MYPP-trial

Myo-inositol supplementation to Prevent Pregnancy Complications in Women with Polycystic Ovary Syndrome: a double-blind randomised controlled trial

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MYPP–trial: Myo-inositol Supplementation to Prevent Pregnancy Complications in Women with Polycystic Ovary Syndrome: a multicentre double-blind randomised controlled trial

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	*ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie (ABR-formulier))*
AE	*Adverse Event*
AR	*Adverse Reaction*
BMI	*Body Mass Index (kg/m^2)*
CA	*Competent Authority*
CCMO	*Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek*
CI	*Confidence Interval*
CRF	*Case Report Form*
CV	*Curriculum Vitae*
DBP	*Diastolic Blood Pressure*
DSMB	*Data Safety Monitoring Board*
DSOG	*Dutch Society of Obstetrics and Gynaecology*
eGFR	*estimated Glomerular Filtration Rate*
EC	*European Commission*
ECHA	*European Chemicals Agency*
EU	*European Union*
EQ-5D-5L	*5-levels EuroQol - Five Dimensions (measurement instrument for quality of life)(1)*
FDA	*Food and Drug Administration*
FGR	*Foetal Growth Restriction. As defined in national guidelines: estimated foetal weight <10th percentile and/or abdominal circumference <10th percentile and/or decline of 20 percentile in estimated foetal weight or abdominal circumference.(2)*
GA	*Gestational Age*
GCP	*Good Clinical Practice*
GDM	*Gestational Diabetes Mellitus. As defined in national guidelines: any degree of glucose intolerance with onset or first recognition during pregnancy.(3-5)*
GDPR	*General Data Protection Regulation; in Dutch: Algemene Vordering Gegevensbescherming (AVG)*
GMP	*Good Manufacturing Practice*
HELLP	*Haemolysis Elevated Liver enzymes and Low Platelet count(HELLP): generally diagnosed when at least two of the following criteria compiled by the International Society for the Study of Hypertension in Pregnancy (ISSHP) are present: i) low platelet count of < 100·10^9/L, ii) elevated liver enzyme: aspartate or alanine aminotransferase elevations twofold the upper limit, iii) evidence of haemolysis based on peripheral smear or elevated lactate dehydrogenase levels (LDH, twofold the upper reference limit or >650 IU/l).(6)*
HOMA-IR	*Homeostasis Model Assessment of Insulin Resistance ((glucose x insulin)/22.5 = glucose in molar units mmol/L)*
HTA	*Health Technology Assessment*
IC	*Informed Consent*
IMP	*Investigational Medicinal Product*
ICSI	*IntraCytoplasmic Sperm Injection*
IVF	*In Vitro Fertilization*
IUI	*Intra-Uterine Insemination*
LGA	*Suspected Large-for-gestational-age. According national guidelines an estimated foetal weight and/or abdominal circumference ≥90th percentile for gestational age, using the Dutch Perinatal Registry birth weight reference charts.(7)*

	Born Large-for-gestational-age. According national guidelines a birth weight ≥90th percentile for gestational age, using Dutch Perinatal Registry birth weight reference charts.(8)
METC	*Medical Research Ethics Committee (MREC);* *in Dutch: Medisch Ethische Toetsing Commissie (METC)*
NICU	*Neonatal Intensive Care Unit*
OR	*Odds Ratio*
PCOS	*Polycystic Ovary Syndrome. PCOS is diagnosed according to Rotterdam consensus criteria, endorsed in the new international evidence-based guidelines for the assessment of PCOS (see Appendix III) (9, 10)*
PCOSQ	*Polycystic Ovary Syndrome Health-Related Quality of Life Questionnaire*
PhD	*Philosophiae Doctor (Doctor of Philosophy)*
RCT	*Randomised Controlled Trial*
RDA	*Recommended Dietary Allowance*
RR	*Relative Risk*
(S)AE	*(Serious) Adverse Event*
SBP	*Systolic Blood Pressure*
SGA	*Suspected Small-for-gestational age (SGA) infant: estimated foetal weight <10th percentile and/or abdominal circumference <10th percentile and/or decline of 20 percentile points in estimated foetal weight or abdominal circumference.(11)* *Born Small-for-gestational-age (SGA): birth weight ≤10th percentile or two standard deviations below the mean birth weight for gestational age, using Dutch Perinatal Registry birth weight reference charts.(2, 12)*
Sponsor	*The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.*
SUSAR	*Suspected Unexpected Serious Adverse Reaction*
UAVG	*Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG*
UMC	*University Medical Center*
Wbp	*Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)*
WMO	*Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen*

SUMMARY

Rationale: Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. PCOS is a heterogeneous condition, characterised by metabolic disturbances, insulin resistance and hyperandrogenism. Pregnancies in women with PCOS have an increased risk of gestational diabetes mellitus, preeclampsia and preterm birth, and their offspring have an increased risk of aberrant birth weight and hospitalization. After pregnancy, PCOS is thought to have an impact on breastfeeding success and breastmilk composition.

Current strategies to improve pregnancy outcome among women with PCOS have not demonstrated significant risk reduction for the major pregnancy endpoints. Myo-inositol is a commonly used dietary supplement with a favourable effect on glucose metabolism and insulin sensitivity. Optimal intake of myo-inositol is associated with a decrease in glucose, lower insulin and lower testosterone levels in women with PCOS. Among women with PCOS-related disorders (e.g. in women with obesity), myo-inositol supplementation in pregnancy has been shown to have clinical benefits in preventing adverse pregnancy outcomes in a number of clinical trials, by reducing the risk of gestational diabetes mellitus, hypertensive complications and preterm birth. However, there are currently no prospective trials to evaluate the effect of myo-inositol supplementation as a nutritional intervention to prevent pregnancy complications among women with PCOS.

Objective: The primary objective of this study is to assess the effectiveness of myo-inositol supplementation to prevent pregnancy complications in women with PCOS. Secondary objectives are to evaluate the impact of supplementation on maternal (mental) and neonatal health and assess cost-effectiveness.

Study design: Prospective multicentre, double-blind, randomised controlled trial.

Study population: 464 women with PCOS after conformation of a viable pregnancy by ultrasound between 8+0 and 16+0 weeks of gestational age.

Intervention: Participants randomly allocated to the intervention group will receive 4 grams myo-inositol added to their routinely recommended folic acid supplement, divided over two daily sachets of sugary powder throughout pregnancy. The control group will receive similar looking sachets of supplements containing only the standard dose of folic acid without the added myo-inositol supplement, as part of the current standard-of-care recommendation.

Main study parameters/endpoints: Primary endpoint will be the incidence of the composite outcome of either gestational diabetes mellitus, and/or preeclampsia and/or preterm birth (i.e. birth before 37 weeks gestational age). Secondary endpoints will include indicators of maternal physical and mental well-being, maternal health-related quality of life, neonatal outcomes, breastfeeding practices and breastmilk composition. In addition, a full cost-effectiveness analysis will be performed.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Myo-inositol supplements have been used in several previous trials in pregnancy and is considered a safe food supplement without any side effects or risks. Myo-inositol is well tolerated at the amounts used in this study. In addition to receiving supplements, participants will be asked to complete three questionnaires, provide blood and urine samples once each trimester of pregnancy, and routine ultrasound scanning will be performed to assess foetal growth. Study visits will be aligned with routine antenatal care appointments and blood tests whenever possible. Additionally, subjects can choose to participate in research on the impact of myo-inositol supplementation on breastfeeding and take part in

the MYPP biobank. The results of this study will provide important novel recommendations for PCOS patients on the importance of optimising life-style and nutrient intake to prevent pregnancy complications.

1. INTRODUCTION AND RATIONALE

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age with a reported incidence of 6-15%.(13) According to the Rotterdam consensus criteria, currently endorsed in the new international evidence-based guidelines for the assessment of PCOS, PCOS is diagnosed when at least two of the following criteria are present and other aetiologies of either one of these criteria are excluded: i) oligo- and/or anovulation (i.e. ovulatory dysfunction and irregular cycles), ii) clinical and/or biochemical hyperandrogenism (i.e. excess androgen production), and iii) polycystic ovaries on ultrasonography.(9, 10) Although PCOS remains a heterogeneous condition, it is generally associated with reproductive (irregular menstrual cycles, infertility), psychological (anxiety, depression) and metabolic features such as insulin resistance, type-2 diabetes mellitus, obesity and dyslipidaemia.(14-19) Reported prevalence of type-2 diabetes mellitus and underlying insulin resistance vary among different populations between 2.3% and 10% (20, 21) and is partly dependent on body-mass index (BMI). However, even after adjustment for BMI, PCOS is still associated with an odds ratio of 2.0 for type-2 diabetes mellitus.(22)

Women with PCOS have an increased risk of complications during pregnancy. Systematic reviews have demonstrated an increased risk of pregnancy-induced hypertension (OR: 3.07 (95%CI: 1.82-5.18)), preeclampsia (OR: 3.47 (95%CI: 1.95-6.17)), gestational diabetes mellitus (GDM: OR: 2.81 (95%CI: 1.99-3.98)) and preterm birth (OR: 1.75 (95%CI: 1.16-2.62)). Offspring of women with PCOS are at increased risk of admission to the neonatal care unit (NICU: OR: 2.31 (95%CI: 1.25-4.26)) and perinatal death (OR: 3.07 (95%CI: 1.03-9.21)).(23, 24) Based on previous case and observational studies there is now reasonable suspicion that PCOS, possibly associated with maternal androgen levels and BMI, may negatively influence breastmilk composition and breastfeeding success.(25-27) However, prospective studies are needed to confirm these preliminary results. To date, little is known about long-term consequences of maternal PCOS for offspring health.

Various other intervention strategies have attempted to reduce pregnancy risks associated with having PCOS, however without much success. Several lifestyle intervention strategies have investigated the effect of changing to a healthy lifestyle on surrogate markers of metabolic disturbance associated with PCOS, including changed body composition, hyperandrogenism and insulin resistance. The results of these strategies have thus far been disappointing due to moderate effect size, compliance issues and insufficient power to include meaningful clinical endpoints. Also, some medication-based interventions have been tried, such as the use of insulin-sensitizing drugs (e.g. metformin). However, in addition to the general concerns related to the use of pharmacotherapy in pregnancy as a preventive strategy, the results of a number of well-conducted randomised trials on metformin and a recent meta-analysis of these studies did not show any significant reductions in the main endpoints, including no reductions in the rates of GDM, preeclampsia and preterm birth.(28, 29) Taken together, there is a need for improved, safe, and more effective life-style interventions to improve pregnancy outcomes in women with PCOS.

Myo-inositol is a naturally occurring substance, with a structure quite similar to glucose, which forms an essential component of the cell membrane and is known to support several cellular processes in all living organisms. It is present in nature in high abundance and can be found in many food products, in particular in fruit such as oranges and cantaloupe melons. Myo-inositol was historically considered part of the vitamin-B complex and can safely be used as a dietary supplement. Because myo-inositol can be synthesized endogenously, however, it is strictly speaking not classified as a vitamin and is no longer considered an essential nutrient. In humans, it is synthesized *de novo* from glucose-6-phosphate in many tissues, mainly in the kidney. Under normal circumstances, the kidneys produce large quantities of myo-inositol, estimated at about 4 grams of endogenous production per day.(30) Myo-inositol has been shown to have a physiological role in supporting the effects of insulin in all living beings. It fulfils a second messenger role in the insulin signalling pathway by enhancing the translocation of GLUT-4 receptors on the plasma membrane and thereby facilitates the intra-cellular uptake of glucose. In animal models the potential value of myo-inositol supplementation has been demonstrated in its ability to decrease insulin and blood glucose levels.(31-33)

There are several lines of evidence that support the concept that women with PCOS may need more myo-inositol than endogenously produced and derived from the natural diet. In women with PCOS, intracellular depletion of myo-inositol has been described.(34, 35) This may clarify the high prevalence of insulin resistance among women with PCOS, as well as the compensatory hyperinsulinemia, which contributes to the excessive androgen synthesis.(36) This observation led to the hypothesis that increasing the dietary intake of myo-inositol may increase insulin sensitivity. Several studies have indeed demonstrated a decrease in glucose, insulin and testosterone levels (and consequently improve ovarian function) after restoring myo-inositol levels through supplementation in women with PCOS.(37) In addition, in a randomised trial by Gerli et al., in which the effect of inositol versus placebo was assessed in PCOS patients with oligo-/amenorrhea, significant weight loss (BMI from 35.2kg/m^2 to 34.6kg/m^2, p<0.05 during treatment) and significantly increased levels of circulating high-density lipoproteins (HDL; often referred to as “good cholesterol”) were recorded in the inositol group, compared with significant weight gain in the placebo group (BMI from 35.3kg/m^2 to 35.6kg/m^2, p<0.05 during treatment).(38)

In the past few years, evidence has emerged to support a role for myo-inositol supplementation as a safe nutritional intervention to reduce the risk of a number of adverse pregnancy outcomes.

