve the metabolic and hormonal parameters and restore ovulation. A well-founded hypothesis argues that such problem can derive from the reduced or absent absorption of inositol due to unclear or unpredictable conditions (e.g. obesity, chronic intestinal diseases, dysbiosis).

Unfer, based on some date already available, had suggested to use alpha-lactalbumin, a whey protein, to improve MI absorption (Unfer 2018). Two studies confirmed the validity of this proposal. One was carried out *in vivo* and *in vitro* (Monastra et al. 2018b). In the first phase, a single dose of 6 g of MI was administered to 18 healthy volunteers and, after a week of washout, 6 g of MI + 150 mg of alpha-LA in single dose were given to the same subjects. The average peak plasma concentration (Cmax) and the area under the time-course curve (AUC) of plasma concentration after the combined treatment were found significantly higher (+32.4% and +27.5%, respectively) compared to the results obtained with 6 g MI alone. Then, the authors examined *in vitro* the underlying mechanism(s) with the aim to explain this effect. They tested the MI transport alone and together with alpha-LA (as biopeptides, i.e. 'digested' protein) using the human intestinal Caco-2 cell monolayer, a usually adopted *in vitro* model of gut mucosa (Sambuy et al. 2005; Lemmer and Hamman 2013). In this case, an increased passage of MI in the presence of alpha-LA was found and, concomitantly, a lowering of the Trans-Epithelial Electrical Resistance (TEER) was detected. This means that alpha-LA induced the opening of the tight junctions between the cells allowing a 'passive' passage, in addition to the active one, due to transporters. This effect is transient, i.e. reversible, hence physiological, and not toxic. MI and alpha-LA, at effective doses, are not harmful to Caco-2 cells. A successive study on PCOS patients was carried out to confirm clinically the effects of the alpha-LA together with MI, having as primary outcome the ovulation restoration. The trial supported the efficacy of the new formulation (Montanino Oliva et al. 2018). The number of inositol-resistant patients was drastically reduced. Initially, 37 anovulatory PCOS women received orally 2 g of MI, twice a day for 3 months. After this treatment, only 23 subjects (62%) ovulated, while 14 (38%) did not. Of note, they had no rise in MI plasma levels, so demonstrating a reduced absorption of the administered compound. This group of patients was

further treated with the same daily dose of MI with the addition of 50 mg alpha-LA twice a day, for an additional period of 3 months. At the end, 12 (86%) patients ovulated, displaying significantly increased level of plasmatic MI and a better hormone and lipid profile with respect to the baseline. This clinical trial showed that a reduced intestinal MI absorption may explain the inefficacy observed in some PCOS patients treated with inositols. Therefore, together with the study by Monastra et al. (Monastra et al. 2018b), it allowed to improve some disappointing results of this therapy, which until recently were unexplained.

The "litmus test" for the 40:1 ratio in PCOS therapy

Many preclinical researches and clinical trials supported Unfer's assertions on the 40:1 plasma ratio as parameter to establish the optimal therapy. Two studies deserve to be mentioned, being the most significant.

A preclinical study was recently carried out with a standardised mouse model of PCOS (Bevilacqua et al. 2019). Female mice were exposed to continuous light for 10 weeks and developed an androgenic-like phenotype of the ovaries as in PCOS women. The experiment gave the first evidence that the efficacy exerted by various MI/DCI molar ratio (5:1; 20:1; 40:1; 80:1) changes, supporting the metabolic link between the two stereoisomers, specifically for PCOS. Mice treated daily with 420 mg/kg MI/DCI in a 40:1 molar ratio obtained a rapid and almost full recovery from PCOS signs and symptoms, whereas the other MI/DCI ratios were found less effective or even harmful for the pathology. In particular, the formulation with the higher DCI dose worsened the PCOS pathological features. The other study, carried out in humans, provided additional support to define the best posology with inositols in the treatment of PCOS (Nordio et al. 2019). Nordio conducted a randomised, interventional, open-label study, that for the first time, directly evaluated the efficacy of seven different MI/DCI ratios administered to PCOS patients. Fifty-six women entered the trial and were randomly allocated into seven groups of eight patients each. They were treated for 3 months with different formulations of inositols: DCI alone, and in 1:3.5; 2.5:1; 5:1; 20:1; 40:1, 80:1 MI/DCI ratios (in total, 2 g of inositols twice daily). The primary outcome was ovulation whereas the secondary outcomes included BMI, menses, basal and postprandial insulin levels, HOMA-IR index, FSH, LH, sex hormone binding globulin (SHBG), E2, free testosterone. Among the seven tested formulations, the 40:1 ratio achieved the best results, restoring ovulation in 62.5% of women. The higher DCI dose was unable at all to restore ovulation, whereas the 5:1, 20:1, and 80:1 ratios induced ovulation however in a lower percentage respect to the 40:1. Furthermore, such ratio more effectively normalised basic parameters such as progesterone, LH, SHBG, E2, testosterone, whereas HOMA-IR index similarly improved in all the groups. The results, achieved using the 40:1 ratio, were significantly better in comparison to the other formulations, except for HOMA-IR index. These results very well agree with the study carried out with the PCOS mice (Bevilacqua et al. 2019).

