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# The impact of a nutritional supplement (Impryl®) on male fertility – study protocol of a multicentre, randomized, double-blind, placebo-controlled clinical trial (SUMMER-trial)

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## ABSTRACT

**Introduction** Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children. In approximately 50% of infertile couples a male factor is involved. Most of the male infertile cases are characterized as “idiopathic”, except for a small percentage of cases which are causative by a genetic etiology. In the past decade, the role of oxidative stress related to sperm quality has been researched thoroughly and estimated to be the problem in 30% to 80% of male infertility cases. Impryl® is a nutritional supplement which works on the metabolic system and regulation of oxidative stress by activating the 1-Carbon cycle and therefore recycling of homocysteine.
We hypothesize that the nutritional supplement Impryl® in men of infertile couples might improve the ongoing pregnancy rate.

**Methods and analysis** We designed a multicentre, randomized, double-blind, placebo-controlled clinical trial. We aim to include 1200 male adults, age 18-50 years, part of a couple that is diagnosed with infertility. The couple will either start or has already been started with fertility treatment, i.e. expectative management (EM, duration 6 months), intra-uterine insemination (IUI) with or without mild ovarian stimulation (MOH) or ovulation induction (OI), either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment. Male participants will be randomized in either Impryl® or placebo group, with identical appearance of the tablets to be distributed (doses: one tablet each day), for a total duration of maximal 6 months. Intervention has to be followed for at least 3 consecutive months before using semen for IVF or ICSI. In case of EM or IUI, patients can start directly with fertility treatment and/or natural conception. The primary outcome is the number of ongoing pregnancies confirmed by ultrasound at ≥ 10-12 weeks, and conceived in the time window between randomization up to and including month 6 of intervention use. Secondary outcomes are change in semen parameters between baseline and after 3 months intervention in IUI/IVF/ICSI group, based on (pre-wash) total motile sperm count (TMSC). Furthermore the number of pregnancies conceived in the optimal intervention time window, overall number of pregnancies, time to pregnancy, embryo fertilization rate in IVF/ICSI, embryo-utilization rate in IVF/ICSI, number of miscarriages, live birth rate and adverse events are documented within the study period of 15 months.

**Ethics and dissemination** The protocol is approved by the local medical ethical review committee (METC) at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects (CCMO). Findings will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of medical care and quality of life for patients with infertility.

**Trial registration number** NCT03337360 / NTR6551


**Keywords** nutritional supplements, fertility, male infertility, oxidative stress, clinical pregnancy rate, live birth rate

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- This multicentre randomized double-blind, placebo-controlled trial will provide important information to patients with diagnosis of male infertility and clinicians about the efficacy of nutritional supplement Impryl® with combined antioxidants
- With a sample size of 1200 patients this is up to date the largest randomized study with the use of nutritional supplements in fertility patients
- Permuted block randomization ensures treatment group numbers are evenly balanced, with stratification for centre and type of infertility treatment
- The use of diagnostic tests to screen for oxidative stress in semen samples (like sperm DNA-fragmentation tests or 8-OH-dG levels) could be of use in predicting the effect of Impryl® in different patient groups. However, these tests are not used in this study due to their complexity and lack of standardization and validation

## INTRODUCTION

Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children.[1] In approximately 50% of infertile couples a male factor is involved.[1,2] In the past decade, the role of oxidative stress on sperm has been researched thoroughly and found to be the problem in 25% to 87% of male infertility cases.[3-9]