To date, three randomised-controlled trials on the prevention of pregnancy complications have been conducted using myo-inositol supplementation. All three trials were conducted in a Southern European population, among women prone to abnormal glucose-regulation, i.e. overweight pregnant women, pregnant women with obesity and in pregnancy in women with a family history positive for type-2 diabetes mellitus. Trials were set up to add 4 grams of myo-inositol supplements to the standard recommended supplementation of 0.4 mg folic acid per day, throughout pregnancy. Outcomes of these trials showed a

significant relative risk reduction of 52-60% for GDM, without safety concerns.(39-41) Two studies reported on the endpoints preterm delivery and hypertensive disorders.(39, 40) One of these two studies showed a significant reduction in gestational hypertension (0% in the intervention group versus 6% in the control group), while in both studies a non-significant relative risk reduction for preterm birth was seen. In all three trials myo-inositol supplementation was well tolerated and no side effects were reported.(39, 40, 42)

In addition, a non-randomised study in pregnancies with continued use of myo-inositol supplements among women with PCOS, continuation of myo-inositol supplementation during pregnancy was estimated to lead to a relative risk reduction of 68% for GDM.(43)

Apart from this retrospective study, there are no current randomised studies aimed specifically at pregnant women with PCOS, to estimate the potential effectiveness of myo-inositol supplementation to prevent all three major pregnancy complications associated with PCOS (i.e. GDM, preeclampsia and preterm birth), which is the subject of our study, the MYPP-trial.

2. OBJECTIVES

Primary Objective:

To assess whether myo-inositol supplementation, added to standard recommended folic acid supplementation, compared with routine folic acid supplements only, is effective in reducing the number of pregnancies complicated with the composite endpoint of either i) gestational diabetes, and/or ii) preeclampsia and/or iii) preterm birth, among women with polycystic ovary syndrome.

Secondary Objectives:

Secondly, this study will evaluate the potential effect of myo-inositol supplementation on:

- Maternal physical and mental health, and health-related quality of life during pregnancy and the first six weeks postpartum.
- Foetal growth, as assessed by ultrasound and birth weight.
- Neonatal outcomes, morbidity and burden of care (e.g. hospital admissions).
- Cost-effectiveness and budget-impact analysis of myo-inositol supplementation from both a societal and patient perspective.

Additional Objectives:

Additionally, this study will monitor the potential effect of myo-inositol supplementation on:

- Maternal outcomes, including maternal morbidity and outcome of labour and delivery.
- Maternal nutrient intake.
- Maternal blood levels of markers representative of glucose homeostasis, endocrine balance and lipid profile.
- Neonatal blood levels of markers indicative of glucose homeostasis and endocrine balance in cord blood.
- Breastfeeding and breastmilk composition.

Informed consent for biobanking (maternal blood, urine and breastmilk samples, umbilical cord blood, umbilical cord and placental tissue) is asked separately.

We will seek permission for additional follow-up of children until school-age to monitor the long-term effects on development, metabolic changes and epigenetic changes (subject to additional protocols and funding).

3. STUDY DESIGN

3.1 Study design

The proposed study is designed as a prospective multicentre, double-blind, randomised controlled nutritional intervention trial in women with PCOS. In this trial we will compare the effect of myo-inositol supplementation, added to standard recommended folic acid supplementation (intervention group), with routine folic acid supplements only (control group), on: maternal and neonatal clinical outcomes, markers representative of blood glucose homeostasis, endocrine balance and lipid-profile, breastfeeding and breastmilk composition and safety. In addition, a full cost-effectiveness analysis will be performed. Women with a diagnosis of PCOS and a singleton pregnancy between 8+0 and 16+0 weeks of gestational age are eligible and will be recruited by their treating fertility physician, obstetrician or midwife. After inclusion participants will be allocated at random (using a web-based computerised program) to either the intervention group or the control group and will receive supplementation up until delivery. Trial participants, care providers and investigators will be blinded for a participant's allocated intervention during the trial. Participants will be followed-up until 6 weeks postpartum. We will seek permission for follow-up of breastfeeding mothers until 6 months postpartum.

An overview of the study design is summarised in the flow chart provided in *Appendix I.*

3.2 Setting and duration of the study

Women with PCOS will be enrolled to the RCT in the following three University Medical Centres (UMCs) and one non-academic teaching hospital: the Erasmus University Medical Center Rotterdam (sponsor), the University Medical Center Utrecht (UMCU), the Amsterdam University Medical Center (Amsterdam UMC) - location VUmc, and the Diakonessenhuis Utrecht.

A time line of the study is provided in *Appendix II.* Based on an expected 60% inclusion-rate we expect to require a 20-months to 2-year recruitment phase to include all participants. Further collection of data and clinical outcomes will be completed in the 3rd year of the study. If needed, i.e. should the study recruitment fall below 30% of the eligible population, the study will be expanded to additional participating centres through protocol amendments.

4. STUDY POPULATION

4.1 Population (base)

Patients eligible to participate will be those women in whom the diagnosis of PCOS has recently been made (e.g. at the fertility clinic) or with known PCOS (i.e. diagnosed earlier on), who meet the Rotterdam consensus criteria (as provided in *Appendix III)* and have a viable pregnancy (i.e. positive heartbeat at ultrasound scanning) confirmed between 8+0 and 16+0 weeks gestational age (GA).

Before participation is requested, information about the study will be provided to women with PCOS who are booked to either the fertility clinic (before conception) or the antenatal care clinic (after conception but before 8 weeks of gestational age).

Following advice of the Dutch PCOS Foundation, patients will receive myo-inositol supplements no earlier than 8+0 weeks of gestational age, and after confirmation of a viable pregnancy (positive heartbeat) by ultrasound to avoid initiating supplementation in women with a first trimester miscarriage.

Most patients with PCOS (~80%) are expected to get pregnant after fertility treatment for PCOS. In that case, the fertility team will confirm the viable pregnancy and organise counselling and informed consent for the study at the fertility clinic, i.e. before booking their first antenatal clinic appointment with their midwife or obstetrician. In addition, patients may be enrolled by their midwife or obstetrician, and receive information to provide informed consent, at any of their antenatal clinic appointments from 8+0 to 16+0 weeks of pregnancy (either after spontaneous conception or after referral from fertility clinics elsewhere), when the midwife/obstetrician notices PCOS in their history at booking.

The recruited research population will be women of fertile age. Due to the geographic distribution of citizens of non-Western origin in the recruitment area, we expect to recruit a diverse and multi-ethnic population, although language barriers may prove to be a challenge to obtain the required informed consent.(44)

A preliminary survey was performed among a focus group organised in collaboration with the Dutch PCOS Foundation, which estimated high willingness to participate in the MYPP-trial. In the survey, the study was briefly explained, after which 8 of 10 participants showed willingness to participate in the trial. This willingness was further supported by a second survey among 20 patients with PCOS attending the outpatient clinic.

The four participating centres have all agreed to participate in the trial and provided letters-of-intent. Based on the conservative estimation of the internationally reported 6-15% incidence of PCOS among women in fertile age(13), PCOS affects about 8% of the 180.000 women that deliver in the Netherlands annually(45). Taken together, the participating centres involved in this trial provide care for a substantial number of these PCOS patients (*n*=250 per year for the UMCs). Taking into account the inclusion criteria and anticipated number of patients, we estimate an inclusion rate of 60% of eligible candidates.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- ≥ 18 years of age
- Diagnosis of PCOS according to the Rotterdam consensus criteria and confirmed by a gynaecologist (see *Appendix III*)
- A viable singleton pregnancy confirmed by ultrasound
- Being able to initiate the use of study supplements between 8+0 and 16+0 weeks gestational age
- Ability to understand Dutch or English
- Ability to provide written informed consent

4.3 Exclusion criteria

A potential subject will be excluded from participation in this study if any of the following criteria are present:

- Diagnosis of pre-existent type-1 or 2 diabetes mellitus
- Pre-existent renal failure, defined as an estimated glomerular filtration rate (eGFR) less than 50ml/min/1.73m^2
- Use of myo-inositol supplements, other insulin-mimetics, hypoglycaemic agents (e.g. metformin) and/or systemic steroids, that cannot be discontinued at the time of inclusion

4.4 Sample size calculation

The primary composite outcome of the MYPP-trial consists of reaching a diagnosis of one or more of the following three complications of pregnancy: i) gestational diabetes mellitus, ii) preeclampsia and/or iii) preterm birth. Based on recent data from our group, these endpoints are anticipated to occur in 23%, 4% and 9% of patients respectively.(46) Using this data, we estimate the overall incidence of the composite outcome at 33% for our study population.

Three previous randomised controlled trials have been conducted to estimate the beneficial effects of myo-inositol supplementation on identical pregnancy outcomes.(39-41) Trials were conducted among pregnant women with PCOS-related disorders (i.e. prone to glucose-dysregulation), namely: women with overweight (BMI >27kg/m^2), obesity or with a family history of type-2 diabetes mellitus. Results of these studies showed a relative risk reduction of 52-60% in incident GDM. Two of these studies, by D'Anna et al.(40), reported data on hypertensive disorders and preterm delivery. In one of these studies, among pregnant obese women, a significant reduction in gestational hypertension (0% in the intervention group versus 6% in the control group, no risk reduction provided) and a non-significant relative risk reduction of 68% for preterm birth was seen. In a retrospective (non-randomised) study addressing myo-inositol supplementation in pregnant women with PCOS, a relative risk reduction of GDM was estimated at 68%.(43)

The above-mentioned studies of myo-inositol supplementation in pregnancy were conducted in the Italian population with limited diversity. In our study, we expect a more diverse population.

In conclusion, to avoid overestimation and taking into account therapy compliance and differences in study population, we aim for a more conservative goal of 35% reduction in the composite outcome for pregnancy complications.

Based on a 35% relative risk reduction of the composite outcome of GDM, preeclampsia and preterm birth among pregnant women with PCOS and a statistical power of 80% (α=0.05), the required sample size was calculated at *n*=464 (*n*=232 in each study arm).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The nutritional intervention will consist of either supplementation with 4 grams myo-inositol added to the standard recommended supplementation of 0.4 mg folic acid daily (intervention group), or standard supplementation of 0.4 mg folic acid daily only (control group), from inclusion (i.e. between 8+0 and 16+0 weeks of gestational age) until delivery.

Folic acid supplements are part of routine pregnancy care and frequently used as background supplements in previous trials on myo-inositol supplementation in pregnancy. The majority of participants are expected to already use the recommended daily dose of folic acid (0.4 mg). Participants will be asked to discontinue their regular supplements containing any folic acid and/or myo-inositol and to switch to the supplement provided by the study team.

The supplements for the intervention group are provided as a neutral tasting powder in sachets containing 2 grams myo-inositol plus 0.2 mg folic acid each, whereas the control group receives sachets of neutral tasting powder containing 0.2 mg folic acid only (see *Appendix IV* for more details on the composition of the supplements). The sachets of powder will be advised to be used twice a day and can be added to food, diluted in a glass of water/juice or consumed directly. The supplements for the two groups will have resembling sensory characteristics and will both be produced by GYNOV SAS, France. The flow of the trial including the standard antenatal care, blinding and allocation of supplements is further specified in Chapter 8.

The nutritional intervention in this study is considered a primary prevention tool for improving pregnancy outcome for women with PCOS. Following the recommendations of the CCMO and previous trials using myo-inositol in pregnancy, the nutritional intervention is not considered an 'investigational product' or 'Investigational Medicinal Product (IMP)'. Myo-inositol and folic acid are registered as dietary (food) supplements in European Union countries. The quantities of myo-inositol supplementation proposed are considered standard amounts, as part of normal dietary intake and endogenous production, in nutritional safety guidelines and current clinical trials in pregnancy. For these trials, confirmation that the supplement is not considered an IMP has been secured by several independent agencies, including the Medicines and Healthcare products Regulatory Agency (UK), MedSafe (New Zealand), Health Science Authority (Singapore), and the Health Products Regulatory Authority (Ireland).(47, 48)

This recommendation is compliant with Directive 2002/46/EC of the European Parliament and of the Council of June 2002 on the approximation of the laws of the Member States relating to food supplements, in which a food supplement is defined as: 'foodstuffs, the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other

similar forms of liquids and powders designed to be taken in measured small unit quantities', and compliant to the U.S. Food & Drug Administration (FDA) definition for *special dietary uses: '*Uses for supplying particular dietary needs which exist by reason of a physical, physiological, pathological or other condition' (Article 105 Code of Federal Regulations).

Preparation and labelling of the supplements used in this study (intervention and control group)

Manufacturer and product stability

The supplements, formulated as a white-beige neutral tasting powder, will be produced by GYNOV SAS, France and are registered as food supplements in European countries. Despite not being considered an IMP in the context of this study, the manufacturing process of the supplements will be performed in accordance with the European Union regulations to Good Manufacture Practice (GMP).

All of the raw materials have been qualified and meet European regulation requirements. The end product was analysed by an independent ISO 17025 certified laboratory and issued as meeting defined specifications. The qualified person certifies that each production batch satisfies these specifications.

Based on stability studies, the supplements are stable under normal and accelerated (temperature 40 +/- 2 °C) conditions. The products shelf life was evaluated at three years. The supplements should be stored in a cool, dry place.

Labelling of the supplements

Given the nature of the trial, using dietary supplements as a nutritional intervention rather than an IMP, the supplements do not need to be labelled in accordance with EC directives or respective national laws. For instance, the Council Directive 90/496/EEC on nutrition labelling for foodstuffs does not apply for dietary supplements. Neither does Commission Regulation (EC) no. 1272/2008 on classification, labelling and packaging of substances and mixtures apply, as myo-inositol and folic acid are not classified as a hazardous substance.

For the purpose of Directive 2002/46/EC on the approximation of the laws of the Member States to food supplements however, patients will be informed of dose and nutrient content of both supplements and will be provided with instructions for appropriate use of the supplements.