We have seen that the inositol(s) dose and posology slowly changed over the years. In particular, MI dose was increased based on the clinical experience demonstrating that 4 g/die, with or without DCI depending on the therapeutic target, is the best posology to achieve the most significant improvement in PCOS patients.

In recent years, Unfer published some reviews and meta-analysis that gathered and evaluated the most robust scientific data available, which confirmed again his positions on PCOS therapy (Unfer et al. 2016, 2017; Gateva et al. 2018; Facchinetti et al. 2019).

Moreover, some reviews (two Consensus Conferences and a very recent Expert's Opinion, edited by Unfer and Facchinetti and written with the collaboration of several experts of international level in this field) confirmed Unfer's ideas and studies (Bevilacqua et al. 2015; Facchinetti et al. 2015, 2020).

A new chapter still to be developed …

The research on these fascinating molecules never stops and new discover recently allowed to increase the knowledge on the DCI activity with reference to a new target, the aromatase enzyme, also called oestrogen synthetase, that was detected in fat tissue, ovaries, testicles, placenta, brain, bone, etc. (Stocco 2012). Its inhibition can be therapeutically very important for the treatment of several disorders. All the aromatase inhibitors entail a systemic increase of testosterone levels and a parallel reduction of oestrogens. It was shown that DCI reduces in dose–response manner the expression of aromatase gene (CYP19A1) (Sacchi et al. 2016), and consequently the conversion of testosterone to oestrogen. These data, with those previously mentioned regarding the stimulatory effect of DCI-IPG on testosterone biosynthesis in human theca cells, demonstrate that DCI can increase the androgen levels and reduce the oestrogen levels through different pathways. Unfer suggested that DCI at high dose may be used in clinical conditions which need the oestrogen reduction. In other words, DCI would work as a brake. Therefore, DCI may be very appropriate to treat endometriosis as well as oestrogen-dependent breast and endometrial cancers. Likewise, the administration of DCI alone or in combination with other aromatase inhibitors, such as letrozole, may be used to induce ovulation, in some kinds of patients. Obviously, the length of the DCI treatment has to be carefully defined to avoid unintended effects. Furthermore, DCI can be very beneficial in several conditions, such as sexual dysfunctions in man (for instance, hypogonadism) in which androgen levels must be significantly increased (Lagana and Unfer 2019).

The overall idea proposed by Unfer is that MI works in our organism as an accelerator and DCI as a brake, and this is a piece of the new paradigm which helps to identify innovative therapeutic approaches, opening unexplored scenarios for practical applications in several fields.

Some conclusions at the end of this long journey

The research carried out by Unfer on inositols started from MI in PCOS and reproduction disorders and then opened innovative ways, also far beyond the PCOS therapy, in areas hitherto little or nothing explored in connection with MI or MI/DCI in the 40:1 ratio. Now we very briefly mention his studies on the use of MI in combination with oral contraceptive pill, achieving the goal to reduce the dose of the contraceptive pill (Minozzi et al. 2011) or in combination with melatonin (Vitale et al. 2016) to improve the outcome of IVF (Unfer et al. 2011b, Carlomagno et al. 2011a, Carlomagno et al. 2018). Furthermore, he tested MI in gestational diabetes mellitus (GDM), obtaining interesting results (Costabile and Unfer 2017), confirmed by other groups (Santamaria et al. 2016; Fraticelli et al. 2018; Pintaudi et al. 2018; Santamaria et al. 2018; Celentano et al. 2020). Finally, he highlighted the usefulness of MI to regulate iodine organification and thyroid hormone biosynthesis. This MI effect may increase thyroid functionality and possibly allow a quicker recovery from iodine deficiency (Barbaro et al. 2019).

Then, outside the inositol sector, we mention his studies on alpha-lipoic acid to prevent miscarriage or treat peripheral neuropathy in obstetrics (Costantino et al. 2014; Monastra et al. 2016; Parente et al. 2017). Also these studies had several confirmations by other groups of research (Porcaro et al. 2015; Costantino et al. 2016).

