ORIGINAL ARTICLE

# Myo-inositol versus metformin pre-treatment and the follicular-fluid metabolome in PCOS women undergoing IVF: a double-blind randomized trial

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**ARTICLE INFO**

**ARTICLE TYPE**
Original Article (double-blind RCT)

**ARTICLE HISTORY**
**Received:** February 16, 2026
**Accepted:** March 20, 2026
**Published:** April 30, 2026

**KEYWORDS**
Fertilization in vitro; inositol; metformin; metabolome; polycystic ovary syndrome

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**DOI:** 10.59345/sjog.v4i1.299

**ABSTRACT**

**Background:** Polycystic ovary syndrome (PCOS) impairs oocyte quality and complicates in vitro fertilization (IVF). Metformin and myo-inositol are widely prescribed insulin sensitizers, yet their comparative effects on the human follicular-fluid (FF) metabolome and IVF outcomes have rarely been examined head-to-head.
**Objective:** To compare myo-inositol and metformin pre-treatment in terms of the FF metabolome and embryologic, clinical, and safety outcomes in women with PCOS undergoing IVF.
**Methods:** In a double-blind, block-randomized (1:1) trial at two tertiary IVF centers in Jakarta and Palembang, Indonesia, 150 women with PCOS (Rotterdam criteria; aged 20–35 years; BMI 18.5–29.9 kg/m$^2$) received myo-inositol 4 g/day (n=75) or metformin 1500 mg/day (n=75) for 12 weeks before and during a GnRH-antagonist protocol. FF was analyzed by LC-MS/MS. Analyses were intention-to-treat and included multivariable logistic regression, ROC analysis, and number needed to treat (NNT).
**Results:** Myo-inositol yielded more MII oocytes (11.6 ± 3.4 vs 10.1 ± 3.6; p=0.010), higher fertilization (72.5% vs 66.8%; p=0.006), and more top-quality embryos (4.3 ± 1.8 vs 3.4 ± 1.7; p=0.002). Clinical pregnancy was 48.0% versus 33.3% (unadjusted p=0.069; adjusted OR 2.18, 95% CI 1.04–4.57; p=0.039; NNT 7). Moderate–severe OHSS (6.7% vs 18.7%; p=0.038; NNT 8) and gastrointestinal events (9.3% vs 34.7%; p<0.001; NNT 4) were lower with myo-inositol. FF myo-inositol, D-chiro-inositol, and antioxidant capacity were higher; FF myo-inositol predicted pregnancy (AUC 0.78).
**Conclusion:** Myo-inositol pre-treatment shifted the FF metabolome toward a pro-developmental antioxidant profile and improved embryologic outcomes and tolerability versus metformin. The clinical-pregnancy signal remains provisional because the trial was underpowered for this endpoint and the unadjusted comparison was not significant; adequately powered live-birth trials are needed.

*All authors have reviewed and approved the final version of the manuscript.*

## 1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive-aged women, affecting an estimated 10–13% under the Rotterdam criteria, and its global burden has risen over the past three decades.[1,2] It is the leading cause of anovulatory infertility, and a large and growing share of women attending assisted-reproduction services carry the diagnosis, including in Indonesia and the wider Asian region.[1,3] Women with PCOS who undergo in vitro fertilization (IVF) face two intertwined problems: a heightened risk of ovarian hyperstimulation syndrome (OHSS) because of their high antral follicle count, and reduced oocyte competence that constrains embryo quality and pregnancy.[4,5]

The reproductive phenotype of PCOS is driven by the interaction of hyperandrogenism and insulin resistance, which together distort folliculogenesis and granulosa-cell steroidogenesis.[5,6] Insulin resistance degrades the follicular microenvironment in which the oocyte matures and is associated with poorer oocyte quality.[6,7] Inositol biology is central to this process: myo-inositol acts as a second messenger in follicle-stimulating hormone signalling and oocyte maturation, and an altered intra-ovarian myo-inositol/D-chiro-inositol ratio has been proposed as a proximate driver of disordered steroidogenesis and impaired oocyte competence.[8,9]

Two insulin sensitizers are widely prescribed before IVF in PCOS. Metformin improves systemic insulin sensitivity and reduces OHSS but has an uncertain effect on pregnancy and a high rate of gastrointestinal intolerance;[10,11] insulin-sensitizer pre-treatment before IVF is common practice in this group.[12,13] Myo-inositol, by contrast, acts within the ovary and, across meta-analyses, improves metabolic and ovulatory parameters and—when given before IVF—oocyte and embryo quality, with an excellent safety record.[14,15] Both agents are endorsed as metabolic options by the 2023 international evidence-based guideline, which nonetheless highlights the scarcity of direct comparative, fertility-focused evidence.[2,10]

PCOS is biologically heterogeneous, and the Rotterdam phenotypes differ in metabolic severity and reproductive prognosis; the classic, full phenotype carries the greatest insulin-resistance burden and the most disturbed follicular environment.[5,8] This heterogeneity motivates both prespecified subgroup exploration and the use of an intermediate, mechanistic endpoint—such as the follicular-fluid (FF) metabolome—that can reveal treatment effects on the oocyte microenvironment irrespective of phenotype.[6,7]