In addition, since it concerns a double-blinded trial, adequate labelling is required in the context of product traceability and participants' safety. After preparation, the supplements will be distributed by Gedeon Richter Benelux bvba. All supplements (including the supplements for the control group) will be supplied to the Erasmus Medical Center Rotterdam as identical sachets of powder, packed in indistinguishable boxes containing 60 sachets each. The boxes will report the expiry date and the batch number only, the latter being different for the interventional and control supplements. At the Erasmus Medical Center, the supplements will be further processed by an independent research staff member, not involved in the study, being the only one informed about the meaning of the batch numbers. This staff member will randomly

number the boxes according to the computer-generated scheme provided by the trial statistician (which integrates block-randomisation and stratification by site) and will create a list of batch-numbers and their corresponding randomisation numbers. The supplements, and enclosed randomisation lists, will then be distributed over the participating centres. After randomisation, local investigators will supply participants with the appropriate supplements according to the provided randomisation list. The process of randomisation and blinding is explained in more detail in Section 8.2 'Randomisation, blinding and treatment allocation'.

5.2 Use of co-intervention

During the trial, routine obstetrical care and treatment of pregnancy complications following national guidelines will not be different from non-participants.

As part of standard antenatal care and regardless of randomisation group, all pregnant women receive nutritional and lifestyle advice in agreement with Dutch guidelines. This normally includes the use of folic acid supplementation at the standard recommended daily dose advised in this trial.(49, 50) In addition, following national guidelines, women are advised to: pursue a variable diet, avoid raw meat or fish products and unpasteurised milk or cheese, minimize the intake of vitamin A, avoid excessive sugar and caffeine intake and to quit smoking, refrain from drug use and abstain from alcohol consumption.(51)

Regarding dietary supplements, patients may use regular over-the-counter products, such as multivitamins and iron supplements, except for those supplements containing additional myo-inositol and/or folic acid.

Patients using insulin-mimetics, hypoglycaemic agents (e.g. metformin) and/or systemic corticosteroids that cannot be discontinued at the time of inclusion will be excluded. A patient will not be excluded if an indication for these products arises during follow-up, as our primary objective (and endpoint) is to explore the effectiveness of myo-inositol in preventing such complications.

Further, patients are advised to report any other medication use during pregnancy to the study team.

5.3 Escape medication

Not applicable.

6. INVESTIGATIONAL PRODUCT

Myo-inositol, the nutritional intervention, is not considered an 'Investigational Medicinal Product (IMP)' in the context of this intervention trial, or in any other context, but classifies as a food supplement (or food product). This is explained in more detail in Section 5.1. To verify this point, our group has sought the expertise of the CCMO, who have confirmed the nature of this study as a nutritional intervention trial and consider myo-inositol a food product classified as an 'investigational product' in the context of this study, and not as a pharmacological substance or 'Investigational Medicinal Product (IMP)', as explained in more detail in the additional document K6. of the METC dossier. Previous knowledge on myo-inositol is summarised below.

Myo-inositol is present in many food products and natural resources. Supplements are authorised to supply particular dietary needs, such as those which exist during pregnancy and/or PCOS, to optimise a healthy diet and thereby improve general health. In previous trials using myo-inositol supplementation in pregnancy, confirmation of myo–inositol being a food product (not considered an IMP) has been given by several independent international healthcare product regulatory agencies.(47, 48) Similar to our study protocol, myo-inositol is usually added to the accepted folic acid supplements advised in clinical practice, as part of routine pregnancy care.

6.1 Name and description of investigational product(s)

Dietary supplement intervention group: sachets containing 2 grams myo-inositol added to a standard dose of 0.2 mg folic acid.

Dietary supplement control group: sachets containing 0.2 mg folic acid only.

Both supplements are provided as sachets of neutral tasting powder and advised to be used twice a day. The supplements will have resembling sensory characteristics and will both be produced by GYNOV SAS, France. For a more detailed description of the composition of the supplements, see *Appendix IV* '*Composition of the dietary supplements'.* General information on myo-inositol is provided in *Appendix V.*

6.2 Summary of findings from non-clinical studies

This is not relevant since myo-inositol and folic acid supplements are currently registered as dietary supplements in European countries and its use in human has been researched extensively. For the results of preclinical studies on myo-inositol, see *Appendix V.*

6.3 Summary of findings from clinical studies

Summary of findings from clinical studies on myo-inositol supplementation

There is extensive experience with myo-inositol supplementation (alone or combined with folic acid) in non-pregnant and pregnant individuals.

Summary of findings from clinical studies on myo-inositol supplementation in non-pregnant women

In clinical studies, intracellular myo-inositol depletions have been described in human subjects with metabolic disorders related to insulin resistance, such as type-2 diabetes mellitus and PCOS.(34, 35, 52)

The effect of supplementation in PCOS patients in improving dietary intake to restore hormonal and metabolic impairments by restoring myo-inositol concentrations, has been well established. Unfer et al.(37) summarized nine RCTs in which a total of 247 women with PCOS receiving myo-inositol (alone or combined with folic acid or *D*-chiro-inositol) were compared with 249 patients receiving folic acid or other treatments (e.g. oral contraceptives). Although heterogeneity across the studies was found, overall a significant decrease in fasting insulin (standard mean difference (SMD) −1.021 µU/mL, 95% CI: −1.791 to -0.251, *p*=0.009) and the HOMA-index (SMD = −0.585, 95% CI: −1.145 to −0.025, *p*=0.041) was seen. Although less clear changes were demonstrated on androgenic levels, there was a trend towards a decrease of total testosterone (SMD = −0.482 nmol/L, 95% CI: −1.052 to 0.088, *p*=0.097). Myo-inositol supplementation up to 24 weeks revealed a significant increase in SHBG levels in the myo-inositol group (SMD = 0.418 nmol/L, 95% CI: 0.049–0.786, *p*=0.026). For its effects on criteria that compromise the metabolic syndrome, the evidence in PCOS patients is less abundant. However, in the included RCT by Gerli et al. in which the effect of inositol (versus placebo) was assessed, a significant weight loss (BMI from 35.2kg/m^2 to 34.6kg/m^2, *p*<0.01 during treatment) and an increase in circulating HDL-cholesterol was recorded in the inositol group, whereas in the placebo group only an increase in weight (BMI from 35.3kg/m^2 to 35.6kg/m^2, *p*<0.05 during treatment) was seen.(38) In addition, Costantino et al. described a significant decrease of triglyceride below the cut-off level for defining metabolic syndrome in PCOS patients receiving supplements with additional myo-inositol versus folic acid supplements alone (195 +/- 20 to 95 +/- 17 mg/dl (intervention group) versus 166 +/- 21 to 148 +/- 19 mg/dl (placebo-group); *p*=0.001).(53)

Taken together, there are multiple studies supporting the concept that myo-inositol supplementation can decrease glucose, insulin and testosterone levels and restore hormonal balance and metabolic function in women with PCOS.

Summary of findings from clinical studies on myo-inositol supplementation in pregnant women

In summary, studies focussing on nutritionally derived myo-inositol in pregnancy show equally promising results with respect to improving glucose homeostasis and insulin resistance.

Corrado et al.(54) performed a RCT in 69 women with GDM (without PCOS) and showed a significant decline in fasting glucose, insulin and consequently in HOMA-IR in the myo-inositol group compared to the control group (decline of 50% versus 29%, *p*=0.0001). Consistent with restoration of insulin sensitivity, adiponectin levels increased in the myo-inositol group compared to decreasing levels in the control group (*p*=0.009).

Another RCT by Matarelli et al.(55) demonstrated the effect of myo-inositol supplementation to prevent the development of GDM in women with elevated fasting glucose in early pregnancy. Whereas 71% women developed GDM in the control group, only 6% of the women supplemented with myo-inositol developed GDM (p=0.001). The increase in mean BMI was significantly lower in the intervention group (2.3 kg/m^2 versus 3.8 kg/m^2, p=0.001). In addition, neonatal hypoglycaemia was only recorded in neonates born to women in the control group (10 controls and no cases in the myo-inositol group, relative risk 0.052, p=0.038*).* In both RCTs, women in the intervention group received 4 grams myo-inositol plus 0.4 mg folic acid*,* whereas controls received 0.4mg folic acid only.

Three recent randomised controlled trials (total n=502) comparing daily supplementation with 4 grams of myo-inositol added to 0.4 mg folic acid, to supplements containing 0.4 mg folic acid only, observed a relative risk reduction of 52-60% in incident GDM.(39, 40, 42) Two studies reported on preterm delivery and hypertensive disorders.(39, 40) One of the two studies reported a significant reduction in gestational hypertension (0% in the intervention group versus 6% in the control group), while in both studies a non-significant relative risk reduction for preterm birth was seen. These studies, summarized in Cochrane review by Crawford et al.(56), included both obese patients and patients with normal body weight. Importantly, no side effects or safety issues of myo-inositol supplementation in pregnancy were observed.

Although these results are promising, evidence to show the potential beneficial effects of myo-inositol supplementation on pregnancy outcome in women with PCOS, is currently based on a single non-randomised retrospective study. This study showed a significant relative risk reduction of 68% for GDM, even after adjusting for covariates, without any sign of adverse outcomes or side-effects.

There are currently no RCTs to provide robust evidence for the use of nutritionally derived myo-inositol for supplying the dietary need in pregnant women with PCOS, which is the subject of our study. In addition, previous trials have been conducted mainly in women of Caucasian origin, thus there is a need for prospective studies in more diverse populations to determine the efficacy of myo-inositol supplementation in improving pregnancy outcome in women with PCOS across different ethnicity groups.

<u>Summary of findings from clinical studies on folic acid supplementation</u>

This is not relevant since, following extensive research on its use in women trying to conceive and during pregnancy, daily consumption of 0.4 mg folic acid is now recommended worldwide for the purpose of reducing the risk of having a pregnancy affected with spina bifida or other neural tube defects (NTDs).(57-61)

Both dietary supplements used in this study will contain the routine dose of folic acid supplementation as part of the standard-of-care recommendation.

6.4 Summary of known and potential risks and benefits

Myo-inositol is considered safe to use in pregnancy and supplementation may have a number of potential benefits, as explained in more detail in Section 6.3.

In summary, the beneficial effects of myo-inositol supplementation are likely due to improvements in insulin sensitivity and by restoring hormonal imbalance in women with PCOS.(37, 38) For participants, the hypothesis is that the supplements may potentially be of benefit in preventing adverse pregnancy outcomes, i.e. gestational diabetes, preeclampsia and preterm birth, which is the subject of the MYPP-trial. Myo-inositol is considered a safe food supplement without any known side effects or serious risks. Myo-inositol is generally well tolerated. Mild (mainly gastro-intestinal) side effects have only been reported at excessive intake above 12 grams per day.(62-64) Further knowledge on the use and safety of myo-inositol is summarised in Appendix V.

6.5 Description and justification of route of administration and dosage

Participants randomised to the intervention-group will receive 4 grams myo-inositol added to their routinely recommended folic acid supplement, divided over two sachets of neutral tasting powder per day. Participants will be advised to take the supplements orally from the moment of inclusion until delivery.

Initiation is not started earlier than 8+0 weeks of gestational age, and after confirmation of a viable pregnancy (positive heartbeat) by ultrasound, to avoid initiating supplementation in women with a first trimester miscarriage. The route of administration and used dosage in this study is based on previous research and does not exceed physiological levels. Myo-inositol taken orally, being the least invasive route of administration, is generally well tolerated in this effective dose.

The control group will follow a similar regime of supplements with resembling sensory characteristics, containing only the standard dose of folic acid, as part of the current standard-of-care recommendation.

6.6 Dosages, dosage modification and method of administration

All participants, irrespective of randomisation group, will be advised to take the dietary supplement orally twice a day. The sachets of powder can be added to food, diluted in a glass of water/juice or consumed directly. Dosage modifications are not required or performed.

6.7 Preparation and labelling of Investigational Medicinal Product

This is not applicable since the nutritional intervention in this study is not considered an Investigational Medicinal Product. For a description of the preparation and labelling of the dietary supplements by the manufacturer, please refer to Section 5.1.

6.8 Drug accountability

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Not applicable. Please refer to the explanatory text in Chapter 6.

7.2 Summary of findings from non-clinical studies

Not applicable.

7.3 Summary of findings from clinical studies

Not applicable.

7.4 Summary of known and potential risks and benefits

Not applicable.

7.5 Description and justification of route of administration and dosage

Not applicable.

7.6 Dosages, dosage modifications and method of administration

Not applicable.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable.

7.8 Drug accountability

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary outcome of this study is the number of PCOS patients in the two study arms that develop one or more of the following pregnancy complications:

- Gestational Diabetes Mellitus (GDM), as defined in national guidelines: any degree of glucose intolerance with onset or first recognition during pregnancy.(3-5) In practice, GDM is diagnosed using an oral glucose tolerance test (OGTT) with cut-off values as described in *Table I* below.(3, 4)
- Preeclampsia, as defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP)(65): the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation and the coexistence of one or more of the following new onset conditions:
  i) Proteinuria: 24 hour urinary protein ≥ 300 mg per day.
  ii) Other maternal organ dysfunction:
    - renal insufficiency: creatinine ≥ 90 µmol/L.
    - liver involvement: elevated transaminases (at least twice upper limit of normal) and/or sever right upper quadrant or epigastric pain.
    - neurological complications, e.g. eclampsia, altered mental status, blindness, hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata.
    - haematological complications: thrombocytopenia (platelet count < $150 \cdot 10^9$/L), disseminated intravascular coagulation, haemolysis.
  iii) Uteroplacental dysfunction: foetal growth restriction.
- Preterm birth, internationally defined as any birth before 37 completed weeks of gestational age.