Another important field of research where we found the involvement of Unfer concerns the identification of vitamin D as a progesterone-like hormone, very helpful to improve pregnancy (Monastra et al. 2018a, Colonese et al. 2015).

Summarising what we wrote previously concerning the core of his research, Unfer was the first to administer specifically MI in PCOS and to carry out researches and studies from a scientifically new point of view. His scientific vision has allowed to realise an innovative therapy for PCOS.

Incidentally, we emphasise that until the year 2006 some very valid scholars wrote that MI is "an inositol not believed to influence insulin sensitivity" (Baillargeon et al. 2006). However, soon enough they changed their mind. Therefore, at that time Unfer was a precursor of the MI use, going against the mainstream. The aspiration to link the therapeutic treatment, based on natural molecules, to their different concentrations (reciprocal equilibrium) in our organism was always one of Unfer's guidelines. Hence, the introduction of MI and DCI in the 40:1 ratio for PCOS treatment was a step forward a more advanced therapy, derived not only from their role as insulin mimetic (or ''insulin sensitising'') agents and their effectiveness in improving metabolism, but also from the knowledge of their physiological plasma ratio. As we have seen, Unfer's lifetime has been devoted to inositol use in obstetrics and gynaecology, but also far beyond, towards the discovery of new therapeutic territories to explore. Retracing the path of Unfer's scientific research has allowed us to re-read, rethink and reconsider together with him, some important pages of recent studies in the field of obstetrics, gynaecology and endocrinology, reminding to all researchers how the desire to know and investigate allows to go further and build the future of health.

Disclosure statement

Patrizia Logoteta is employee at Lo.Li.Pharma Company. The other authors declare no competing interests.

Author contribution

All authors contributed equally to the writing of the manuscript.

ORCID

Mariano Bizzarri http://orcid.org/0000-0003-0408-4136
Giovanni Monastra http://orcid.org/0000-0003-2012-1435
Antonio Simone Laganà http://orcid.org/0000-0003-1543-2802

References

Artini PG, Casarosa E, Carletti E, Monteleone P, Di Noia A, Di Berardino OM. 2017. *In vitro* effect of myo-inositol on sperm motility in normal and oligoasthenospermia patients undergoing *in vitro* fertilization. Gynecological Endocrinology 33:109–112.

Baillargeon JP, Diamanti-Kandarakis E, Ostlund RE, Jr., Apridonidze T, Iuorno MJ, Nestler JE. 2006. Altered D-chiro-inositol urinary clearance in women with polycystic ovary syndrome. Diabetes Care 29:300–305.

Barbaro D, Orru B, Unfer V. 2019. Iodine and Myo-Inositol: a novel promising combination for iodine deficiency. Frontiers in Endocrinology 10: 457.

Bevilacqua A, Bizzarri M. 2018. Inositols in insulin signaling and glucose metabolism. International Journal of Endocrinology 2018:1968450.

Bevilacqua A, Carlomagno G, Gerli S, Montanino Oliva M, Devroey P, Lanzone A, et al. 2015. Results from the International Consensus Conference on myo-inositol and D-chiro-inositol in Obstetrics and Gynecology–assisted reproduction technology. Gynecological Endocrinology 31:441–446.

Bevilacqua A, Dragotto J, Giuliani A, Bizzarri M. 2019. Myo-inositol and D-chiro-inositol (40:1) reverse histological and functional features of polycystic ovary syndrome in a mouse model. Journal of Cellular Physiology 234:9387–9398.

Bizzarri M, Fuso A, Dinicola S, Cucina A, Bevilacqua A. 2016. Pharmacodynamics and pharmacokinetics of inositol(s) in health and disease. Expert Opinion on Drug Metabolism & Toxicology 12: 1181–1196.

Burghen GA, Givens JR, Kitabchi AE. 1980. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. The Journal of Clinical Endocrinology and Metabolism 50:113–116.

Calogero AE, Gullo G, La Vignera S, Condorelli RA, Vaiarelli A. 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.

Carlomagno G, De Grazia S, Unfer V, Manna F. 2012. Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use. Expert Opinion on Drug Delivery 9:267–271.

Carlomagno G, Minini M, Tilotta M, Unfer V. 2018. From Implantation to Birth: Insight into Molecular Melatonin Functions. International Journal of Molecular Sciences. 19:2802.

Carlomagno G, Nordio M, Chiu TT, Unfer V. 2011a. Contribution of myo-inositol and melatonin to human reproduction. European Journal of Obstetrics, Gynecology, and Reproductive Biology 159:267–272.