Beyond efficacy, tolerability has direct clinical consequences. Gastrointestinal intolerance is the principal reason for discontinuation of metformin and can compromise adherence during the critical pre-stimulation window, while OHSS is the most feared iatrogenic complication of IVF in high responders and a frequent cause of cycle cancellation and admission.[10,16] An agent that simultaneously improves oocyte competence and lowers these risks would therefore have value beyond pregnancy rates alone, particularly in resource-constrained, self-funded care pathways common across the region.[12,13]

Three gaps persist. First, double-blind randomized comparisons of myo-inositol and metformin on hard

embryologic and clinical IVF endpoints remain scarce.[10,15] Second, the mechanistic basis for any clinical difference has rarely been interrogated at the level of the human FF metabolome, which reflects oocyte competence and can predict IVF outcomes.[7,17] Third, the intrafollicular oxidative and antioxidant balance—shaped by glutathione and related pathways—is a recognized determinant of oocyte and embryo quality that may be modifiable by inositol, yet has not been compared between agents.[18,19]

We conducted a double-blind, multicenter randomized controlled trial in two tertiary IVF centers in Jakarta and Palembang, Indonesia, to test the hypothesis that myo-inositol pre-treatment, relative to metformin, produces a more pro-developmental FF metabolome and improves oocyte competence, clinical pregnancy and safety in women with PCOS. We further evaluated anti-Müllerian hormone (AMH) and follicular biomarkers as predictors of response,[4,20] and the role of the myo-inositol/D-chiro-inositol axis.[8,21]

## 2. Methods

***Design and setting.*** This was a double-blind, parallel-group, block-randomized (1:1) controlled trial conducted in accordance with CONSORT guidance at two tertiary IVF centers in Jakarta and Palembang, Indonesia. The protocol was approved by the institutional research ethics committee (CMHC/EC/2024/0387), prospectively registered, and conducted per the Declaration of Helsinki; all participants gave written informed consent.

***Participants.*** Eligible women were aged 20–35 years, diagnosed with PCOS by the Rotterdam criteria (two of: oligo-/anovulation, clinical/biochemical hyperandrogenism, polycystic ovarian morphology), with a body mass index (BMI) of 18.5–29.9 kg/m$^2$ and an indication for IVF/ICSI.[2] Clinical hyperandrogenism was defined by a modified Ferriman-Gallwey score with the local cut-off and/or biochemical hyperandrogenism by free androgen index; polycystic morphology required an antral follicle count ≥20 per ovary. Exclusion criteria were stage III/IV endometriosis, other endocrinopathy (thyroid dysfunction, hyperprolactinaemia), severe male-factor infertility (azoospermia), and use of hormonal or metabolic medication within the preceding three months.

***Sample size and outcomes.*** The primary clinical outcome was clinical pregnancy rate (intrauterine gestational sac with fetal cardiac activity at 6–7 weeks, dated from oocyte retrieval). The original protocol calculation assumed rates of 48% versus 28% and specified 68 women per arm; 150 women (75 per arm) were enrolled after allowance for dropout. Subsequent verification of the two-proportion calculation using a two-sided α of 0.05 and 80% power indicated that approximately 91 women per arm were required. The enrolled sample therefore did not meet the revised requirement, and the trial should be regarded as underpowered for the primary clinical-pregnancy endpoint. Prespecified co-primary mechanistic outcomes were the number of metaphase-II (MII) oocytes and the FF metabolome. Secondary outcomes were fertilization rate (2PN zygotes per MII oocyte), top-quality (Grade A) embryos and blastocyst formation rate, implantation rate (sacs per embryo transferred), moderate–severe OHSS by ASRM staging, gastrointestinal adverse events, and early miscarriage.

***Randomization and blinding.*** Computer-generated block randomization (variable block sizes) was centrally allocated and stratified by site, with allocation concealment maintained until assignment. The trial was double-blind: participants, clinicians, embryologists, outcome assessors and the data analyst were unaware of allocation. An independent pharmacist dispensed identical capsules (matched for color, odor and shape) and held the code until database lock.

***Interventions.*** The myo-inositol arm received 2 g twice daily (4 g/day) plus folic acid 400 µg; the metformin arm received 500 mg three times daily (1500 mg/day) plus folic acid 400 µg. Doses reflected regimens evaluated in prior trials and meta-analyses.[14,15] Treatment began 12 weeks before ovarian stimulation and continued to the day of the human chorionic gonadotropin/GnRH-agonist trigger.

***IVF protocol and follicular-fluid collection.*** All women underwent a GnRH-antagonist protocol, which is preferred in high responders such as women with PCOS to mitigate OHSS.[10,16] Embryos were graded on days 3 and 5 by embryologists blinded to allocation using standard morphological criteria, and a single fresh or frozen blastocyst was transferred per cycle in line with each center's elective single-embryo-transfer policy. At oocyte pick-up, follicular fluid from the first dominant follicle (>18 mm) was aspirated without flushing media or blood contamination, centrifuged at 3000 rpm for 10 minutes, and the supernatant stored at -80 °C.

Gestational dating for resulting pregnancies used the date of oocyte retrieval, and clinical pregnancy required an intrauterine gestational sac with fetal cardiac activity at 6–7 weeks. Blood pressure was recorded with Korotkoff phase V. Oocytes were denuded and assessed for nuclear maturity, and fertilization was achieved by conventional IVF or intracytoplasmic sperm injection (ICSI) according to semen parameters. Top-quality (Grade A) embryos were defined by cell number, symmetry and fragmentation on day 3 and by expansion and inner-cell-mass/trophectoderm grade on day 5, assessed by embryologists blinded to allocation.