A composite of the above complications, ultimately scored one week postpartum (as to include postpartum preeclampsia), will form the primary outcome to assess the effectiveness of myo-inositol supplementation.

Table I - *Cut-off values for the oral glucose tolerance test*
(World Health Organisation, 1999 criteria; current Dutch National Guidelines, NVOG/NIV)

75-grams OGTT	**Venous plasma (mmol/L)**	**Capillary blood sample (mmol/L)**
Fasting	≥ 7,0	≥ 6,1
After 2 hours	≥ 7,8	≥ 7,8
*GDM diagnosis: at least one value is abnormal		

8.1.2 Secondary study parameters/endpoints

Secondary, this study will compare the following parameters between the two groups:

1. Maternal physical health by the occurrence of pregnancy related hypertensive disorders associated with PCOS:
   - *Pregnancy-induced/Gestational Hypertension,* as defined by the ISSHP as the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks of gestation.(65)
   - *Haemolysis Elevated Liver enzymes and Low Platelet count (HELLP):* generally diagnosed when at least two of the following criteria compiled by the ISSHP are present(6):
     i) low platelet count of $<100 \cdot 10^9$/L.
     ii) elevated liver enzymes: aspartate or alanine aminotransferase elevations twofold the upper limit.
     iii) evidence of haemolysis based on peripheral smear or elevated lactate dehydrogenase levels (LDH, twofold the upper reference limit or >650 IU/l).

   Cut-off values may differ slightly per centre and the syndrome may be partial or complete.
2. Maternal mental health: using a composite questionnaire (see *F1. Vragenlijst I-III*) specifically developed for this study, to be completed at study inclusion, 36 weeks of pregnancy and 6 weeks postpartum. Questions for the purpose of assessing maternal mental health were derived from the *Beck Depression Inventory (BDI) Scale II Netherlands.*

   The BDI-II-NL is the Dutch and revised version of the self-report inventory that was first developed by Beck et al.(66) It measures characteristic attitudes and symptoms of depression and contains 21 items, subdivided in cognitive, affective and somatic factors. Patients rate themselves on these depressive symptoms by endorsing the most relevant statement (ranging in four steps of intensity from minimal to severe) covering a time frame of the past 2 weeks.(67)
3. Maternal health-related quality of life: assessed at study inclusion, 36 weeks of gestational age and 6 weeks postpartum. For this purpose, in the study questionnaire (see *F1. Vragenlijst I-III*) questions were incorporated based on the following two validated questionnaires:
   - *5-Level EuroQol - five dimension (EQ-5D-5L)*

     This standardized instrument was developed by the EuroQol group as a measure of health-related quality of life and consists of a vertical visual analogue scale (EQ VAS), recording the patient's self-rated health, and a descriptive system. The descriptive system distinguishes five health domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Patients are asked to indicate their health state for each dimension in five levels (no problems, slight problems, moderate problems, severe problems and extreme problems) which will result in a 5-digit number that describes the patient's overall health state.
   - *Polycystic Ovary Syndrome Health-Related Quality of Life Questionnaire (PCOSQ)*

The PCOSQ is the only existing validated, disease-specific, health related quality of life assessment questionnaire. As stated in the new evidence-based PCOS guidelines, this questionnaire could be clinically useful to highlight PCOS features causing greatest distress and was developed to evaluate the effect of treatment in clinical trials in PCOS.(68, 69) The PCOSQ includes five domains, each related to a common symptom of PCOS, namely: emotions, body hair, infertility, weight and menstrual problems. The five domains compromises 26 items, which were labelled as important by PCOS patients in structured interviews, supplemented by items based on a review of the medical literature and a survey of health professionals experienced in management of PCOS women. In the PCOSQ patients are asked to rate their health for each item over the past 2 weeks on a 7 point scale in which 7 represents optimal function (no problem) and 1 represents the poorest function (severe problem).(68)

4. Ultrasound parameters:
   - *Foetal biometry* (growth scans): foetal abdominal circumference, femur length, head circumference, estimated foetal weight (Hadlock) at routine intervals, as per clinical protocol.
     - *Suspected small-for-gestational age (SGA) infant*: estimated foetal weight <10th percentile, and/or abdominal circumference <10th percentile and/or decline of 20 percentile points in estimated foetal weight or abdominal circumference.(11)
     - *Suspected large-for-gestational-age (LGA) infant*: estimated foetal weight and/or abdominal circumference ≥90th percentile.(7, 8)
   - *Polyhydramnios*, defined as a single deepest pocket (SDP) ≥ 8cm and/or amniotic fluid index (AFI) > 24cm.
   - *Congenital abnormalities*
5. Neonatal health
   - *Small-for-gestational-age (SGA):* birth weight ≤10th percentile or two standard deviations below the mean birth weight for gestational age, using Dutch Perinatal Registry birth weight reference charts.(2, 8, 12)
   - *Large-for-gestational-age (LGA):* birth weight ≥90th percentile for gestational age, using Dutch Perinatal Registry birth weight reference charts.(8)
   - *NICU admission (yes/no),* reason for admission and time to discharge or transfer to another unit.
   - *Neonatal hypoglycaemia (yes/no):*
     - subdivided in *moderate (*serum glucose levels ≤2.6 mmol/L) and *severe* (<2.0 mmol/L)
     - requiring intravenous glucose therapy (yes/no)
6. Cost-effectiveness: from both a societal and patient perspective (explained in more detail in Section 10.2).

8.1.3 Other study parameters

1. Additional maternal (baseline) characteristics
   - *Age* at the time of inclusion
   - *Gravidity, parity*
   - *Number of previous miscarriages* (miscarriage defined as the spontaneous loss of a pregnancy before 20 weeks gestation(70))
   - *Intoxications*: drug, tobacco and/or alcohol use at time of inclusion
   - *Medication and/or dietary supplements* at time of inclusion
   - *Nutrition and lifestyle*: dietary intake and physical activities (Information gathered through questionnaires. Questions for the purpose of assessing physical activity were derived from the Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH)(71))
   - *Ethnicity*
   - *Socio economic status*
   - *Positive family history of* cardiovascular disease, (gestational) diabetes mellitus, congenital abnormalities and/or PCOS
   - *Diagnosis of PCOS*: age at time of diagnosis, diagnostic criteria and setting
   - *Symptoms associated with PCOS* (information gathered through questionnaires and medical records)
     - Irregular menstrual cycles: <21 days or >35 days, or <8 cycles per year, or >90 days for any one cycle(9, 10)
     - Fertility problems
     - Acne and androgenic alopecia
     - Sleep apnoea
     - Psychological features: e.g. anxiety, depression
     - Biochemical hyperandrogenism: defined as elevated Calculated Free Testosterone or a Free Androgen Index > 4.5 (FAI = (total testosterone x 100)/SHBG)(10, 72)
     - Clinical hyperandrogenism: hirsutism (Ferriman-Gallwey score 4-6)(9, 10)
     - Hypercholesterolemia
     - Polycystic ovaries on ultrasonography: defined according to the Rotterdam consensus criteria as either i) volume: one or two ovaries >10cm^3 and/or ii) follicle count (2-9mm): one or two ovaries ≥ 12 follicles per ovary(9)
   - *PCOS phenotype*
   - *History of:*
     - Pre-existent hypertension, following national and ISSHP guidelines: >140 mmHg systolic or >90 mmHg diastolic preconception or before 20 weeks gestational age(65)
     - Other cardiovascular disease

- Thyroid abnormalities: hypo- or hyperthyroidism
- Psychological problems: burn-out/stress, anxiety symptoms, depression, eating disorders
- Liver and/or kidney disease
- Abdominal surgery and/or gynaecological operations

- *History of metabolic syndrome* (preconception), diagnosed when 3 of the following 5 criteria are present(73):
  i) elevated waist circumference (≥ 88cm)
  ii) elevated triglycerides (≥150 mg/dL = 1.7 mmol/L) or on drug-treatment for elevated triglycerides
  iii) reduced HDL-cholesterol (<50 mg/dL = 1.3 mmol/L) or on drug-treatment for reduced HDL-cholesterol
  iv) elevated blood pressure (≥ 130mmHg systolic or ≥ 85mmHg diastolic) or on antihypertensive drug-treatment
  v) elevated fasting glucose: glucose ≥ 5.6 mmol/L
- *Obstetric history*:
  - History of pregnancy-related hypertensive disorders (preeclampsia, gestational hypertension, HELLP as defined in Section 8.1.1. and 8.1.2.)
  - History of gestational diabetes mellitus (as defined in Section 8.1.1.)
  - History of preterm birth (as defined in Section 8.1.1.)
  - History of LGA or SGA born babies (as defined in Section 8.1.2.)
  - Previous section caesarean
- *Family history of cardiovascular risk factors*
- *Method of conception*: spontaneous, assisted reproductive treatment (clomiphene citrate, gonadotropins, intra-uterine insemination (IUI), in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI))
- *Mean arterial blood pressure* at intake
- *Maternal BMI* preconception and at intake, using: maternal weight preconception (self-report), maternal weight at intake (measured), length (measured/self-report)
- *Maternal waist-hip ratio*, using waist and hip circumferences (cm) at preconception (if available) or at intake

2. Maternal characteristics recorded during pregnancy and delivery
   - *Intoxications:* drug, tobacco and/or alcohol use during pregnancy and up until six weeks postpartum.
   - *Dietary supplements* used during pregnancy and up until six weeks postpartum.
   - *Nutrition intake* during pregnancy and up until six weeks postpartum (information gathered through questionnaires).

- *Mean arterial blood pressure* during pregnancy and postpartum, taken at regular care visits around 24-28 weeks and 32-34 weeks of gestation, at time of labour and six weeks postpartum (as per local protocol).
- *Gestational weight gain*, by maternal weight (and BMI) at inclusion, 24-28 and 32-34 weeks of gestation and six weeks postpartum (aligned with blood tests).
- *Hospital admissions* for GDM, pregnancy related hypertensive disorders and/or threatened preterm labour, or other reasons.
- *Any pharmacological treatment* for GDM and/or pregnancy related hypertensive disorders or the use of corticosteroids during pregnancy, or any other medication use.
- *Thromboembolic events*
- *Induction of labour* (yes/no) and reason for induction
- *Meconium-stained liquor* (yes/no)
- *Mode of delivery*: spontaneous vaginal delivery, assisted vaginal delivery (using vacuum or forceps), primary caesarean section, secondary caesarean section.
- *Sentinel events*: umbilical cord prolapse, uterine rupture, placental abruption, shoulder dystocia.
- Post-partum haemorrhage (i.e. blood loss of >1000ml within 24 hours of delivery(74)), third or fourth degree of perineal tears, any other major complications.

3. Neonatal outcomes
   - *Gestational age at birth*
   - *Sex*
   - *Birth weight* (grams)
   - *Apgar score* at 5 and 10 minutes after birth
   - *Umbilical artery pH*
   - *Paediatric consultation postpartum*
   - *(Total) length of hospitalization* (days)
   - *Neonatal morbidity*
     - Birth injury: brachial plexus injury, clavicle/humerus fracture, other.
     - Perinatal asphyxia, diagnosed by the following: i) 5-minute Apgar score ≤ 5, ii) umbilical artery pH <7.0 and/or a base-excess ≥16 mmol/l), iii) neonatal neurologic sequelae (seizures, coma, hypotonic), iv) multi-organ system injury or failure.(75)
     - Respiratory problems: Respiratory Distress Syndrome (RDS), Meconium Aspiration Syndrome (MAS), Persistent Pulmonary Hypertension of the Newborn (PPHN).
     - Infection: proven by positive culture.
     - Neurological sequelae: seizures confirmed on electroencephalography (aEEG), cerebral bleeding.

  - Metabolic disorders other than hypoglycaemia: hyperbilirubinemia requiring phototherapy, anaemia, thyroid abnormalities, thrombocytopenia.
  - Other
- *Congenital abnormalities*
- *Neonatal mortality* (and cause)

4. Laboratory tests obtained during pregnancy, labour and postpartum:
   - Measured at the time of inclusion (blood tests, unless otherwise stated)
     - CRP, Albumin
     - Creatinine, eGFR, Thyroid-Stimulating Hormone (TSH), Free Thyroxine (FT4)
     - Total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol
     - HbA1c, insulin, glucose
     - Folic acid, vitamin B6, vitamin B12, 25-hydroxy vitamin D
     - Steroid profile, Sex Hormone Binding Protein (SHBG), oestrogen
     - Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Anti-Müllerian Hormone (AMH), human Chorionic Gonadotrophin (hCG)
     - Leptin, RBP-4, DPP-IV and adiponectin
     - Urine: creatinine, micro-albumin, protein/creatinine-ratio
     - Urine*:* myo-inositol excretion*
   - Measured at 12-14 weeks
     - 75-grams OGTT (and corresponding blood glucose levels)
   - Measured at 24-28 weeks
     - 75-grams OGTT (and corresponding blood glucose levels). In case an OGTT is indicated for other reasons, the indication, gestational age at time of testing and corresponding blood glucose levels are reported
   - Measured at time 32 weeks
     - Albumin, Creatinine, eGFR
     - HbA1c, glucose, insulin
     - Total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol
     - Folic acid
     - Steroid profile, SHBG, oestrogen
     - AMH, hCG
     - Leptin, adiponectin
     - Urine: creatinine, micro-albumin, protein/creatinine-ratio
     - Urine*:* myo-inositol excretion*
   - Measured at time of labour (Erasmus MC Rotterdam only)

- HbA1c, glucose, insulin
- Steroid profile, SHBG, oestrogen

- Measured at 5-6 weeks postpartum
  - CRP, Albumin, Creatinine, eGFR
  - HbA1c, glucose, insulin
  - Total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol
  - Folic acid
  - Steroid profile, SHBG, oestrogen
  - Leptin, adiponectin
  - LH, FSH, AMH, hCG
  - Urine: creatinine, micro-albumin, protein/creatinine-ratio
  - Breast milk composition (in case of breastfeeding and after obtaining separate permission): myo-inositol levels, nutrient analysis and hormone levels

* Urine samples will be collected to analyse myo-inositol excretion. All samples will be tested at the end of the study (after the last visit of the last included participant) to avoid associations with randomisation group during the study.