Carlomagno G, Unfer V. 2011. Inositol safety: clinical evidences. European Review for Medical and Pharmacological Sciences 15:931–936.

Carlomagno G, Unfer V, Roseff S. 2011b. The D-chiro-inositol paradox in the ovary. Fertility and Sterility 95:2515–2516.

Celentano C, Matarrelli B, Pavone G, Vitacolonna E, Mattei PA, Berghella V, et al. 2020. The influence of different inositol stereoisomers supplementation in pregnancy on maternal gestational diabetes mellitus and fetal outcomes in high-risk patients: a randomized controlled trial. The Journal of Maternal-Fetal & Neonatal Medicine 33:743–751.

Chatree S, Thongmaen N, Tantivejkul K, Sitticharoon C, Vucenik I. 2020. Role of inositols and inositol phosphates in energy metabolism. Molecules 25:5079.

Chauvin TR, Griswold MD. 2004. Characterization of the expression and regulation of genes necessary for myo-inositol biosynthesis and transport in the seminiferous epithelium. Biology of Reproduction 70: 744–751.

Cheang KI, Baillargeon JP, Essah PA, Ostlund RE, Jr., Apridonize T, Islam L, 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.

Chiu TT, Rogers MS, Briton-Jones C, Haines C. 2003. Effects of myo-inositol on the *in-vitro* maturation and subsequent development of mouse oocytes. Human Reproduction 18:408–416.

Chiu TT, Rogers MS, Law EL, Briton-Jones CM, Cheung LP, Haines CJ. 2002. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: relationship with oocyte quality. Human Reproduction 17:1591–1596.

Chiu TT, Tam PP. 1992. A correlation of the outcome of clinical *in vitro* fertilization with the inositol content and embryotrophic properties of human serum. Journal of Assisted Reproduction and Genetics 9: 524–530.

Colone M, Calcabrini A, Unfer V, Stringaro A. 2017. Contribution of electron microscopy to study *in vitro* inositol effects on human spermatozoa. European Review for Medical and Pharmacological Sciences 21: 4–9.

Colone M, Marelli G, Unfer V, Bozzuto G, Molinari A, Stringaro A. 2010. Inositol activity in oligoasthenoteratospermia–an *in vitro* study. European Review for Medical and Pharmacological Sciences 14: 891–896.

Colonese F, Lagana AS, Colonese E, Sofo V, Salmeri FM, Granese R, et al. 2015. The pleiotropic effects of vitamin D in gynaecological and obstetric diseases: an overview on a hot topic. BioMed Research International 2015:986281.

Condorelli RA, La Vignera S, Bellanca S, Vicari E, Calogero AE. 2012. Myoinositol: does it improve sperm mitochondrial function and sperm motility? Urology 79:1290–1295.

Condorelli RA, La Vignera S, Di Bari F, Unfer V, Calogero AE. 2011. Effects of myoinositol on sperm mitochondrial function *in-vitro*. European Review for Medical and Pharmacological Sciences 15:129–134.

Condorelli RA, La Vignera S, Mongioi LM, Vitale SG, Lagana AS, Cimino L, et al. 2017. Myo-inositol as a male fertility molecule: speed them up. European Review for Medical and Pharmacological Sciences 21:30–35.

Correia J, Michelangeli F, Publicover S. 2015. Regulation and roles of Ca2+ stores in human sperm. Reproduction 150:R65–76.

Costabile L, Unfer V. 2017. Treatment of gestational diabetes mellitus with myo-inositol: analyzing the cutting edge starting from a peculiar case. European Review for Medical and Pharmacological Sciences 21: 73–76.

Costantino D, Minozzi G, Minozzi E, Guaraldi C. 2009. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. European Review for Medical and Pharmacological Sciences. 13:105–110.

Costantino M, Guaraldi C, Costantino D. 2016. Resolution of subchorionic hematoma and symptoms of threatened miscarriage using vaginal alpha lipoic acid or progesterone: clinical evidences. European Review for Medical and Pharmacological Sciences 20:1656–1663.

Costantino M, Guaraldi C, Costantino D, De Grazia S, Unfer V. 2014. Peripheral neuropathy in obstetrics: efficacy and safety of alpha-lipoic acid supplementation. European Review for Medical and Pharmacological Sciences 18:2766–2771.

Cotrozzi G, Matteini M, Relli P, Lazzari T. 1983. Hyperinsulinism and insulin resistance in polycystic ovarian syndrome: a verification using oral glucose, I.V. Glucose and tolbutamide. Acta Diabetologica Latina 20: 135–142.

Croze ML, Soulage CO. 2013. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie 95:1811–1827.