All cells in the human body use oxygen to survive. In this process, toxic radicals with high reactive activity, so called reactive oxygen species (ROS), are produced as a consequence.[10] To scavenge these toxins the body has developed a defense mechanism in which antioxidants play an important role. The production of ROS in sperm is a normal physiological process mostly regulated by cellular en extracellular antioxidative factors. However, when the ROS production overwhelms the natural antioxidant defense, increase of apoptosis is observed. Increased oxidative stress can occur due to environmental and lifestyle factors such as smoking, stress, obesity and nutrition.[10] In the reproductive tract, semen leukocytes and immature spermatozoa are major sources of ROS production.[11] However, a low production of ROS is also physiological and needed for adequate sperm functioning by supporting capacitation, maturation and hyperactivation.[12] Spermatozoa are more vulnerable and prone to oxidative stress compared to other body cells because of the lack of cytoplasm.[13] Cytoplasmic antioxidant enzymes are important for directly scavenging and repairing the damage of ROS. However, in the spermatozoa the cytoplasm is removed during the final stages of spermatogenesis, leaving them without these important enzymes to protect them from ROS altering the sperm deoxyribonucleic acid (DNA). Furthermore, the membrane of spermatozoa is rich in fatty acids, making them vulnerable for lipid peroxidation by ROS resulting in decreased flexibility of the sperm membrane and reduction of tail motion.[13] For these reasons, spermatozoa are dependent on seminal plasma which is rich in antioxidants. Examples of antioxidants already present within seminal plasma are ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione, amino acids (taurine, hypotaurine), albumin, carnitine and carotenoids.[14] Despite the common association between male infertility and oxidative damage, there is no World Health Organization (WHO) recommendation about which test to use for detecting sperm DNA damage and there are no standardized protocols which could help global interpretation of the results. Men with sperm subject to increased oxidative stress may have normal seminal parameters, however with a poor chance of natural conception [15,16] Sperm DNA fragmentation (SDF) tests are available, however most of them are expensive (roughly 200-300 USD per test), complex and lack standardization and validation.[17] Furthermore, a recent meta-analysis showed that an association does not imply an actual predictive value.[17] Examples of SDF test are deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), the COMET assay or sperm chromatin structure assay (SCSA) and another option is measuring the byproduct of DNA oxidation called 8-hydroxydeoxyguanosine (8-OHdG).

A recent Cochrane review suggested that antioxidant supplementation in male infertility might improve the outcomes in assisted reproductive technologies (ART).[18] However, the

evidence was rated as low and clinical studies showed contradictory results with sometimes even a negative effect of high doses of antioxidants due to reductive stress as a rebound effect.[19] Alternatives were therefore explored, to support the natural antioxidant defenses that are predicted to act within the modulation of the natural cellular homeostasis without generating rebound effects. For example, the use of nutritional supplements supporting DNA methylation and the homocysteine pathway. Homocysteine is the end-product of the 1-Carbon cycle, feeding DNA methylation, and the starting substrate for the thiol glutathione (GSH, l-γ-glutamyl-l-cysteinyl-glycine) de novo biosynthesis. GSH is the most important endogenous antioxidant, involved in maintaining the antioxidant balance in human tissues and directly involved in the elimination of ROS.[20] Homocysteine is an inhibitor of the methylation process and a powerful pro-oxidant. It has a negative effect on spermatogenesis and its concentration in the ejaculate is inversely correlated with fertility outcome.[21,22] Dattilo et al stated that the ideal supplement should work by favoring homocysteine recycling by restoring the efficiency of the 1-Carbon cycle, therefore ensuring the availability of activated methyl groups for DNA methylation and feeding the intracellular antioxidant system by supporting GSH synthesis.[23] In non-randomized pilot studies with such a 1-Carbon cycle supporting nutritional supplement Condensyl®, the precursor of Impryl®, there was a significant decline of DNA fragmentation index leading to an improvement of the clinical pregnancy rate. However, the quality of these studies for the impact on pregnancy rate is rather low due to the non-randomized nature and having no control group.[21,24,25] Furthermore, the ideal parameters to measure DNA-damage might not be DNA-fragmentation, but sperm-oxidation level which indirectly affects DNA-damages.[26,27]

Impryl® is a nutritional supplement mainly consisting of vitamin B, which works on the metabolic system by activating the 1-Carbon cycle and recycling of homocysteine without the use of any direct strong antioxidant. Therefore it is could be more effective than other nutritional supplements. The cost of Impryl® is 30 euro for each 30 days of use.