5. <u>Cord blood laboratory tests to test neonatal biochemical factors and hormone levels</u>
   - Blood gas analysis (as per clinical protocol)
   - Albumin
   - Steroid profile (including Androstenedione), SHBG
   - Oestrogens: Estrone (E1), Estradiol (E2), Estriol (E3), Estretrol (E4)
   - LH, FSH, AMH, Progesterone
   - Inhibin A and B
   - Cortisol
   - C-peptide (as a marker of overall glycaemia during gestation)
   - Glucose, Insulin
6. <u>Compliance and tolerability of the nutritional intervention</u>
   - *Compliance with supplementation,* assessed by:
     - self-reported supplement use
     - counting the number of supplements returned during a regular antenatal care visit between 24-28 weeks gestational age and 6 weeks postpartum. Good adherence is defined as a total number of supplements consumed of at least 80%.
     - Eight-Item Morisky Medication Adherence Scale (MMAS-8)(76): incorporated in the study questionnaire at 36 weeks of pregnancy and 6 weeks postpartum (see *F1. Vragenlijst II and III*).

- *Patients' overall satisfaction* with supplementation and any issues encountered with consumption, examined using study questionnaires at 36 weeks of gestation and 6 weeks postpartum.
- *Reported side effects* of supplementation.

7. Breastfeeding and breastmilk
   - Breastfeeding rate among participants
   - All participants: to assess breastfeeding practices among participants the questionnaires used at 36 weeks of pregnancy and 6 weeks postpartum will contain questions regarding breastfeeding intentions and practices.
   - In case of breastfeeding 6 weeks postpartum (after obtaining separate permission as indicated on the informed consent form): additional validated questionnaire on breastfeeding around 6 months postpartum and analysis of breastmilk composition (myo-inositol levels, hormone levels and nutrient analysis).
8. Biobanking (separate informed consent required)
   - Maternal serum and urine samples at time of inclusion, around 32 weeks of gestational age, at time of labour and 6 weeks postpartum
   - Umbilical cord blood
   - Placental tissue
   - Umbilical cord tissue
   - Breast milk, to be collected from a subset of participants early postpartum for hormone levels and nutrient analysis
9. Budget-impact analysis (for a detailed description see Section 10.3)

8.2 Randomisation, blinding and treatment allocation

Participants will be enrolled between 8+0 and 16+0 weeks of gestation after confirmation of a viable pregnancy by ultrasound. The process of recruitment and the resulting study population is described in more detail in Chapter 4 and Section 11.2.

After inclusion, patients will be randomised between the intervention group and the control group.

To ensure that the study is blinded to patients, physicians and researchers, labelling and randomisation is carried out by an independent research staff member in collaboration with an independent statistician at the Erasmus Medical Center Rotterdam, both not involved in further research or patient care. Randomisation is centrally controlled by the independent statistician who will generate a randomisation list using the online software tool ALEA (accessible at https://nl/tenalea/net). Block randomisation will be performed on a 1:1 basis, with permuted blocks of random block size (sizes 4 and 6). To prevent any imbalance between groups in aspects of care that may differ between participating sites, randomisation will be stratified by site to ensure balanced allocation of participants across the two arms at each of the four participating sites.

As explained in more detail in Section 5.1, the independent research staff member will number all supplement boxes used in this study with nonspeaking codes according to the generated randomisation sequence. The supplement boxes, identical in appearance, will ultimately only display a randomisation number. Until blinding is broken, the meaning of the randomisation numbers is solely accessible to the independent research staff member and is centrally held in an access controlled folder at the Erasmus Medical Center Rotterdam.

After labelling, the boxes with supplements and enclosed randomisation lists will be distributed over the participating centres.

At the clinics, local investigators will allocate patients to either the intervention group or control group and will provide participants with a sufficient supply of supplements (up to 42 weeks of gestational age plus a few spare ones) after inclusion. For this, local investigators will take the first box of supplements in row and will note down the participants ID next to the corresponding randomisation number on the provided randomisation list. Next, the investigator will note down the allocated randomisation number on the participants enrolment form.

The randomisation code will only be broken in case of severe/adverse events.

8.3 Study procedures

During the trial, standard obstetrical care and treatment of pregnancy complications following national guidelines will be assumed. Upon advice of the Dutch PCOS Foundation, study related examinations, such as blood sampling, are aligned with routine antenatal care visits and tests from inclusion, throughout pregnancy and up until 6 weeks postpartum, as much as possible.

For a schematic representation of the study timeline, routine and study specific procedures see *Appendix VI.*

Routine antenatal care

In the Netherlands, women are advised to consult a midwife, obstetrician or their fertility physician as soon as possible after confirming a pregnancy (usually with a urinary self-test).

As most patients will be recruited from outpatient fertility clinics, the majority of participants will have had a comprehensive physical and medical assessment. During a *first antenatal care visit*, preferably between 6 and 8 weeks of gestational age, a standard comprehensive obstetrical intake interview (compromising medical, obstetric and family history, intoxications etc.) will be taken and physical examinations (blood pressure, weight and length) and baseline blood tests (e.g. blood type, antibodies, viral status) will be performed. Subsequent risk assessment is made and a principal maternity care provider (midwife or obstetrician) assigned.

Upon advice of the Dutch Society of Obstetrics and Gynaecology (NVOG), pregnant women will then be seen between *10 and 13 weeks of gestational age* for determination of a reliable estimated date of delivery (EDD) using first-trimester ultrasonography.
In addition, information with regard to healthy nutrition and lifestyle (e.g. regular physical activity) is provided by nurses as part of routine antenatal care.

Next, as set-out in regular care paths outlined in national guidelines, about 6-9 routine antenatal care visits are recommended in low-risk and uncomplicated pregnancies. Check-ups will take place at an interval of 4-8 weeks, increasing to weekly appointments when approaching the due date.(49, 50)
For these low-risk care paths, the following recommendations are made by the NVOG:

- Measure blood pressure, check foetal heartbeat and assess foetal growth (by physical examination) during every visit.
- Ask for foetal movements during every visit from a gestational age of 24 weeks and on.
- Give instructions to contact a physician in case of decreased foetal movements, symptoms associated with hypertensive disorders, blood or fluid loss and/or regular contractions/backache.
- Routine ultrasounds (amniotic fluid or biometry), vaginal swabs or urine checks are not recommended.
- A third-trimester ultrasound is only indicated if there is any uncertainty about foetal growth.
- Perform an OGTT in case of a polyhydramnios or foetal abdominal circumference >95th percentile.

Customized care paths will be formed by supplementing indicated examinations guided by medical conditions and arising complications. Following the new international evidence-based guideline for assessment and management of PCOS 2018, a 75-grams OGTT should be offered before 20 weeks of gestation (if not performed preconception), and all women with PCOS should be offered the test at 24 weeks of gestation. The latter being in line with national antenatal care guidelines.

<u>Study-related examinations aligned with routine care</u>

After the conformation of a viable pregnancy, participants proceed through the process of recruitment and allocation as described above.
Following informed consent, concomitantly with the regular obstetrical intake at *first antenatal care visits around 8 weeks of gestational age*, the first study procedures will be executed: maternal waist-hip ratio will be noted, baseline blood samples will be taken together with standard blood tests and urine will be collected for storage (to be tested at the end of the study). If separate permission is obtained, maternal blood and urine samples are stored according to the biobank regulations of the Erasmus MC. As explained in more detail in Section 8.2., allocated supplements will be supplied by a local investigator. Patients will be asked to discontinue their regular folic acid supplements and any supplements containing myo-inositol and to switch to the provided supplements. At the same time, patients will receive instructions for appropriate use of the supplements and information on when and how to contact the research team. At least, patients

will be asked to complete the first study questionnaire (see *F1.Vragenlijst I)* addressing nutritional status and use of dietary supplements, lifestyle (e.g. physical activities), PCOS diagnosis and associated symptoms, maternal mental health status (BDI-II-NL) and health related quality of life (EQ-5D-5L, PSOSQ).

As part of standard antenatal care, pregnant women will be seen between *10 and 13 weeks gestational age for their first-trimester ultrasonography* to calculate the estimated day of delivery.

Around the same time, preferable between *12 and 14 weeks gestational age*, an oral glucose tolerance test is performed (per local protocol).

A standard or advanced *20-week ultrasound* will be performed per clinical protocol. Any congenital abnormalities or polyhydramnios identified will be recorded.

Preferably along with an *appointment at 24-28 weeks of gestational age,* blood pressure (per protocol) and maternal weight is recorded and the oral glucose tolerance test is repeated (per local protocol) to ascertain the primary outcome. If oral glucose tolerance testing is indicated for other reasons, the indication and gestational age at time of performance is recorded.

At their regular appointment around 32 weeks, blood pressure (per protocol) and maternal weight is recorded. Blood and urine sampling will be repeated (and stored for biobanking if permitted) and it is ensured that foetal ultrasound examinations (foetal biometryand amniotic fluid) will be performed (if not indicated).

Around 36 weeks of gestational age, using the second study questionnaire (see *F1.Vragenlijst II),* tolerability of the nutritional intervention (e.g. any experienced side effects, any encountered issues in consumption and/or reasons for non-compliance with the supplement), maternal mental health and health related quality of life, will be (re)assessed.

During the study, foetal biometry ultrasounds will be performed per clinical protocol. In case of any arising complication during pregnancy, treatment following national guidelines will be allowed and patients will be allowed to continue their study supplement. Patients are advised to report any medication use during pregnancy to the study team and are regularly asked if they are taking other dietary supplements (which will also be addressed in the study questionnaires).

At birth maternal blood and offspring umbilical cord blood is taken. If separate permission is obtained, a maternal blood and cord blood sample, and cord and placental tissue, is collected for biobanking.

After giving birth participants can stop using the dietary supplements for this study.

Neonatal glucose monitoring to detect neonatal hypoglycaemia in the first 12-24 hours postpartum is performed in accordance with local protocol (usually performed in case of prematurity, asphyxia, SGA/LGA born babies, maternal GDM or maternal use of hypoglycaemic medication or in case of symptoms associated with hypoglycaemia etc.).

A *postpartum visit* is scheduled per local protocol around 5-6 weeks postpartum. During this appointment patients will be acknowledged for their participation and the remaining supplements will be collected. Maternal weight and blood pressure will be recorded. Blood and urine samples will be taken (and stored

for biobanking if permitted). Participants receive their last study questionnaire (see *F1.Vragenlijst III)* which consist of questions regarding tolerability of the nutritional intervention, compliance and barriers to adherence, maternal mental health status (BDI-II-NL), health related quality of life (EQ-5D-5L, PSOSQ) and breastfeeding in women with PCOS. This questionnaire will also be used to assess the cost-effectiveness of the nutritional intervention as explained in more detail below and in Section 10.2 If separate consent is obtained previously, breastfeeding mothers are asked to bring a breastmilk sample at their postpartum visit and to complete a validated questionnaire on breastfeeding around 6 months postpartum.

During the study, compliance with the nutritional intervention will be assessed by counting the number of sachets returned during a antenatal care visit between 24-28 weeks of gestation and 6 weeks postpartum. Good adherence is defined as a total number of supplements consumed of at least 80%. Tolerability of the nutritional intervention (e.g. any experienced side effects, any encountered issued with the consumption of the supplements and/or reasons for non-compliance with the intervention) will be examined using study questionnaires at 36 weeks of gestation and six weeks postpartum.

Any arising pregnancy complication will be recorded in the eCRF. To ensure that preeclampsia arising in the postnatal period will be scored, the definitive composite outcome will be assessed no earlier than one week postpartum.

To evaluate the cost-effectiveness of the nutritional intervention from a societal perspective, all resources used in maternity and neonatal care are recorded from initiation of supplementation up until 6 weeks postpartum. Using questions derived from the Medical Consumption Questionnaire (MCQ), medical consumption outside the hospital will be included.(77) To evaluate the patient perspective, questions derived from the EQ-5D-5L and the Productivity Cost Questionnaire (PCQ) are incorporated in the study questionnaire.(1, 78) The process of cost-effectiveness analysis will be explained in more detail in Section 10.2.

<u>Data and blood sample collection</u>

All other study data can be collected retrospectively from the medical records. Data collection will be done by trained research staff in accordance with the General Data Protection Regulation.

The urine samples, collected at different times during the study, will be stored and tested at the end of the study (after the last visit of the last included participant) to avoid associations with randomisation group during the study. Blood and urine samples will be processed using standardized equipment and protocols across the participating institutions.

Materials taken for biobanking will be encoded and stored at the biobank of the Erasmus MC in accordance with the code of conduct for responsible use (2011).

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

For withdrawal by the investigator the following specific criteria will be applied:

- A participant is unwilling or unable (e.g. because of vomiting) to take more than 60% of the supplements, evidenced by sachet counting.
- A participant suffers and adverse event which is assessed as possibly related to the intervention by the investigator.
- Based on other medical reasons.