De Grazia S, Carlomagno G, Unfer V, Cavalli P. 2012. Myo-inositol soft gel capsules may prevent the risk of coffee-induced neural tube defects. Expert Opinion on Drug Delivery 9:1033–1039.

Dinicola S, Chiu TT, Unfer V, Carlomagno G, Bizzarri M. 2014. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. Journal of Clinical Pharmacology 54: 1079–1092.

Dinkova A, Martinov D, Konova E. 2017. Efficacy of myo-inositol in the clinical management of patients with asthenozoospermia. European Review for Medical and Pharmacological Sciences 21:62–65.

Eisenberg F, Jr., Bolden AH. 1964. Reproductive tract as site of synthesis and secretion of inositol in the male rat. Nature 202:599–600.

Facchinetti F, Appetecchia M, Aragona C, Bevilacqua A, Bezerra Espinola MS, Bizzarri M, et al. 2020. Experts' opinion on inositols in treating polycystic ovary syndrome and non-insulin dependent diabetes mellitus: a further help for human reproduction and beyond. Expert Opinion on Drug Metabolism & Toxicology 16:255–274.

Facchinetti F, Bizzarri M, Benvenga S, D'Anna R, Lanzone A, Soulage C, et al. 2015. Results from the International Consensus Conference on Myo-inositol and d-chiro-inositol in Obstetrics and Gynecology: the link between metabolic syndrome and PCOS. European Journal of Obstetrics & Gynecology and Reproductive Biology 195:72–76.

Facchinetti F, Orru B, Grandi G, Unfer V. 2019. Short-term effects of metformin and myo-inositol in women with polycystic ovarian syndrome (PCOS): a meta-analysis of randomized clinical trials. Gynecological Endocrinology 35:198–206.

Facchinetti FDG, Neri I. 2016. The Ratio of MI to DCI and Its Impact in the Treatment of Polycystic Ovary Syndrome: Experimental and Literature Evidences. Frontiers in Gynecological Endocrinology 3: 103–109.

FDA. 2017. Opinion: Inositol [Online]; [cited 2021 Apr 21]. Available from: http://wayback.archive-it.org/7993/20171031060738/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm260436.htm.

Fraticelli F, Celentano C, Zecca IA, Di Vieste G, Pintaudi B, Liberati M, et al. 2018. Effect of inositol stereoisomers at different dosages in gestational diabetes: an open-label, parallel, randomized controlled trial. Acta Diabetologica 55:805–812.

Garzon S, Lagana AS, Monastra G. 2019. Risk of reduced intestinal absorption of myo-inositol caused by D-chiro-inositol or by glucose transporter inhibitors. Expert Opinion on Drug Metabolism & Toxicology. 15:697–703.

Gateva A, Unfer V, Kamenov Z. 2018. The use of inositol(s) isomers in the management of polycystic ovary syndrome: a comprehensive review. Gynecological Endocrinology 34:545–550.

Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. 2008. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. Gynecological Endocrinology 24:139–144.

Gerli S, Mignosa M, Di Renzo GC. 2003. Effects of inositol on ovarian function and metabolic factors in women with PCOS: a randomized double blind placebo-controlled trial. European Review for Medical and Pharmacological Sciences. 7:151–159.

Gerli S, Papaleo E, Ferrari A, Di Renzo GC. 2007. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. European Review for Medical and Pharmacological Sciences. 11:347–354.

Ghafoorunissa 1975. Effect of dietary protein on the biosynthesis of inositol in rat testes. Reproduction, Fertility and Development 42: 233–238.

Gulino FA, Leonardi E, Marilli I, Musmeci G, Vitale SG, Leanza V, et al. 2016. Effect of treatment with myo-inositol on semen parameters of patients undergoing an IVF cycle: *in vivo* study. Gynecological Endocrinology 32:65–68.

Heimark D, McAllister J, Larner J. 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. Endocrine Journal 61:111–117.

Hinton BT, Setchell BP. 1981. Micropuncture and microanalytical studies of rhesus monkey and baboon epididymis and the human ductus deferens. American Journal of Primatology 1:251–256.

Huang LC, Fonteles MC, Houston DB, Zhang C, Larner J. 1993. Chiroinositol deficiency and insulin resistance. III. Acute glycogenic

and hypoglycemic effects of two inositol phosphoglycan insulin mediators in normal and streptozotocin-diabetic rats *in vivo*. Endocrinology 132:652–657.

Iuorno MJ, Jakubowicz DJ, Baillargeon JP, Dillon P, Gunn RD, Allan G, et al. 2002. Effects of d-chiro-inositol in lean women with the polycystic ovary syndrome. Endocrine Practice 8:417–423.