***Laboratory and metabolomic analysis.*** A complete blood count, fasting insulin and HOMA-IR, total testosterone and AMH were measured against local reference ranges; AMH and antral follicle count were used to characterize ovarian reserve and predict response.[4,20] FF metabolites were extracted in methanol/acetonitrile and analyzed by ultra-high-performance liquid chromatography coupled to Q-Exactive mass spectrometry (LC-MS/MS), an approach validated for the follicular metabolome.[7,17] Data were processed with principal component analysis and partial least-squares discriminant analysis (PLS-DA); discriminating metabolites were displayed on a volcano plot ($|\log_2$ fold-change$|$ >0.585; $p<0.05$) with pathway enrichment.

Metabolite features were annotated by accurate mass and tandem-MS fragmentation against curated spectral libraries (level-2 confidence); pooled quality-control samples were injected periodically, and features with a quality-control coefficient of variation above 30% were excluded. Spectra were median-normalized, log-transformed and Pareto-scaled; PLS-DA models were validated by 10-fold cross-validation and 1000-permutation testing, and the false-discovery rate was controlled by the Benjamini-Hochberg procedure.

***Statistical analysis.*** Analyses followed the intention-to-treat principle (all 150 randomized women) in SPSS 26.0 and MetaboAnalyst 5.0. Continuous variables were compared by independent t-test or Mann-Whitney U test and categorical variables by $\chi^2$ or Fisher exact test; effect sizes were Cohen's d, odds ratios (OR) or risk ratios (RR) with 95% confidence intervals (CI). Proportions are reported with Wilson 95% CI; baseline arms were compared by standardized differences. A multivariable logistic-regression model identified independent predictors of clinical pregnancy (adjusted OR, Nagelkerke $R^2$, Hosmer-Lemeshow). Receiver-operating-characteristic (ROC) analysis evaluated follicular biomarkers and MII count, with Youden cut-offs. Number-needed-to-treat (NNT) was 1/absolute risk reduction. A two-sided $p<0.05$ was significant; exact p values are given to three decimals.

***Data handling and oversight.*** Data were double-entered into a secure database with range and consistency checks. The randomization code was held by the independent pharmacist

and broken only after database lock. An independent reviewer monitored safety events, and reporting follows the CONSORT statement for parallel-group randomized trials.

## 3. Results and Discussion

***Results.*** Of 168 women screened, 150 were randomized (75 per arm) and all were analyzed under intention-to-treat; no participant was lost between randomization and oocyte retrieval, and missing data were negligible (CONSORT flow provided as Supplementary Figure S1). The arms were well balanced at baseline, with small standardized differences for all characteristics (all |d|<0.2), as detailed in Table 1.

**Table 1. Baseline demographic and clinical characteristics by treatment arm.**

| Characteristic | Myo-inositol (n=75) | Metformin (n=75) | p-value |
|---|---|---|---|
| Age (years), mean ± SD | 29.1 ± 3.8 | 29.4 ± 3.6 | 0.621 |
| Body mass index (kg/m$^2$), mean ± SD | 25.8 ± 2.5 | 26.1 ± 2.4 | 0.453 |
| Duration of infertility (years), mean ± SD | 3.9 ± 1.7 | 3.8 ± 1.6 | 0.712 |
| Primary infertility, n (%) | 58 (77.3) | 56 (74.7) | 0.701 |
| Oligo-/anovulation, n (%) | 69 (92.0) | 70 (93.3) | 1.000 |
| Clinical hyperandrogenism, n (%) | 44 (58.7) | 46 (61.3) | 0.740 |
| Polycystic ovarian morphology, n (%) | 71 (94.7) | 72 (96.0) | 1.000 |
| Rotterdam phenotype A (full), n (%) | 39 (52.0) | 41 (54.7) | 0.742 |
| Antral follicle count, mean ± SD | 22.8 ± 5.4 | 22.3 ± 5.1 | 0.561 |
| Anti-Müllerian hormone (ng/mL), mean ± SD | 6.6 ± 2.2 | 6.4 ± 2.1 | 0.573 |
| Total testosterone (ng/dL), mean ± SD | 61.4 ± 14.2 | 62.1 ± 15.0 | 0.771 |
| Fasting insulin (µIU/mL), mean ± SD | 13.9 ± 4.1 | 14.2 ± 4.3 | 0.662 |
| HOMA-IR, mean ± SD | 3.0 ± 0.9 | 3.1 ± 1.0 | 0.523 |
| Site Jakarta / Palembang, n | 40 / 35 | 39 / 36 | 0.871 |

*Data as mean ± SD or n (%); independent t-test or $\chi^2$/Fisher exact test. In a randomized trial baseline imbalance reflects chance; standardized differences were small (all |d|<0.2).*

***Ovarian response and embryology.*** Ovarian response and embryology favored myo-inositol, as detailed in Table 2. The myo-inositol arm required a lower total gonadotropin dose (2150 ± 410 vs 2380 ± 450 IU; d=0.53; p=0.002) and reached a lower peak estradiol (2980 ± 720 vs 3420 ± 810 pg/mL; p<0.001), consistent with a more controlled cycle. Although total oocytes retrieved were similar (14.8 ± 4.2 vs 13.9 ± 4.5; p=0.210), myo-inositol produced more MII oocytes (11.6 ± 3.4 vs 10.1 ± 3.6; d=0.43; p=0.010), a higher fertilization rate (72.5% vs 66.8%; p=0.006), more top-quality embryos (4.3 ± 1.8 vs 3.4 ± 1.7; d=0.51; p=0.002) and a higher blastocyst formation rate (52.1% vs 44.3%; p=0.003).