It is well known that assisted reproduction technologies are expensive with the cost per in vitro fertilization (IVF) treatment in the Netherlands estimated to be between 2,885 and 5,259 euro (€) and the cost of an intra-uterine insemination (IUI) cycle between 497€ and 1123€, depending on the use and doses of medication during ovarian stimulation.[28-30] Therefore, substantial cost savings could be made if the use of relatively inexpensive nutritional supplements would lead to a shorter time to pregnancy with less treatment cycles necessary or even better, when the use of more expensive invasive reproductive techniques can be avoided at all.

## METHODS AND ANALYSIS

### Study design

This investigator-initiated, multicentre, placebo-controlled study has a double-blinded design, with blinding of participants and investigators. We conduct this study in 15 academic and non-academic hospitals in The Netherlands, at the Department of Gynaecology and/or Reproductive Medicine.

The study protocol was designed using the recommendations of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[31]

The study is currently ongoing; the first participant was included on 30 May 2018. We expect to include the last participant in June 2022 and study completion by December 2023.

### Participants

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

Inclusion criteria:
- Couples with failure to conceive for at least 12 months and starting with expectative management (EM) **or** couples starting with 1st/ 2nd/3rd cycle of IUI (with or without mild ovarian stimulation) **or** 1st/ 2nd/3rd cycle of IVF or intracytoplasmic sperm injection (ICSI) - Male with age 18-50 years
- Female partner with age 18-43 years
- Both male and female are willing and able to give informed consent

Exclusion criteria:
- Planned or performed TEsticular Sperm Extraction (TESE) or Percutaneous Epididymal Sperm Aspiration (PESA)
- Use of donor-, cryopreserved- or electro-ejaculated semen or donor oocytes
- Ovulation induction (OI) without IUI
- IVF for an absolute tubal factor
- Embryo-transfer after pre-implantation genetic diagnosis
- Known endocrine abnormalities related to male infertility, or use of fertility enhancing drugs such as clomiphene citrate or follicle-stimulating hormone (FSH)
- Known genetic abnormalities related to male infertility
- Known urological abnormality such as a varicocele or bilateral cryptorchism
- Use of other vitamins or nutritional supplements with antioxidant effect

Withdrawn or unblinded participants will not be replaced. They will not be re-included in the study once they dropped out, and their identification number and treatment will not be reused.

**Sample size calculation**

We aim to recruit 1200 male participants as a part of an infertile couple, based on the following data and power calculation.

Overall, the number of ongoing pregnancies in IVF and ICSI in the Netherlands was respectively 19.8% and 21.5% per started fresh cycle in 2015.[32] The number of ongoing pregnancies in IUI is estimated to be around 5 to 13% per cycle, around 18% after three cycles and 20-30% after six cycles.[33-35] Based on Radboudumc data, the ongoing pregnancy rate in EM is estimated around 20% after 6 months.

A Cochrane review estimated the increase in clinical pregnancy rates after use of antioxidants with an Odds Ratio (OR) of 3.43 (95% CI 1.92 to 6.11) based on 7 RCTs with in total 522 men.[36] However, this concerns low quality evidence, and the included population in this Cochrane review was couples with male factor subfertility, whereas in our study we also include couples with unexplained infertility. Therefore, we assumed a more conservative OR of 1.5. Assuming a 20% ongoing pregnancy rate in the placebo group, this reflects in an expected pregnancy rate of 27.3% in the Impryl® group.

The study is designed as a superiority trial. Based on the above mentioned data, we expect a 7.3% increase in ongoing pregnancy number when men are treated with Impryl® compared to placebo. However, in the EM group we expect a difference of 5% and in the IUI group of 6.5%, due to the fact that the participants can simultaneously start with intervention and start to conceive. In the first months the effect of the intervention is still suboptimal due to the duration of the spermatogenesis (72 days before whole renewal). Because the first IVF/ICSI-cycle in this study is only allowed in the optimal time-window after 3 months post-randomization, we assume an increase in this group of 7.5%.