Kamenov Z, Kolarov G, Gateva A, Carlomagno G, Genazzani AD. 2015. Ovulation induction with myo-inositol alone and in combination with clomiphene citrate in polycystic ovarian syndrome patients with insulin resistance. Gynecological Endocrinology 31:131–135.

Kumar N, Singh AK. 2015. Trends of male factor infertility, an important cause of infertility: a review of literature. Journal of Human Reproductive Sciences 8:191–196.

Lackey KH, Pope PM, Johnson MD. 2003. Expression of 1L-myoinositol-1-phosphate synthase in organelles. Plant Physiology 132:2240–2247.

Lagana AS, Barbaro L, Pizzo A. 2015. Evaluation of ovarian function and metabolic factors in women affected by polycystic ovary syndrome after treatment with D-Chiro-Inositol. Archives of Gynecology and Obstetrics 291:1181–1186.

Lagana AS, Garzon S, Casarin J, Franchi M, Ghezzi F. 2018a. Inositol in polycystic ovary syndrome: restoring fertility through a pathophysiology-based approach. Trends in Endocrinology and Metabolism. 29: 768–780.

Lagana AS, Pizzo A. 2015. Authors' reply to: "Empiric" inositol supplementation in normal-weight non-insulin resistant women with polycystic ovarian disease: from the absence of benefit to the potential adverse effects. Archives of Gynecology and Obstetrics 291:959–960.

Lagana AS, Rossetti P, Buscema M, La Vignera S, Condorelli RA, Gullo G, et al. 2016. Metabolism and Ovarian function in PCOS women: a therapeutic approach with inositols. International Journal of Endocrinology. 2016:1–9.

Lagana AS, Rossetti P, Sapia F, Chiofalo B, Buscema M, Valenti G, et al. 2017. Evidence-based and patient-oriented inositol treatment in polycystic ovary syndrome: changing the perspective of the disease. International Journal of Endocrinology and Metabolism 15:e43695.

Lagana AS, Unfer V. 2019. D-Chiro-Inositol's action as aromatase inhibitor: rationale and potential clinical targets. European Review for Medical and Pharmacological Sciences. 23:10575–10576.

Laganà AS, Vitagliano A, Noventa M, Ambrosini G, D'Anna R. 2018b. 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. Archives of Gynecology and Obstetrics 298:675–684.

Larner J, Allan G, Kessler C, Reamer P, Gunn R, Huang LC. 1998. Phosphoinositol glycan derived mediators and insulin resistance. Prospects for diagnosis and therapy. Journal of Basic and Clinical Physiology and Pharmacology 9:127–137.

Larner J, Huang LC, Tang G, Suzuki S, Schwartz CFW, Romero G, et al. 1988. Insulin mediators: structure and formation. Cold Spring Harbor Symposia on Quantitative Biology 53: 965–971.

Lemmer HJ, Hamman JH. 2013. Paracellular drug absorption enhancement through tight junction modulation. Expert Opinion on Drug Delivery 10:103–114.

Leung KY, Mills K, Burren KA, Copp AJ, Greene ND. 2011. Quantitative analysis of myo-inositol in urine, blood and nutritional supplements by high-performance liquid chromatography tandem mass spectrometry. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences 879:2759–2763.

Lewin LM, Yannai Y, Melmed S, Weiss M. 1982. myo-inositol in the reproductive tract of the female rat. The International Journal of Biochemistry 14:147–150.

Maffucci T, Falasca M. 2020. Signalling properties of inositol polyphosphates. Molecules 25:5281.

Milewska EM, Czyzyk A, Meczekalski B, Genazzani AD. 2016. Inositol and human reproduction. From cellular metabolism to clinical use. Gynecological Endocrinology 32:690–695.

Minozzi M, Costantino D, Guaraldi C, Unfer V. 2011. The effect of a combination therapy with myo-inositol and a combined oral contraceptive pill versus a combined oral contraceptive pill alone on metabolic, endocrine, and clinical parameters in polycystic ovary syndrome. Gynecological Endocrinology 27:920–924.

Minozzi M, D'Andrea G, Unfer V. 2008. Treatment of hirsutism with myo-inositol: a prospective clinical study. Reproductive Biomedicine Online 17:579–582.

Monastra G, De Grazia S, Cilaker Micili S, Goker A, Unfer V. 2016. Immunomodulatory activities of alpha lipoic acid with a special focus on its efficacy in preventing miscarriage. Expert Opinion on Drug Delivery 13:1695–1708.