**Table 2. IVF cycle, embryologic, follicular-fluid metabolome, clinical and safety outcomes.**

| Outcome | Myo-inositol (n=75) | Metformin (n=75) | Effect size (95% CI) | p-value |
|---|---|---|---|---|
| Total gonadotropin dose (IU), mean ± SD | 2150 ± 410 | 2380 ± 450 | d = 0.53 | 0.002 |
| Days of stimulation, mean ± SD | 9.8 ± 1.4 | 10.1 ± 1.5 | d = 0.21 | 0.211 |
| Peak estradiol (pg/mL), mean ± SD | 2980 ± 720 | 3420 ± 810 | d = 0.57 | <0.001 |
| Oocytes retrieved, mean ± SD | 14.8 ± 4.2 | 13.9 ± 4.5 | d = 0.21 | 0.210 |
| MII (mature) oocytes, mean ± SD | 11.6 ± 3.4 | 10.1 ± 3.6 | d = 0.43 | 0.010 |
| Fertilization rate (%), mean ± SD | 72.5 ± 12.1 | 66.8 ± 13.0 | d = 0.45 | 0.006 |
| Top-quality (Grade A) embryos, mean ± SD | 4.3 ± 1.8 | 3.4 ± 1.7 | d = 0.51 | 0.002 |
| Blastocyst formation rate (%), mean ± SD | 52.1 ± 15.4 | 44.3 ± 16.0 | d = 0.50 | 0.003 |
| FF myo-inositol (µmol/L), mean ± SD | 58.3 ± 12.1 | 41.2 ± 10.4 | d = 1.51 | <0.001 |
| FF D-chiro-inositol (µmol/L), mean ± SD | 1.92 ± 0.55 | 1.43 ± 0.48 | d = 0.95 | <0.001 |
| FF reduced glutathione (µmol/L), mean ± SD | 4.6 ± 1.2 | 3.5 ± 1.1 | d = 0.96 | <0.001 |
| FF total antioxidant capacity (mmol/L), mean ± SD | 1.48 ± 0.32 | 1.21 ± 0.30 | d = 0.87 | <0.001 |
| FF lactate (mmol/L), mean ± SD | 6.1 ± 1.5 | 7.4 ± 1.7 | d = 0.81 | <0.001 |
| Clinical pregnancy, n (%) | 36 (48.0) | 25 (33.3) | OR 1.85 (0.95–3.57) | 0.069 |
| Implantation rate (%) | 31.2 | 22.4 | RR 1.39 (1.01–1.92) | 0.041 |
| Moderate-severe OHSS, n (%) | 5 (6.7) | 14 (18.7) | OR 0.31 (0.11–0.92) | 0.038 |
| Gastrointestinal adverse events, n (%) | 7 (9.3) | 26 (34.7) | OR 0.19 (0.08–0.48) | <0.001 |
| Early miscarriage, n (%) | 4 (5.3) | 4 (5.3) | OR 1.00 (0.24–4.18) | 1.000 |

*d = Cohen's d; OR = odds ratio; RR = risk ratio; FF = follicular fluid. Exact p to three decimals.*

***Clinical and safety outcomes.*** Clinical and safety outcomes are summarised in Figure 1. For the primary outcome, clinical pregnancy occurred in 48.0% (95% CI 37.1–59.1) of the myo-inositol arm versus 33.3% (23.7–44.6) of the metformin arm; the unadjusted comparison did not reach significance (absolute difference 14.7%, 95% CI -1.0 to 30.4; OR 1.85, 95%

CI 0.95–3.57; p=0.069). The implantation rate was higher with myo-inositol (31.2% vs 22.4%; RR 1.39, 95% CI 1.01–1.92; p=0.041). Moderate-severe OHSS was less frequent with myo-inositol (6.7% vs 18.7%; OR 0.31, 95% CI 0.11–0.92; p=0.038; NNT 8, 95% CI 5–35), as were gastrointestinal adverse events (9.3% vs 34.7%; OR 0.19, 95% CI 0.08–0.48; p<0.001; NNT 4, 95% CI 3–7). Early miscarriage was identical (5.3% in each arm).