EM: increase from 20% to 25% (cumulative incidence after 6 months)
IVF/ ICSI: increase from 20% to 27.5% (after 1 cycle with fresh embryo-transfer)
IUI: increase from 20% to 26.5% (cumulative incidence after 6 inseminations)

Based on the number of new patients treated at Radboudumc and the other sites each year, we expect a ratio between the 3 fertility treatment groups (EM: IVF/ICSI: IUI) of 1:2:3. To test the effect of Impryl® on the probability of ongoing pregnancy, a sample size of 600 men per treatment group (1200 in total) is needed, assuming differences in ongoing pregnancy rates of 7.5% (IVF/ICSI), 5% (EM) and 6.5% (IUI) and an expected ongoing pregnancy rate of 20% in the placebo group, a two-sided alpha of 5% and a beta of 24% (i.e. 76% power). As we expect the number of participants lost to follow up to be minimal, we will allocate 600 patients to both the intervention and the placebo arm. Figure 1 shows the distribution of the number of participants for the 3 different types of fertility treatment (EM, IVF/ICSI and IUI).

**Investigational product/intervention**

Impryl® tablets will be produced by Labomar SRL (compliant with European Good Manufacturing Practice (GMP) who will also produce an identical looking placebo tablet. Impryl® is a food supplement and is therefore not considered an investigational medicinal product. To assess product quality, we have quality assessment documents of both the food supplement and placebo: Summary of Product Characteristics (SPC, see supplementary file), Certificates of Analysis (CoA), certificate of Good Manufacturing Practice (GMP) and ISO 9001 form. Intervention will either start directly after completing diagnostic work up in new couples (IUI) or after at least 3 months of using study medication before using semen for a new IVF/ICSI cycle. When EM is applied, patients start directly with study medication for a total duration of maximum 6 months.

All couples will receive our standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology. After diagnostic work up couples will either start with EM (6 months), IUI, IVF or ICSI. Participants will take study supplement for a maximum of 6 months, even if the fertility intervention (6 x IUI of 1 x IVF/ICSI cycles) is not completed.

Co-treatment with other vitamins or supplements is prohibited. If vitamins or supplements are already used by men, a wash-out period of 3 months is recommended. Patients using other supplements will be excluded or reported as protocol violation If after randomization it appears that a patient still uses other supplements (reported in the online questionnaire), they will be telephonically contacted and asked to stop use of other supplements. After this stop, there is a wash-out period of 72 days (duration of spermatogenesis). If no pregnancy occurs within this wash-out period the patient and data can still be included.

**Randomization and treatment allocation**

All couples visiting a fertility specialist for the first time or for evaluation of fertility treatment will be informed on this study. Prior to or at their actual appointment they will receive the patient information (PIF) from the fertility specialist (specialised nurse or doctor). Randomization will be performed, after signed informed consent has been obtained, using a web-based application (Castor). We will use permuted block-design, stratified for fertility treatment (1st) and recruiting centre (2nd). Block sizes are flexible. Participants will be randomized in a 1:1 ratio to study medication (Impryl®) or placebo. Assignment to medication, using the randomization list, will either performed at the local centre or at Radboudumc by an independent person. This person is neither participating in clinical treatment nor processing the study data. Randomization outcome is either A (Impryl®) or B (placebo).

Coding starts immediately after informed consent has been obtained. In Castor each patient will receive a combination of one letter and 3 numbers. The code will not provide any information about the received intervention (Impryl® or placebo). The list of codes corresponding with patient information and received medication will be saved in an separate

file, locked (either digital with password or a locked cabinet), only accessible for the independent person who did the randomization.