If a participant is unable to attend study visits (for example if maternity care is relocated to midwives or outside the Netherlands) she will not be withdrawn from the study if consent is obtained to follow up on key outcome measures.

8.5 Replacement of individual subjects after withdrawal

Not applicable.

8.6 Follow-up of subjects withdrawn from treatment

In case of withdrawal of a subject, the reason for withdrawal will be documented. As explained in Section 8.4, participants are withdrawn if they do not give permission for follow-up. Therefore, no follow-up of withdrawn subject with ongoing pregnancies will take place.

8.7 Premature termination of the study

The study is classified as of 'negligible risk' requiring 'minimal monitoring', in line with previous studies in pregnancy and the minimal risks associated with the use of myo-inositol supplements.

Despite the minimal risk status of the intervention, the study will be additionally monitored by an independent Data and Safety Monitoring Board (DSMB) given the fact that the intervention is aimed at a vulnerable patient group (i.e. pregnant women). The DSMB will be asked to evaluate safety outcomes following the pre-specified risk-based monitoring plan, explained in more detail in Chapter 9. All serious adverse events will be reported to the DSMB and an interim-analysis on safety will be performed after 100 included participants have given birth. Premature termination of the study will be decided if there is sufficient ground that continuation of the study will jeopardise subject health or safety.

In case of premature termination of the study, advice of the DSMB and Medical Ethics Committee will be followed carefully. The intervention will be stopped in all participants and they will subsequently be treated according to standard patient care protocols.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor (Erasmus MC) will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the nutritional intervention.

All of the following adverse events reported spontaneously by the subject or observed by the investigator or his staff up until 6 weeks postpartum will be recorded:

- All complications of pregnancy.
- All adverse events that have a causal relationship with study procedures or are considered related to the nutritional intervention.
- Congenital anomalies or birth defects.
- Any other undesirable experience occurring to a subject that
  - is life threatening (at the time of the event);
  - requires hospitalisation or prolongation of existing inpatients' hospitalisation;
  - requires medical or surgical intervention, such as antibiotics, intravenous fluid therapy, surgical correction etc.; or
  - results in moderate to severe (temporary) disability or incapacity (e.g. temporary disability to work) or death.

Any other undesirable experiences occurring to a subject (e.g. mild contusions or superficial injuries), that do not meet the above criteria, do not require reporting.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation: an elective hospital admission or hospitalization for delivery will not be considered as a serious adverse event.

- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

The investigator will report all of the above SAEs to the sponsor without undue delay after obtaining knowledge of the events. SAEs will be reported through the web portal *ToetsingOnline* to the accredited METC that approved the protocol. SAEs that result in death or are life threatening will be reported within 7 days of first knowledge followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. The DSMB will be informed after each 5 SAEs.

However, so-called 'context-specific SAEs', that are inherent to the study population/setting, have no causal relationship with study procedures, and are expected to be equally distributed over both groups, do not require immediate reporting by the investigator to the sponsor. The following SAE's are considered context-specific:

- All SAE's that occur after recruitment, but before randomisation of the participant (e.g. late pregnancy loss).
- Hospitalisation of patients for common pregnancy complications not related to the intervention, such as: anaemia requiring blood or iron transfusion, sleep deprivation requiring sedation, (abdominal) trauma requiring foetal-maternal monitoring, congenital abnormalities requiring foetal monitoring etc.
- Hospitalisation of patients for complications not related to pregnancy, such as: pneumonia, traumata, pyelonephritis or gastro-enteritis etc.
- Hospitalisation of patients for common perinatal complications and postpartum indications, such as: hospitalisation < 48 hours for routine observation, urine retention, postpartum fever, postpartum haemorrhage, pre-existent cardiac/pulmonary problems requiring maternal monitoring, social/psychological reasons or hospitalisation of their new born child etc.
- Neonatal Medium care / NICU admission for common non-life-threatening conditions (not per se related to the intervention) and less than 48 hours, such as: hypo- or hypertension, hyperbilirubinemia, moderate hypoglycaemia, thyroid abnormalities, infection, respiratory problems.

Context-specific SAE's are included in the secondary and additional outcomes of this study and thereby recorded in the Case Report Form. They will be reported to the DSMB by line listening every half year and to the METC every year.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

In line with previous examples of earlier trials(47) and given the fact that the study is an interventional trial among pregnant women, an external, independent DSMB will be established to monitor the safety of participants in this study.

The DSMB will include experts in, or representatives of, the fields of relevant clinical expertise, clinical trial methodology and biostatistics. Members will be appointed for the duration of the clinical trial and are independent, thus have no conflict of interest with the sponsor or outcome of the study, as confirmed by a signed conflict of interest form. The members observe confidentiality with regard to all study information. The DSMB can request advice from additional experts (e.g. physicians, methodologists, epidemiologists), who need to sign a confidentiality statement. Before the study is started, the principal investigator will provide the DSMB with an overview of the basic characteristics of the study population (inclusion criteria), the main primary outcomes and the main safety data (AEs and SAEs). Based on this, a DSMB Charter describing the exact composition, tasks and responsibilities of the DSMB is established by common agreement between the two parties. A draft version of the DSMB Charter is provided in Section K of the METC dossier (*K5. Data Safety Monitoring Board Charter MYPP-trial).*

During the study, the principal investigator will report the external DSMB periodically in accordance with this charter. Based on its interim evaluation of the accumulating data with regard to the progress and safety of the study, the DSMB advises the sponsor via the principal investigator of modifications, continuation or termination of the study. Criteria on which the DSMB may decide to terminate the trial prematurely are defined in the DSMB Charter.

The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to (fully) implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

The independent external DSMB reports to the Execute Board at least once a year and ensures that the METC is informed promptly by the principle investigator.

Following completion of the study, the principal investigator informs the DSMB on the conclusions of the study (aim: within a month after completion of the last study procedures).

10. STATISTICAL ANALYSIS

Data will be recorded by trained research staff, using the eCRF, and collected in an access-controlled web-based database (OpenClinica), as explained in more detail in Section 12.1. The database will be validated by checking internal consistency and by the identification of data outliers. Prior to database closure, the project team of the study will finalise a Statistical Analysis Plan (SAP). Any revision in planned analyses will be clearly identified in the final SAP and issued prior to database lock.

After database closure, SPSS (SPSS version 21, IBM, Armonk, New York) and Excel will be used to perform statistical analysis and *p*-values of <0.05 are considered significant. For missing data, complete case analysis will be used as this achieves unbiased results with reasonable statistical coverage.(79) Only in the event of high missing rates or in case missing's are not at random, the multiple imputation methods will be used.

Analysis of the trial results will primarily be performed according to the intention-to-treat principle.

Secondly, a priori sensitivity analysis (per protocol) will be executed, omitting participants whom are withdrawn because of reluctance to continue with the trial or because they are unwilling or unable to take more than 60% of the capsules. When appropriate, and upon advice of the statistician within the project group, a per protocol analysis is performed.

10.1 Primary study parameter(s)

To achieve the primary objective of this study, the number of pregnancies complicated with either: i) gestational diabetes mellitus, ii) preeclampsia and/or iii) preterm birth, will be compared between the two study arms. The primary outcome will be the composite of the above quantitative parameters, as defined in Section 8.1.1. The potential benefits of myo-inositol supplementation (added to standard recommended folic acid supplementation) will subsequently be assessed by calculating the event rate of the primary outcome in the two groups using a chi-square test (or Fishers Exact Test as appropriate). Effectiveness of the nutritional intervention will be presented as absolute and relative risks, the relative risk reduction (along with 95% confidence intervals) and by assessing the number needed to treat (if applicable).

10.2 Secondary study parameter(s)

Secondary parameters concerning maternal physical and neonatal health, and most ultrasound parameters, are categorical and therefore described quantitatively by exact numbers and percentages and analysed in the same way as primary study parameters.

Continuous ultrasound parameters will be presented as medians (with interquartile ranges) if data is skewed and as means (with standard deviations) if normally distributed.

As described in Section 8.1.2., maternal mental health and health-related quality of life will be assessed in both groups using validated questionnaires. Results will be presented per group as means (with standard deviations) of total scores and sub-scores of the different dimensions/domains. Since it concerns

continuous variables, we will use the independent *t*-test (if the outcome is normally distributed) or a non-parametric Mann-Whitney U-test (if the outcome is skewed) to assess the potential differences between the groups.

Cost-effectiveness

In addition to this clinical evaluation, an economic evaluation will be conducted using Health Technology Assessment (HTA) analyses performed under the supervision of the HTA specialist within the project team, according to guidelines issued by the National Health Care Institute(80). This analysis will be performed from a societal and patient perspective and will therefore encompass the inclusion of both medical and non-medical costs, from inclusion until 6 weeks postpartum. We forecast that the nutritional intervention will result in two categories of cost-savings: i) decrease in costs for the treatment of pregnancy related complications and neonatal morbidity/mortality, ii) decrease in productivity impairment of mothers and/or fathers.

Cost-effectiveness analysis will be conducted as follow:

1) Data collection:

To analyse the cost-effectiveness of myo-inositol supplementation (added to standard recommended folic acid supplementation) compared to standard care (standard folic acids supplements only), future prospective data and baseline data previously collected, is demanded.

- Future prospective data collection: in the trial cohort of 464 PCOS patients we aim to collect all resources used from inclusion up until 6 weeks postpartum.
- Baseline/Additional data collection: to complement the future prospective data and validate our cost analysis of the control group, data will be collected from the COPPER study.(46) In this study the costs of PCOS-related complications (gestational diabetes, pregnancy induced hypertension, preeclampsia, HELLP-syndrome, preterm birth, aberrant birth weight, perinatal death) and long-term outcomes, were collected prospectively in at least 180 PCOS patients. Using this data, we will be able to improve costs analyses of pregnancy complications and long-term outcomes in the PCOS patients at baseline (control group).
- Total costs: the units of maternity care provided to PCOS patients in both cohorts will be multiplied by the cost per unit. For this, reimbursement prices issued by the Dutch Healthcare Authority (NZA) and national reference prices will be used as outlined in current Dutch pharmaco-economic guidelines.
- Quality of Life: the benefit of the nutritional intervention in patients interest's will be measured by assessing its effect on maternal health-related quality of life using questions derived from the EQ-5D-5L and the PCOSQ, examined at study inclusion, 36 weeks of pregnancy and 6 weeks postpartum.

2) *Cost-effectiveness analysis:*

Both costs and effect measures will be combined in a so-called decision tree to calculate incremental cost utility ratios. Eventually, deterministic and probabilistic sensitivity analyses will be performed to demonstrate and visualize uncertainty of our outcome measures. This will also result in the costs averted per complication and the total costs in both groups.

10.3 Other study parameters

Additional (baseline) maternal characteristics, including both descriptive and prognostic factors, will be compared between groups and presented in a baseline table.

Maternal characteristics recorded during pregnancy and delivery, parameters addressing perinatal and neonatal outcomes, blood and urine test results, will be presented and compared between the groups in the same manner as primary and secondary study parameters.

As described in more detail in Section 8.3, patient compliance will be assessed by sachet counting between 24-28 weeks of gestation and 6 weeks postpartum. Good adherence is defined as a total number of sachets consumed of at least 80% and the percentage of women affirmed to be 'compliant' will be compared between the groups. Any differences between the groups in tolerability of the nutritional intervention and/or barriers to adherence will be assessed using questionnaires and reported in a descriptive manner.

Breastfeeding rates will be calculated in both groups and presented as exact numbers and percentages. Any differences between the groups regarding breastfeeding success, duration and problems encountered during breastfeeding, will be assessed using questionnaires and reported in a descriptive manner.

Subgroup analysis

Subgroup analysis will be performed comparing women with hyperandrogenic PCOS and normoandrogenic PCOS.

Budget impact analysis

Budget impact analysis (BIA) of implementing the nutritional intervention (i.e. myo-inositol supplementation in addition to standard recommended folic acid supplementation) into the Dutch healthcare system will be calculated in line with the new guidelines (Zorginstituut Nederland NZa). The BIA will be assessed through (decision analytical) modelling and analysed in a probabilistic way, addressing the following perspectives:

- Societal perspective, using societal-CEA based prices.
- Health insurance/third party perspectives, using NZa average rates and other applicable rates such as specific passenger rates in case of local health care providers.
- Health care perspective (Budgettair Kader Zorg BKZ), using BKZ average rates according to NZa.

10.4 Interim analysis

An interim-analysis on safety will be performed when 100 included participants have given birth to evaluate the safety of the trial. This analysis will be executed by an independent statistician, blinded for the treatment allocation, whom will report to the DSMB. The independent DSMB will have unblinded access to all data and advises the sponsor via the principal investigator of modifications, continuation or termination of the study as described in the DSMB Charter (see K5 *Data and Safety Monitoring Board Charter MYPP-trial*).

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study involving human subjects will be conducted according to the ethical principles of the Declaration of Helsinki (Adopted by the 18th WMA General Assembly in June 1964 and amended by the 64th WMA general Assembly in October 2013), in accordance with the Medical Research Involving Human subject Act (WMO), ICH guidelines for GCP and other applicable regulations and Acts.

Local regulatory requirements include investigator reporting requirements and institutional review board approval. Data will be handled in compliance with Directive 95/46/EC (General Data Protection Regulation "GDPR"). Materials taken for biobanking will be stored and handled in compliance with the Code of Conduct for Responsible Use (2011).