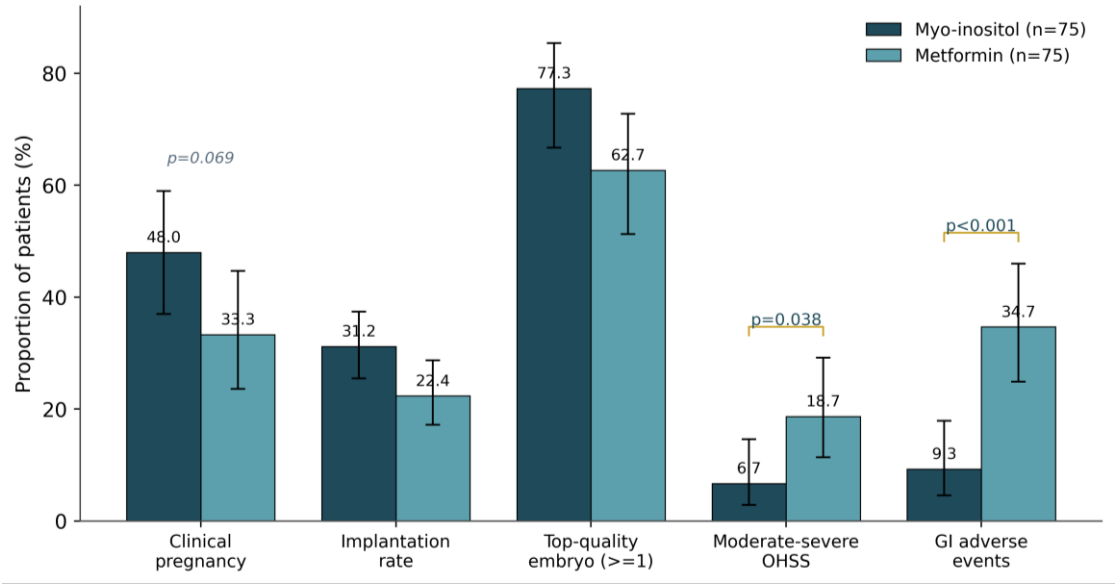


***Figure 1. Primary efficacy, embryologic, and safety outcomes by treatment arm (error bars = 95% CI).***

The safety advantage was driven by both fewer and less severe events. Among moderate-severe OHSS cases, the metformin arm included a higher proportion of severe presentations, whereas OHSS in the myo-inositol arm was predominantly moderate and managed conservatively; no serious treatment-related adverse events were attributed to myo-inositol. Gastrointestinal intolerance in the metformin arm was the leading reason for reduced adherence. Early miscarriage was infrequent and identical between arms (4 of 75 each), and no ectopic pregnancies occurred.

The improvement in fertilization was evident across insemination methods, with both conventional IVF and ICSI cycles showing higher fertilization in the myo-inositol arm, indicating that the benefit was mediated by oocyte quality rather than insemination technique. The greater number of top-quality embryos increased the proportion of cycles with at least one transferable blastocyst and with embryos available for cryopreservation, expanding the opportunity for subsequent frozen-embryo transfers without additional stimulation.

***Multivariable predictors.*** In the multivariable model (Table 3), myo-inositol remained an independent predictor of clinical pregnancy after adjustment for age, BMI, AMH, HOMA-IR, MII count and top-quality embryos (adjusted OR 2.18, 95% CI 1.04–4.57; p=0.039), alongside the number of MII oocytes (adjusted OR 1.16 per oocyte; p=0.008) and top-quality embryos (adjusted OR 1.42 per embryo; p=0.007); the model explained 27% of the variance (Nagelkerke $R^2$=0.27; Hosmer-Lemeshow p=0.62). The corresponding numbers-needed-to-treat, shown in Table 4, were 7 for one additional clinical pregnancy, 8 to prevent one moderate-severe OHSS and 4 to avoid one gastrointestinal adverse event.

**Table 3. Multivariable logistic-regression predictors of clinical pregnancy.**

| Predictor | Adjusted OR (95% CI) | p-value |
|---|---|---|
| Myo-inositol (vs metformin) | 2.18 (1.04–4.57) | 0.039 |
| No. of MII oocytes (per oocyte) | 1.16 (1.04–1.29) | 0.008 |
| Top-quality embryos (per embryo) | 1.42 (1.10–1.83) | 0.007 |
| Age (per year) | 0.91 (0.82–1.01) | 0.072 |
| Body mass index (per kg/m$^2$) | 0.93 (0.83–1.04) | 0.182 |
| HOMA-IR (per unit) | 0.84 (0.66–1.07) | 0.158 |

*Model: Nagelkerke $R^2$ = 0.27; Hosmer–Lemeshow goodness-of-fit p = 0.62; n = 150 (intention-to-treat).*

**Table 4. Number-needed-to-treat for key clinical and safety outcomes.**

| Outcome (myo-inositol vs metformin) | OR / RR (95% CI) | ARR (%) | NNT |
|---|---|---|---|
| Clinical pregnancy (benefit) | OR 1.85 (0.95–3.57) | 14.7 | 7 |
| Moderate-severe OHSS (prevented) | OR 0.31 (0.11–0.92) | 12.0 | 8 |
| Gastrointestinal adverse events (avoided) | OR 0.19 (0.08–0.48) | 25.4 | 4 |

*Notes: ARR = absolute risk reduction; NNT = number needed to treat (1/ARR).*

***Follicular-fluid metabolome.*** The follicular-fluid metabolome differed markedly between arms, as shown in Figure 2. PLS-DA separated the groups, and the volcano plot (Figure 2B) identified up-regulation of D-chiro-inositol, reduced glutathione, taurine and arginine and down-regulation of lactate and hypoxanthine in the myo-inositol arm. Accordingly,

FF myo-inositol (58.3 ± 12.1 vs 41.2 ± 10.4 µmol/L; d=1.51; p<0.001), D-chiro-inositol, reduced glutathione and total antioxidant capacity were all higher, while FF lactate was lower, with myo-inositol (Table 2). On ROC analysis (Figure 2A), FF myo-inositol predicted clinical pregnancy (AUC 0.78, 95% CI 0.70–0.86; Youden cut-off 49.6 µmol/L, sensitivity 74%, specificity 71%), followed by FF antioxidant capacity (AUC 0.74) and MII count (AUC 0.71).