Monastra G, De Grazia S, De Luca L, Vittorio S, Unfer V. 2018a. Vitamin D: a steroid hormone with progesterone-like activity. European Review for Medical and Pharmacological Sciences 22:2502–2512.

Monastra G, Sambuy Y, Ferruzza S, Ferrari D, Ranaldi G. 2018b. Alpha-lactalbumin effect on Myo-inositol intestinal absorption: *in vivo* and *in vitro*. Current Drug Delivery 15:1305–1311.

Monastra G, Unfer V, Harrath AH, Bizzarri M. 2017. Combining treatment with myo-inositol and D-chiro-inositol (40:1) is effective in restoring ovary function and metabolic balance in PCOS patients. Gynecological Endocrinology 33:1–9.

Montanino Oliva M, Buonomo G, Calcagno M, Unfer V. 2018. Effects of myo-inositol plus alpha-lactalbumin in myo-inositol-resistant PCOS women. Journal of Ovarian Research 11:38.

Montanino Oliva M, Minutolo E, Lippa A, Iaconianni P, Vaiarelli A. 2016. Effect of myoinositol and antioxidants on sperm quality in men with metabolic syndrome. International Journal of Endocrinology 2016: 1674950.

Morris RN, Collins AC. 1971. Biosynthesis of myo-inositol by rat testis following surgically induced cryptorchidism or treatment with triethylenemelamine. Journal of Reproduction and Fertility 27:201–210.

Muscogiuri G, Palomba S, Lagana AS, Orio F. 2016a. Current insights into inositol isoforms, mediterranean and ketogenic diets for polycystic ovary syndrome: from bench to bedside. Current Pharmaceutical Design 22:5554–5557.

Muscogiuri G, Palomba S, Lagana AS, Orio F. 2016b. Inositols in the treatment of insulin-mediated diseases. International Journal of Endocrinology 2016:1–1.

Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. 1998. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. The Journal of Clinical Endocrinology and Metabolism 83:2001–2005.

Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. 1999. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. New England Journal of Medicine 340:1314–1320.

Nestler JE, Unfer V. 2015. Reflections on inositol(s) for PCOS therapy: steps toward success. Gynecological Endocrinology 31:501–505.

Nordio M, Basciani S, Camajani E. 2019. The 40:1 myo-inositol/D-chiro-inositol plasma ratio is able to restore ovulation in PCOS patients: comparison with other ratios. European Review for Medical and Pharmacological Sciences 23:5512–5521.

Pak Y, Huang LC, Lilley KJ, Larner J. 1992. *In vivo* conversion of [3H]myoinositol to [3H]chiroinositol in rat tissues. Journal of Biological Chemistry. 267:16904–16910.

Palmieri M, Papale P, Della Ragione A, Quaranta G, Russo G, Russo S. 2016. *In vitro* antioxidant treatment of semen samples in assisted reproductive technology: effects of Myo-inositol on nemaspermic parameters. International Journal of Endocrinology. 2016:1–5.

Papaleo E, Unfer V, Baillargeon JP, Chiu TT. 2009a. Contribution of myo-inositol to reproduction. European Journal of Obstetrics, Gynecology, and Reproductive Biology 147:120–123.

Papaleo E, Unfer V, Baillargeon JP, De Santis L, Fusi F, Brigante C, et al. 2007. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecological Endocrinology 23: 700–703.

Papaleo E, Unfer V, Baillargeon JP, Fusi F, Occhi F, De Santis L. 2009b. Myo-inositol may improve oocyte quality in intracytoplasmic sperm injection cycles: a prospective, controlled, randomized trial. Fertility and Sterility 91:1750–1754.

Parente E, Colannino G, Picconi O, Monastra G. 2017. Safety of oral alpha-lipoic acid treatment in pregnant women: a retrospective observational study. European Review for Medical and Pharmacological Sciences 21:4219–4227.

Paul C, Lagana AS, Maniglio P, Triolo O, Brady DM. 2016. Inositol's and other nutraceuticals' synergistic actions counteract insulin resistance in polycystic ovarian syndrome and metabolic syndrome: state-of-the-art and future perspectives. Gynecological Endocrinology 32:431–438.

Pintaudi B, Di Vieste G, Corrado F, Lucisano G, Giunta L, D'Anna R, et al. 2018. Effects of myo-inositol on glucose variability in women with gestational diabetes. European Review for Medical and Pharmacological Sciences. 22:6567–6572.

Pizzo A, Lagana AS, Barbaro L. 2014. Comparison between effects of myo-inositol and D-chiro-inositol on ovarian function and metabolic factors in women with PCOS. Gynecological Endocrinology 30: 205–208.