11.2 Recruitment and consent

Eligible patients will be approached by their treating obstetrician, midwife or fertility physician for participation in this study. After receiving verbal information about the study and its procedures, potential subjects will be handed a Participant Information Form (PIF) and given sufficient time to read this. Additional information is provided by research staff members, not involved in further patient care, wherever possible. The PIF also contains contact information of local (independent) investigators in case additional information is requested. After reading the information supplied, patients will be asked for informed consent, preferably during their next appointment. Only in exceptional cases, in which in which first consultation takes place just before 16+0 weeks of gestational age, patients will be asked to decide during their first visit. Written informed consent is obtained by GCP trained research midwives, physicians or investigators. Participants will receive a hardcopy of the written informed consent, signed by both the participants and the physician or midwife involved.

Patients who are not willing to participate will be treated according to usual care.

The information letter and informed consent form are attached as documents E1/E2. of the METC dossier.

11.3 Objection by minors or incapacitated subjects

Not applicable.

11.4 Benefits and risks assessment, group relatedness

Despite consistent literature acknowledging the impact of PCOS on pregnancy outcome, due to the lack of supporting evidence, there are currently no effective risk reduction strategies available for pregnant women with PCOS and their offspring.

Myo-inositol supplementation as a nutritional intervention seems a simple, safe and effective first-line strategy to improve pregnancy outcome for women with PCOS. Optimal intake of myo-inositol is associated with reducing insulin levels and improving excessive testosterone synthesis associated with PCOS. In addition, three RCTs have established the effectiveness and safety in preventing pregnancy complications in PCOS-related disorders.(37)

This study will however be the first prospective trial to assess the effectiveness of myo-inositol supplementation as a nutritional intervention to prevent pregnancy complications among women with PCOS. During the trial, routine obstetrical care following national guidelines is recommended. In addition to receiving supplements, subjects will be asked to complete three questionnaires, provide two or three additional blood samples (depending on required samples per local protocol) and to provide two additional urine samples. If not demanded per local protocol, an ultrasound (foetal biometry) will be performed. Participants can provide separate consent to participate in biobanking of blood and urine samples, cord blood, placental and cord tissue for later scientific use in accordance with biobank regulations. All study related examinations are aligned with routine care visits.

Myo-inositol is a commonly used food supplement with no known (serious) adverse effects and reported to be completely free of side effects in the therapeutic dosage used in this study.(62-64). At present, no congenital malformations have been reported in animal and human studies.(81, 82) Given the extensive experience with its safe-use in previous research, we anticipate the risk of participation negligible.

Considering the lack of preventive interventions although the increased risks of patients is widely acknowledged, we are convinced that the results of this study will provide important novel recommendations for PCOS patients on the importance of optimising life-style and nutrient intake to prevent pregnancy complications.

In addition, biosampling and detailed phenotyping as part of this study provides opportunities to discover novel biomarkers of maternal and offspring health. It will proved extensive information on how maternal nutrition and metabolic state can promote offspring wellbeing. Follow-up of children of participants (beyond the first 6 weeks postpartum) is not part of this study protocol, but consent to allow for future follow-up will be sought during this trial.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

Not applicable.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Baseline characteristics, clinical data concerning maternal and neonatal health and blood test results will be recorded by trained research staff using the eCRF and collected using the access-controlled, web-based database OpenClinica.

In this database, data will be encoded using a non-speaking numeric code (randomisation number) that is randomly assigned in the process of treatment allocation. Randomisation lists containing patients' ID and corresponding randomisation number will be stored securely at each participating centre. The encryption key of the randomisation code for linkage to personal information, is only available to the local investigator and data manager of the participating centre. As explained in more detail in Section 8.2, the meaning of the randomisation numbers and corresponding batch-numbers (i.e. information on treatment allocation) is centrally held at the Erasmus Medical Center Rotterdam and solely accessible to the independent research staff member, responsible for labelling of the supplements. Source data can only be accessed by the research team, the datamanager, members of the safety committee, an independent monitor hired by the Erasmus MC and the Health and Youth Care Inspectorate (IGJ).

Further data handling will be done with coded data in compliance with the Dutch Personal Protection Act.

Collected and encoded data will be stored separately at each participating centre. Data extraction from the database will be executed using dedicated scripts at secured computers, directly allocating extracts to a designated access-controlled folder on the server of the institution.

Access to both the database and this folder will be limited to: investigators, research staff, the data manager, monitoring and quality assurance personnel and the external independent DSMB. For central analysis of the data created at the different participating centres, data extracts will be provided by local investigators. As incorporated in the participant information form, patients will give informed consent for future studies to use the generated data.

In addition, to allow for futures studies to follow-up on patients and their off-springs beyond the scope of this study, participants are asked for permission to be approached in the future. After obtaining informed consent, contact details are recorded and stored in a password-protected encrypted data file. This file will be stored at the sponsor site and will only be accessible to the principal investigator.

Data will be preserved for the duration of 15 years.

At least, materials taken for biobanking will be encoded and stored at the biobank of the Erasmus MC in accordance with the Code of Conduct for Responsible Use (2011). Before the use of materials stored at the biobank, future studies have to be approved by the accredited independent Review Committee of the Erasmus MC.

12.2 Monitoring and Quality Assurance

Following the ICH-GCP guidance, since the trial is not a Clinical Trial of an Investigational Medicinal Product but rather a clinical trial using dietary supplements as first-line nutritional intervention, monitoring is not legally obliged in accordance with Commission Directive 2005/28/EC. However, to guarantee high quality research and reliability of data a minimal intensive form of monitoring will be performed in compliance with Good Clinical Practice (GCP) and other rules and regulations.

Monitoring services will be provided by the qualified and independent monitor Julius Clinical. Julius Clinical is a unique academic research organization (ARO) that combines strong scientific leadership and operational excellence to conduct innovative national and global clinical trials. Over the past decade, it has provided monitoring services for 110 Investigator Initiated Trials and are currently involved in 47 studies with up to 900 participants. To guarantee compliance with GCP, project and regulatory requirements, it uses their own Standard Operating Procedures (SOPs). Due to the nature of the intervention, and the risk of (serious) adverse events with myo-inositol supplementation non-existing, this trial was classified as having 'negligible risk' according to the Dutch Federation of Universities (NFU) guidance 'Quality Assurance of Human Subject Research', requiring minimal monitoring. Using guidelines from the NFU, Julius Clinical has established a site specific monitoring program. Based on this plan, monitoring visits in each participating site will be performed every half year on average. To assure the quality of research data at participating sites, this independent monitor will have access to the data and source documents of the trial. For details, see the provided monitor plan in Section K of the METC dossier (*K6. Monitoringplan MYPP-trial)*.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.4 Annual progress report

The principal investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on: the date of inclusion of the first subject, numbers of subjects included, numbers of subjects that have completed the trial, serious adverse events, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The principal investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The investigator will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the investigator will notify the accredited METC, the competent authority and funder within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the principal investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

This trial will be registered in the Dutch public trial registry. In agreement with the funder ZonMw, study results will be disseminated as soon as possible. Results will be presented on international congresses and published in international scientific journals. In addition, upon advice of the Dutch PCOS Foundation, results may be shared with PCOS communities on social media.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable.

13.2 Synthesis

Not applicable.

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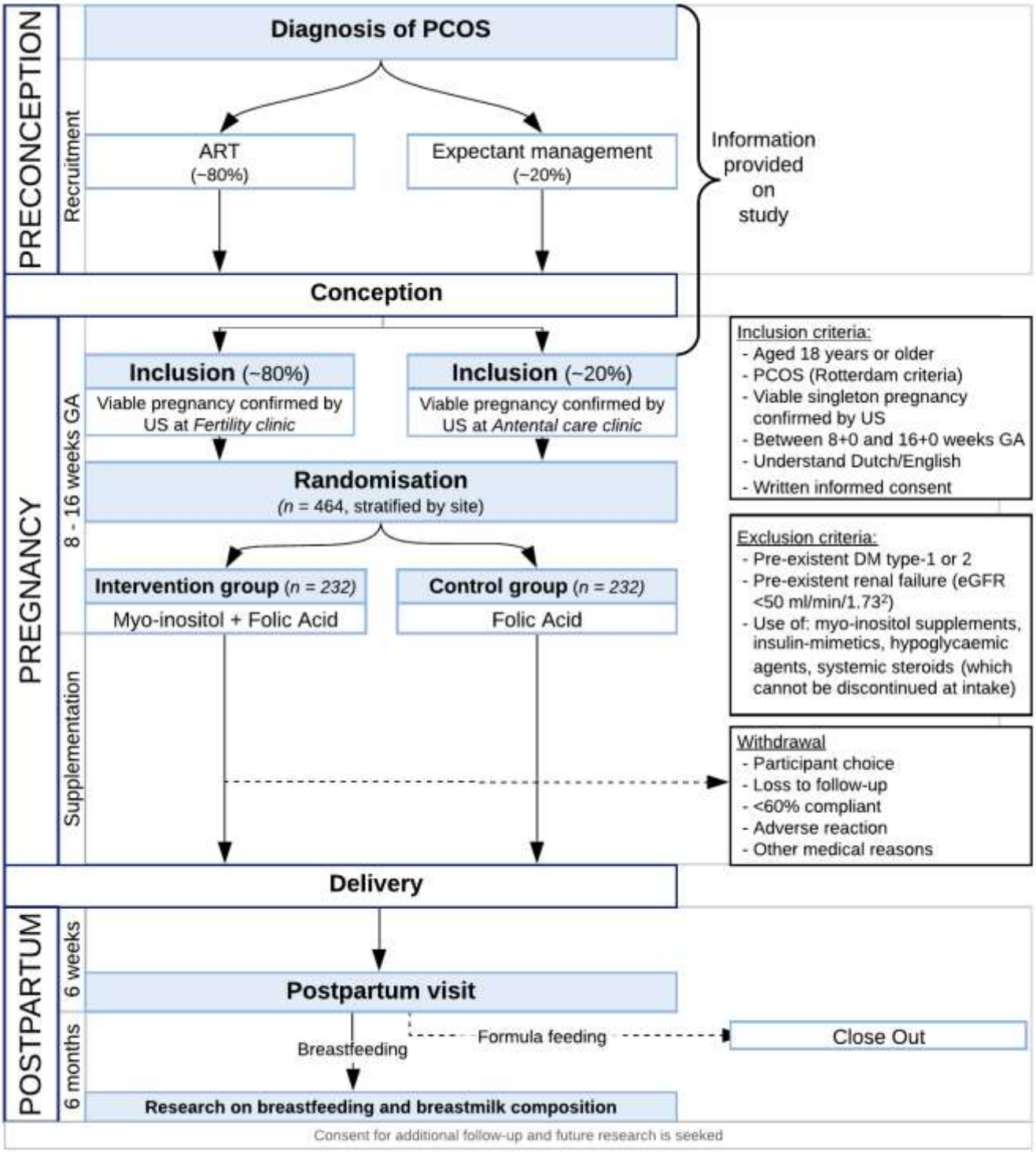
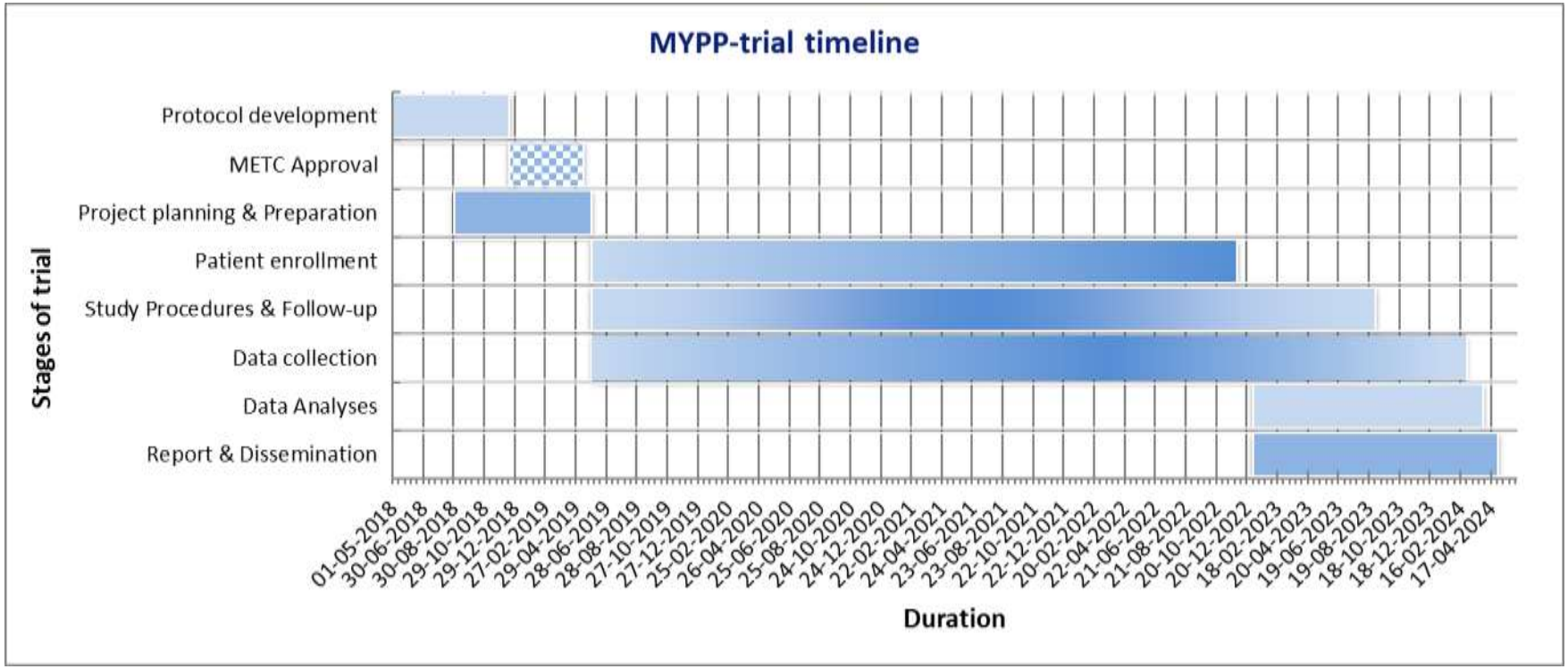
APPENDIX I : STUDY DESIGN



Abbreviations: *GA* Gestational Age, *ART* Assisted Reproductive Treatment, *US* Ultrasound, *DM* Diabetes Mellitus

APPENDIX II : MYPP-TRIAL TIMELINE



APPENDIX III : PCOS DIAGNOSTIC CRITERIA

PCOS is diagnosed according to the Rotterdam consensus criteria(9), when:

A. *At least two of the following three criteria are present:*

1. Oligo-/amenorrhoea	▪ *Oligomenorrhoea*: 35-182 days and ▪ *Amenorrhoea*: >182 days
2. Hyperandrogenism	▪ *Clinical*: hirsutism defined as Modified Ferriman Gallwey score ≥ 4-6 (10) (whether or not accompanied by acne and alopecia) and/or ▪ *Biochemical*: elevated Calculated Free Testosterone or Free androgen index > 4.5 (10) (FAI = total testosterone x 100)/SHBG
3. Polycystic ovaries on transvaginal sonography	▪ *Volume*: one or two ovaries > $10cm^3$ and/or ▪ *Follicle count* (2-9mm): one or two ovaries ≥ 12 follicles per ovary ǂ

AND

B. *Other aetiologies of the above criteria are excluded,* such as thyroid disease, hyperprolactinemia, androgen-secreting tumors, Cushing's syndrome and non-classing congenital adrenal hyperplasia*.