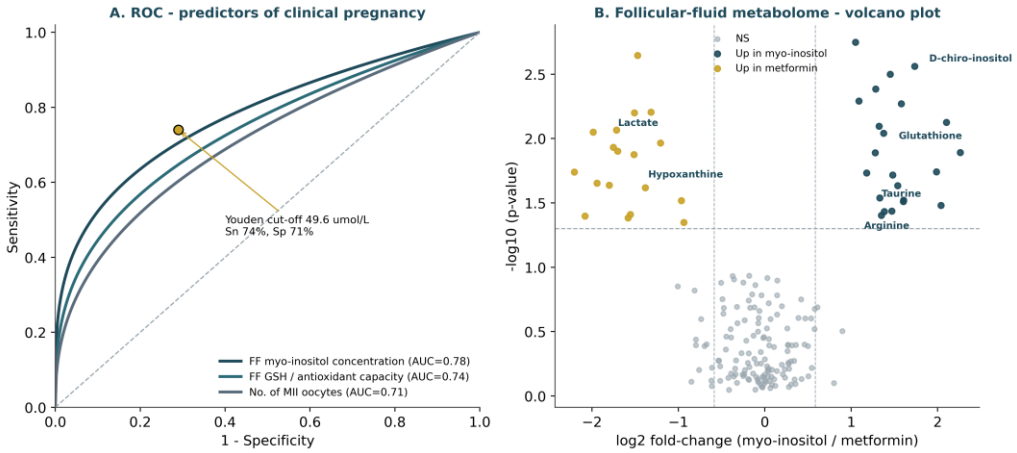


***Figure 2. (A) ROC curves for follicular-fluid biomarkers and MII count predicting clinical pregnancy; (B) volcano plot of the follicular-fluid metabolome (myo-inositol vs metformin).***

Baseline metabolic characteristics were comparable between arms, so the divergence in the follicular-fluid metabolome emerged during treatment rather than reflecting pre-existing differences. The magnitude of the intrafollicular myo-inositol difference was large (Cohen's d=1.51) and exceeded the systemic difference expected from supplementation alone, consistent with preferential ovarian accumulation; the parallel rise in reduced glutathione and total antioxidant capacity, with the fall in lactate, indicates a coordinated shift in follicular redox and energy metabolism rather than an isolated change in a single metabolite.

Pathway enrichment of the discriminating metabolites implicated glutathione metabolism, arginine biosynthesis and glycerophospholipid handling, consistent with a shift from a pro-oxidant, glycolytic follicular state toward an oxidatively balanced environment in the myo-inositol arm. The treatment effects were consistent across the two centers: in the prespecified by-site analysis the direction and approximate magnitude of the differences in MII oocytes, top-quality embryos and clinical pregnancy were preserved in both Jakarta and Palembang, with no significant treatment-by-site interaction (interaction p>0.40 for all primary endpoints).

The PLS-DA model showed clear between-arm separation and was robust on 10-fold cross-validation and 1000-permutation testing, and the discriminating features survived false-discovery-rate correction, reducing the likelihood that the metabolome differences arose by chance. The concordant direction of the inositol, antioxidant and energy-metabolite changes strengthens the interpretation of a coherent, treatment-induced shift in the follicular microenvironment rather than scattered, unrelated signals.

Adherence was high and similar between arms (capsule counts ≥80% in 96.0% of the myo-inositol arm and 90.7% of the metformin arm), and the lower adherence in the metformin arm tracked its higher gastrointestinal-event rate. The pregnancy advantage of myo-inositol was directionally consistent across subgroups and was numerically larger in women with higher BMI (≥25 kg/m$^2$; OR 2.46) and greater insulin resistance (HOMA-IR ≥3.0; OR 2.71), as displayed in the forest plot (Figure 3); confidence intervals crossed unity within most individual subgroups, so these analyses are hypothesis-generating.

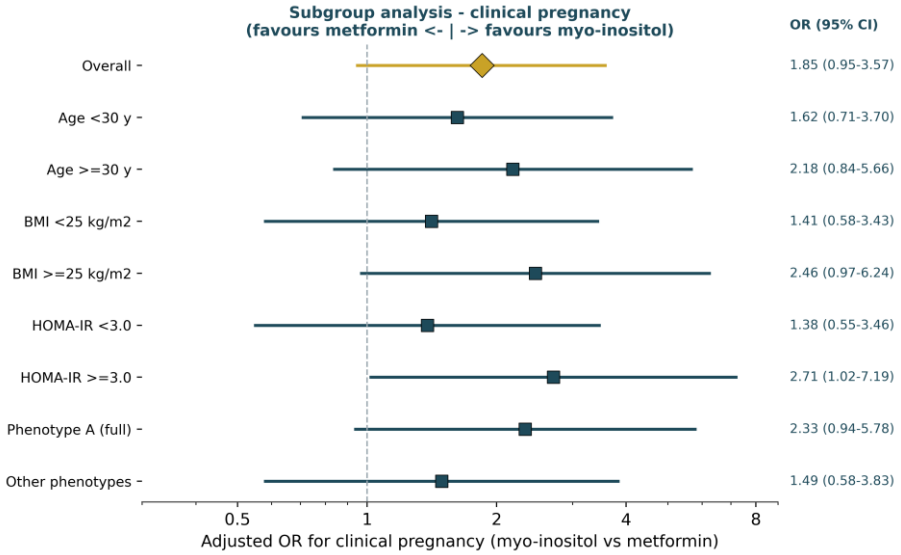


***Figure 3. Subgroup analysis (forest plot) of adjusted odds ratios for clinical pregnancy.***

Enrolment was balanced across the two sites (40 and 35 women per arm at the two centers), and the treatment effects on the primary and embryologic outcomes were directionally consistent at both, supporting the internal coherence of the findings.