Porcaro G, Brillo E, Giardina I, Di Iorio R. 2015. Alpha Lipoic Acid (ALA) effects on subchorionic hematoma: preliminary clinical results. European Review for Medical and Pharmacological Sciences. 19: 3426–3432.

Raffone E, Rizzo P, Benedetto V. 2010. Insulin sensitiser agents alone and in co-treatment with r-FSH for ovulation induction in PCOS women. Gynecological Endocrinology 26:275–280.

Ravanos K, Monastra G, Pavlidou T, Goudakou M, Prapas N. 2017. Can high levels of D-chiro-inositol in follicular fluid exert detrimental effects on blastocyst quality? European Review for Medical and Pharmacological Sciences 21:5491–5498.

Rotterdam EA-SPCWG. 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81:19–25.

Rubino P, Palini S, Chigioni S, Carlomagno G, Quagliariello A, De Stefani S, 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. Journal of Assisted Reproduction and Genetics 32:387–394.

Sacchi S, Marinaro F, Tondelli D, Lui J, Xella S, Marsella T, et al. 2016. Modulation of gonadotrophin induced steroidogenic enzymes in granulosa cells by d-chiroinositol. Reproductive Biology and Endocrinology 14:52.

Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stammati A, Zucco F. 2005. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biology and Toxicology 21:1–26.

Santamaria A, Alibrandi A, Di Benedetto A, Pintaudi B, Corrado F, Facchinetti F, et al. 2018. Clinical and metabolic outcomes in pregnant women at risk for gestational diabetes mellitus supplemented with myo-inositol: a secondary analysis from 3 RCTs. Am J Obstet Gynecol 219:300 e301–300 e306.

Santamaria A, Di Benedetto A, Petrella E, Pintaudi B, Corrado F, D'Anna R, et al. 2016. Myo-inositol may prevent gestational diabetes onset in overweight women: a randomized, controlled trial. The Journal of Maternal-Fetal & Neonatal Medicine 29:3234–3237.

Seli E, Duleba AJ. 2004. Treatment of PCOS with metformin and other insulin-sensitizing agents. Current Diabetes Reports 4:69–75.

Stocco C. 2012. Tissue physiology and pathology of aromatase. Steroids 77:27–35.

Sullivan KM, Busa WB, Wilson KL. 1993. Calcium mobilization is required for nuclear vesicle fusion *in vitro*: implications for membrane traffic and IP3 receptor function. Cell 73:1411–1422.

Unfer V. 2010. Polycystic ovary syndrome: a vitamin deficiency? Floating a new pathogenesis hypothesis. European Review for Medical and Pharmacological Sciences 14:1101–1105.

Unfer V. 2018. Use of alpha-lactalbumin for the management of PCOS in inositols resistant women. European Review for Medical and Pharmacological Sciences 22:1873–1874.

Unfer V, Carlomagno G, Papaleo E, Vailati S, Candiani M, Baillargeon JP. 2014. Hyperinsulinemia alters myoinositol to d-chiroinositol ratio in the follicular fluid of patients with PCOS. Reproductive Sciences 21: 854–858.

Unfer V, Carlomagno G, Rizzo P, Raffone E, Roseff S. 2011a. Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles: a prospective, controlled, randomized trial. European Review for Medical and Pharmacological Sciences 15:452–457.

Unfer V, Facchinetti F, Orru B, Giordani B, Nestler J. 2017. Myo-inositol effects in women with PCOS: a meta-analysis of randomized controlled trials. Endocrine Connections 6:647–658.

Unfer V, Nestler JE, Kamenov ZA, Prapas N, Facchinetti F. 2016. Effects of Inositol(s) in Women with PCOS: a systematic review of randomized controlled trials. International Journal of Endocrinology 2016:1849162.

Unfer V, Porcaro G. 2014. Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome. Expert Review of Clinical Pharmacology 7:623–631.

Unfer V, Raffone E, Rizzo P, Buffo S. 2011b. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous *in vitro* fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. Gynecological Endocrinology 27:857–861.

Vitale SG, Rossetti P, Corrado F, Rapisarda AM, La Vignera S, Condorelli RA, et al. 2016. How to achieve high-quality oocytes? The key role of myo-inositol and melatonin. International Journal of Endocrinology. 2016:1–9.

Voglmayr JK, Amann RP. 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. Biology of Reproduction 8:504–513.

Walensky LD, Snyder SH. 1995. Inositol 1,4,5-trisphosphate receptors selectively localized to the acrosomes of mammalian sperm. The Journal of Cell Biology 130:857–869.