* Exclusion of other causes requires TSH, Prolactin levels, FSH and, if clinical status indicates other causes need to be excluded.

ǂ For this criteria we have not adopted the new recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome (2018), as most patients have been diagnosed using the previous recommendations.

APPENDIX IV : COMPOSITION OF THE DIETARY SUPPLEMENTS

Table I – *Composition of dietary supplement intervention group*

Ingredient	Function	Per sachet (mg)	Daily dose (mg)
Myo-inositol	Ingredient	2000	4000
Folic acid	Ingredient	0.20	0.40
Silicon dioxide	Anti-caking agent (E551)	2.5	5.0
Maltodextrin	Filling agent	97.27	194.54
Total		2100	4200

Table II – *Composition of supplement control group*

Ingredient	Function	Per sachet (mg)	Daily dose (mg)
Folic acid	Ingredient	0.20	0.40
Maltodextrin	Filling agent	2096	4192
Silicon dioxide	Anti-caking agent (E551)	2.29	4.58
Sucralose	Sweetener	1.28	2.56
Total		2100	4200

Myo-inositol has been replaced by maltodextrin. A small amount of sucralose has been added to gain comparable sweetness.

All of the supplements are produced under the same Good Manufacturing Practice conditions and provided as a neutral tasting powder in sachets, packed in boxed containing 60 sachets each. When supplied to the Erasmus Medical Center Rotterdam, the indistinguishable boxes will report the expiry date and batch number only. At the Erasmus MC the packaging will be labelled with individual, traceable codes, as explained in more detail in Section 5.1 of this protocol.

APPENDIX V : GENERAL INFORMATION ON MYO-INOSITOL

Biology and nutrient status

Myo-inositol (EC no. 201-781-2 and CAS no. 87-89-8) is a naturally occurring substance, present in animal, human and plant eukaryotic cells. Myo-inositol is a white, crystalline, odourless powder with a very slight sweet taste. This water-soluble molecule (molecular formula: $C_6H_{12}O_6$) has a structure quite similar to glucose and belongs to the so called 'inositol family'. Inositol occurs naturally in nine stereo-isomers, of which myo-inositol is the most abundant form in nature. Myo-inositol can be absorbed from practically all food derived from plants and animal, but in particular from fresh fruits and vegetables (especially when containing seeds). In the intestinal mucosa, it can be transformed into the different phosphate forms of inositol by enzymes phytases.

A standard diet contains up to 1500 milligrams of myo-inositol daily.(83) Myo-inositol has historically been classified as part of the vitamin-B complex and is considered safe-to-use. Dietary myo-inositol is currently, without prescription, available as an over-the-counter-product in several countries (e.g. the United States) and can be purchased online.

In humans, myo-inositol is synthesised *de novo* from glucose-6-phosphate in many tissues including the testis, brain and liver, but mainly in the kidney. About 4 grams of myo-inositol is daily produced by the kidneys alone.(30) Because in the overall population the endogenously produced amounts are sufficient, myo-inositol is no longer regarded an essential nutrient or vitamin. However, in patients diagnosed with PCOS, as well as in other condition related to insulin resistance, myo-inositol depletion is described.(34, 35, 42)

Myo-inositol is incorporated in the cells of different tissues, especially the thyroid, pituitary, liver, spleen, ovaries and all organs of the male reproductive tract except for the testis.(84) Besides being a component of cell membranes, myo-inositol and its derivates have an important physiological role in various cellular processes by acting as second messengers. Relevant biological functions within the scope of this study are its role in the insulin-signalling pathway, the regulation of free androgens and cholesterol levels and in increasing quality of oocytes and embryos.

Categories

In European countries, myo-inositol is registered as a dietary supplement and is authorised under Commission Regulation (EC) No. 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses.

Safety

Myo-inositol has been reviewed by the FDA, and is in accordance with §184.1 of the Code of Federal Regulations (Title 21, Chapter I, Part 184) affirmed as Generally Recognized as Safe (GRAS) as a direct human

food ingredient. Chemical safety assessments have been performed by the European Chemicals Agency (ECHA). Based on the available data on degradation and mammalian toxicity, the ECHA stated that myo-inositol is *not* Persistent Bioaccumulative Toxic (PBT), *neither* very Persistent and very Bioaccumulative (vPvB). No hazards were identified in case of acute/short term exposure or long term exposure via inhalation, dermal and oral route.(85) Although toxicological studies are limited, based on the results of non-clinical and clinical studies with used dosages ranging from 4 to 30 gram daily, no serious side effects are known, even for the high dosages up to 18 grams daily. (62, 63)
Myo-inositol has not been associated with congenital malformations in animal and human studies.

Recommended dietary allowance

As stated by the U.S. FDA in the code of Federal Regulations (Title 21, Chapter I, Part 184, §184.1) "Myo-inositol can be used in food with no limitations other than current good manufacturing practice".(86)
Despite its wide clinical use, there is currently no Recommended Dietary Allowance (RDA) for myo-inositol. Based on previous research and quantities synthesised *de novo* in healthy human (over 4 grams per days), as a food supplement a daily intake of 500-4000 mg is generally recommended for adults. Neurological usage of inositol tends to require even higher doses (14-18 grams daily).

Side effects

Although toxicological studies are limited, based on the results of non-clinical and clinical studies with used dosages ranging from 4 to 30 gram per day over 1 to 12 months, no serious side effects are known.(62)
Based on the study by Lam et al., in which subjects were administered in increasing doses from 12 to 30 grams per day for lung cancer chemoprevention, the maximum daily dose without adverse effects was established at 18 grams per day.(63)
Myo-inositol is generally well tolerated and the amounts commonly used in clinics (4 grams per day) are reported to be completely free of side effects. Whenever side effects are present (only observed at dosages exceeding 12 grams per day), they are mild, likely to be non-specific (e.g. tiredness) and mainly gastro-intestinal in nature (e.g. nausea, flatus and diarrhoea).(62-64)

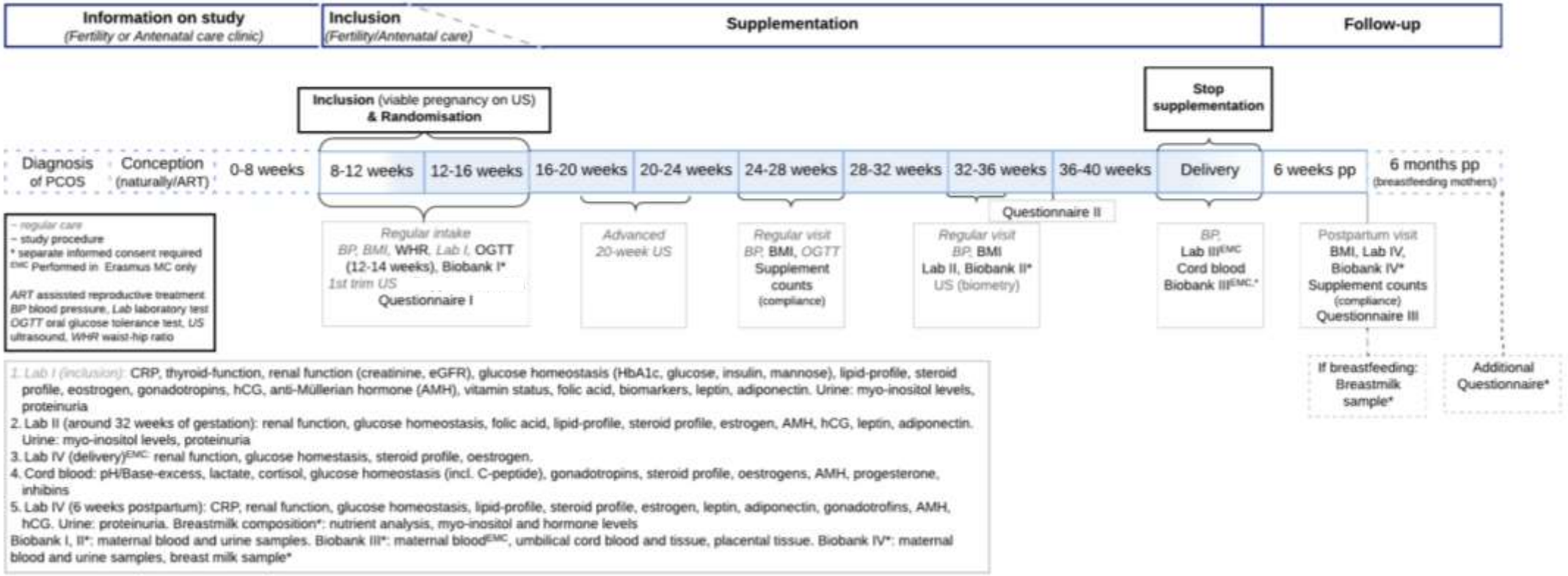
Interactions and/or precautions

Myo-inositol overuse (supplementation) is best avoided in persons with bipolar disorder as concerns exist of an association with excessive use and exacerbation of symptoms, although there is no convincing evidence to support this claim. There are no reported allergies for myo-inositol. There are no known interactions with drugs or food.

Summary of findings from non-clinical studies

In preclinical studies, experimental models in both rhesus monkeys(52) and rats(87) with type-2 diabetes mellitus have confirmed associations of this metabolic disorder with intracellular inositol depletion (i.e. decreased amounts of urinary excretion). A number of studies have shown that administration of inositol stereoisomers was able to lower post-prandial blood glucose levels in animal models mimicking diabetes.(33, 88) Ortmeyer et al. was the first to demonstrate that supplementation of the stereoisomer myo-inositol resulted in improving glucose balance in insulin-resistant rhesus monkeys.(31, 33) This has since been confirmed by others using high doses of myo-inositol, either intermittently or chronically administered.(89) In vivo tests showed that this effect can probably be attributed to improved peripheral insulin sensitivity due to enhanced GLUT-4 translocation to the plasma membrane in response to hyperglycaemia in skeletal muscle cells.(89, 90) Myo-inositol supplementation in animal models is not associated with any signs of harm, and no side effects related to reproduction, congenital defects or other deformities have been reported.

APPENDIX VI : STUDY TIMELINE AND PROCEDURES



1. Lab I (inclusion): CRP, thyroid-function, renal function (creatinine, eGFR), glucose homeostasis (HbA1c, glucose, insulin, mannose), lipid-profile, steroid profile, eostrogen, gonadotropins, hCG, anti-Müllerian hormone (AMH), vitamin status, folic acid, biomarkers, leptin, adiponectin. Urine: myo-inositol levels, proteinuria
2. Lab II (around 32 weeks of gestation): renal function, glucose homeostasis, folic acid, lipid-profile, steroid profile, estrogen, AMH, hCG, leptin, adiponectin. Urine: myo-inositol levels, proteinuria
3. Lab IV (delivery)[EMC]: renal function, glucose homestasis, steroid profile, oestrogen.
4. Cord blood: pH/Base-excess, lactate, cortisol, glucose homeostasis (incl. C-peptide), gonadotropins, steroid profile, oestrogens, AMH, progesterone, inhibins
5. Lab IV (6 weeks postpartum): CRP, renal function, glucose homeostasis, lipid-profile, steroid profile, estrogen, leptin, adiponectin, gonadotrofins, AMH, hCG. Urine: proteinuria. Breastmilk composition*: nutrient analysis, myo-inositol and hormone levels

Biobank I, II*: maternal blood and urine samples. Biobank III*: maternal blood[EMC], umbilical cord blood and tissue, placental tissue. Biobank IV*: maternal blood and urine samples, breast milk sample*