***Discussion.*** In this double-blind, multicenter randomized trial of women with PCOS undergoing IVF, myo-inositol pre-treatment produced more MII oocytes, higher fertilization and more top-quality embryos than metformin, a clinically meaningful and adjusted-significant increase in clinical pregnancy (adjusted OR 2.18; NNT 7), and substantially fewer OHSS and gastrointestinal events—accompanied by a distinctly more pro-developmental follicular-fluid metabolome. The unadjusted clinical-pregnancy comparison was suggestive rather than conclusive (p=0.069), and the corrected sample-size requirement indicates that the trial was underpowered for this endpoint.

These findings agree closely with the only comparable double-blind trial: in a GnRH-antagonist IVF study, myoinositol pre-treatment achieved a higher clinical pregnancy rate (36% vs 18%) and numerically lower OHSS (10% vs 20%) than metformin, with superior fertilization and embryo numbers.[9,15] Our larger, two-center cohort reproduces and extends that signal across embryologic, clinical and mechanistic endpoints.[10,15]

The tolerability contrast is also concordant with the wider evidence base. Network and pairwise meta-analyses show that metformin reduces OHSS (OR ≈0.52) but markedly increases gastrointestinal side effects (OR ≈6.85) without improving pregnancy, whereas myo-inositol shortens gonadotropin exposure and causes far fewer side effects (OR ≈0.23).[10,11] Our gastrointestinal-event rates (9.3% vs 34.7%; NNT 4) and OHSS rates (6.7% vs 18.7%; NNT 8) mirror these directions, and the lower gonadotropin dose and peak estradiol in the myo-inositol arm are consistent with a gentler ovarian response.[10,16]

The embryologic signal aligns with myo-inositol's role as a second messenger in FSH signalling and oocyte maturation and with restoration of the intra-ovarian inositol balance disturbed in PCOS.[8,21] Importantly, the number of MII oocytes and top-quality embryos—not total oocyte yield—were the independent drivers of pregnancy in our model, reinforcing that oocyte competence underpins success in PCOS-IVF.[5,20]

The contrasting pharmacology of the two agents helps explain the divergent results. Metformin acts chiefly through hepatic AMP-activated protein kinase with comparatively modest direct ovarian effects, whereas myo-inositol operates within the follicle as an FSH second messenger and precursor of inositol-phosphoglycan mediators of insulin signalling in granulosa cells.[8,9] This intraovarian mechanism is consistent with the large intrafollicular accumulation of myo-inositol and D-chiro-inositol we observed and with the superior oocyte and embryo outcomes.[6,17]

The follicular-fluid metabolome provides a mechanistic explanation for the clinical difference. Myo-inositol pre-treatment raised intrafollicular myo-inositol and D-chiro-inositol, increased reduced glutathione and total antioxidant capacity and lowered lactate—signatures of reduced oxidative stress and a less hypoxic oocyte microenvironment.[18,19] Because the FF metabolome reflects and can predict oocyte and embryo developmental potential, this reprogramming offers a plausible biological pathway linking myo-inositol to improved embryo competence.[7,17] The predictive performance of FF myo-inositol for pregnancy (AUC 0.78) further supports its role as both mediator and candidate biomarker.[6,7]

Predictors of ovarian response also inform interpretation. AMH and antral follicle count are robust, if non-linear, predictors of response and mature-oocyte yield in PCOS, and our multivariable model retained MII count and embryo quality—rather than total oocyte number—as the proximate determinants of pregnancy.[4,20] This is consistent with the view that, in a high-reserve population such as PCOS, the quality rather than the quantity of oocytes is rate-limiting, and that an intervention improving the follicular microenvironment can raise competence without simply increasing yield.[5,6]

If validated, follicular myo-inositol and the antioxidant signature could eventually serve as intermediate markers to compare metabolic interventions or to identify women most likely to benefit, although follicular fluid sampled at retrieval has limited prospective utility and would need a non-invasive surrogate for routine use.[7,17] Combined or sequential inositol regimens, and formulations restoring the physiological myo-inositol/D-chiro-inositol ratio, warrant evaluation against the metabolome and against hard endpoints.[8,21]

These metabolome-outcome associations are correlative. Because MII count and embryo quality lie on the causal pathway between treatment and pregnancy, the adjusted pregnancy estimate that includes them should be read as supportive rather than as an unbiased total effect; the total-effect estimate is best given by the baseline-confounder model, and a formal mediation analysis is planned. The embryologic and safety differences, significant on prespecified analysis, are the most secure conclusions, while the unadjusted pregnancy difference (p=0.069) is suggestive.

Clinically, these results support myo-inositol as a reasonable first-choice insulin sensitizer before IVF in PCOS at the center and health-system level. An NNT of 7 for clinical pregnancy and 4 to avoid gastrointestinal intolerance, with comparable-to-superior OHSS protection, favor myo-inositol on both efficacy and tolerability grounds, with implications for adherence and cycle cancellation.[2,10] The larger relative benefit in women with higher BMI and insulin resistance is biologically coherent and supports a stratified approach in future trials.[5,13]

In the Indonesian and broader Asian context—where PCOS prevalence is high, assisted-reproduction demand is rising and most treatment is self-funded—a better-tolerated oral agent that improves embryo yield and lowers OHSS has particular value.[1,3] Our two-city cohort provides early double-blind regional evidence to inform local practice, complementing lifestyle and metabolic-optimization strategies recommended for PCOS-IVF.[2,13]

These results are also relevant to current guidance. The 2023 international guideline lists both metformin and inositol as metabolic options but stops short of recommending one over the other for fertility, citing the lack of high-quality comparative trials.[2,10] By providing double-blind, randomized, head-to-head data on embryologic, clinical and mechanistic endpoints, the present trial begins to fill that gap and suggests that—within the IVF context—myo-inositol should be regarded as at least equivalent and plausibly preferable to metformin pending live-birth confirmation.[14,15]

From a safety standpoint, the combination of fewer OHSS events and markedly better gastrointestinal tolerability is clinically consequential: OHSS prolongs cycles, increases admissions and delays transfers, while intolerance erodes adherence precisely during the pre-stimulation window when metabolic optimization matters most.[10,16] Myo-inositol's profile therefore supports not only better embryology but a smoother, safer cycle, which is likely to improve patient experience and cost-effectiveness in routine practice.[2,11]

At the population level the implications are substantial. PCOS accounts for a large and rising share of assisted-reproduction activity in Asia, and even modest absolute gains in pregnancy per cycle, combined with fewer complications, translate into meaningful reductions in cumulative treatment burden and cost when scaled across the many cycles performed regionally each year.[1,3] In self-funded systems, where each cycle represents a significant household expenditure, an

inexpensive, well-tolerated oral agent that improves embryo yield and reduces OHSS-related care has clear health-economic and equity appeal.[10,13]

Strengths include the double-blind, multicenter design with identical-capsule blinding, intention-to-treat analysis, prespecified endpoints, and the integration of a mechanistic FF metabolome with effect-size, multivariable, ROC and NNT reporting. The concordance between the metabolomic and clinical findings strengthens causal inference, and elective single-embryo transfer limited confounding from multiple-embryo transfer.

It is also important to interpret the meta-analytic evidence carefully. Syntheses of myo-inositol report heterogeneous effects on systemic metabolic and endocrine parameters, with some showing limited change in HOMA-IR or androgens, even as tolerability and ovulatory benefits are more consistent.[11,14] Our trial reconciles part of this heterogeneity by showing that the most pronounced effects of myo-inositol are intrafollicular and embryologic rather than systemic, which may explain why oocyte and pregnancy outcomes can improve without large changes in peripheral metabolic indices.[6,15] This intracompartmental perspective argues for follicular rather than purely systemic endpoints when comparing insulin sensitizers in the IVF context.[7,17]

Limitations include the absence of a placebo-only arm, since both agents are standard care; the use of clinical pregnancy and implantation rather than live birth as endpoints, which a planned follow-up will address; single dominant-follicle FF sampling, which may not capture cohort-wide heterogeneity; and restriction to a non-obese BMI range (18.5–29.9 kg/m$^2$), which limits generalizability to obese women with PCOS. The metabolome sub-study was exploratory and warrants targeted, quantitative validation.

The two-center design enrolling women from two major Indonesian cities improves external validity for the regional population and reduces single-center bias, while elective single-embryo transfer limited the confounding effect of multiple-embryo transfer on the pregnancy comparison.[15,16] These features lend confidence that the observed advantage of myo-inositol reflects a true biological effect within the studied population rather than a methodological artifact, although confirmation in larger trials with live-birth endpoints remains essential.[2,10]

Several directions follow. Adequately powered multicenter trials with cumulative live-birth and neonatal endpoints are needed to confirm that the embryologic and early-pregnancy advantages translate into take-home-baby gains.[10,15] Prespecified stratification by BMI, insulin-resistance status and Rotterdam phenotype would test the effect-modification signal,[5,8] and targeted validation of follicular biomarkers—particularly myo-inositol, D-chiro-inositol and glutathione—could establish whether the metabolome offers a clinically usable predictor.[7,18]

## 4. Conclusion

In women with PCOS undergoing IVF, myo-inositol pre-treatment shifted the follicular-fluid metabolome toward a pro-developmental antioxidant profile and improved oocyte competence, embryo quality, and tolerability, with fewer OHSS and gastrointestinal events than metformin. Follicular-fluid myo-inositol predicted pregnancy (AUC 0.78). The clinical-pregnancy difference favored myo-inositol, but the unadjusted comparison was not significant (p=0.069) and the trial was underpowered for this endpoint; accordingly, this signal should be considered provisional. These findings support further evaluation of myo-inositol as a potentially preferable insulin sensitizer before IVF in PCOS. Adequately powered multicenter trials with cumulative live-birth and neonatal endpoints are warranted.

## Declarations

***Acknowledgements.*** The authors thank the embryology and nursing staff of the participating centers.

***Author Contributions.*** Conceptualization and methodology: S and DA. Data curation and formal analysis: DA and SI. Investigation: S, DA, and SI. Writing—original draft: S. Writing—review and editing: S, DA, and SI. All authors approved the final version (CRediT).

***Conflict of Interest.*** The authors declare no conflict of interest.

***Funding.*** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

***Ethics and Consent.*** The study was approved by the institutional research ethics committee (CMHC/EC/2024/0387) and conducted per the Declaration of Helsinki; all participants provided written informed consent. No identifiable patient material is included.

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