The study is double blinded. All personnel, the researchers and patients will remain blinded to the intervention being received, except the personnel performing randomization and distributing the study medication. The indications for breaking the randomization code are: serious adverse event (SAE), serious adverse reaction (SAR) and Suspected unexpected serious adverse reactions (SUSARs) SUSAR, as instructed by the local medical ethical review committee (METC), or in a dire emergency, as directed by the principal investigators or trial manager. Every site has access to a deblinding form in which patients details, reason for deblinding, statement of principal site investigator, date and time of intervention stop and randomization allocation will be reported. The principal site investigator will inform the coordinating investigator about the deblinding.

At all study sites the person distributing the study medication is an unblinded employee of the department of Reproductive medicine or Gynaecology who is not involved in the treatment of the patient nor involved in the data collecting of the research. Drug accountability will be performed in line with Good Clinical Practice (GCP) requirements. The investigator is responsible for drug accountability and these tasks will be delegated to the primary investigators at the sites. We will log all dispensing of the investigational product on a drug accountability log. On every distributing study site there will be a batch of study medication stored at room temperature, no special precautions. An unblinded authorized employee of the department of Reproductive medicine (or Gynaecology) will distribute the study medication after randomization has been performed by Castor. The flag label (identification A = Impryl® or B = placebo) will be removed from the box and the randomization number noted (handwritten) on the medication box. Thereafter, the box will be handed over to the patient. On the patient identification & drug accountability log (either digital or on paper) the following details will be reported: date of issue, study (randomization) number, batch number, expiration date, amount dispensed, current storage amount, randomizer/distributor initials, hospital number, patient name and initials, date of birth and monitoring check.

All unblinded personnel performing randomization will be trained by a site initiation to perform randomization in Castor EDC and distribution of medication. A log list of all personnel involved in the study, with blinding status, responsibilities and signature, will be saved.

Unblinding will be performed when the study has ended, database is locked and protocol violators have been defined.

Patients are not asked to return study medication because this would mean an extra burden for the patients: an extra visit for all EM patients and some IUI/IVF/ICSI patients. Furthermore, patients could also forget a strip at home that they did not collect or put in the box. Since it is a supplement and not medication there are no specials precautions for destruction or disposal.

**Participant timeline and intervention**

All couples will receive standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology (NVOG). After diagnostic work up couples will either start with EM (6 months), IUI, IVF or ICSI, according to the NVOG guidelines. Participants will take study medication (Impryl® or placebo) for a maximum of 6 months, even if the fertility intervention (6 x IUI or 1 x IVF/ICSI cycle) is not completed. Use of the food supplement or placebo will be stopped earlier when a pregnancy is achieved. Participants will be followed for 15 months after randomization for detecting live births. Figure 2 shows the participant timeline.

During the study, participants are asked to report on baseline characteristics, lifestyle changes and pregnancy outcomes by short online questionnaires.

- Baseline: directly after randomization, patients will receive an automatic email invitation to provide baseline information online in the Castor database system.
- Monthly: during use of study medication (six months), participants will be asked to report on occurrence of pregnancy (with date of positive test), changes in lifestyle, consumed total amount of study medication (used boxes and tablets) and occurrence of adverse events. They will receive an automatic email invitation.
- Follow-up: 15 months after randomization participants will receive an automatic email with invitation for the last short questionnaire. They will receive questions about fertility treatment and pregnancy outcome (ability to achieve a pregnancy, occurrence of miscarriage or childbirth, date of delivery, gestational age, mode of delivery, complications of delivery and birth weight, sex, congenital abnormalities and health of the neonate.

At least 2 semen analyses will be performed at two time points in this study: the first semen analysis will be performed during diagnostic work-up (standard care, analysis according to WHO criteria[37]) and the second one after approximately 3 months of using study medication for participants having the IUI or IVF/ICSI treatment. We decided not to perform a second semen analysis in the EM group due to the fact that they present with normal semen parameters at intake and we want to avoid the burden of an extra visit.

If a clinical pregnancy is achieved, a routine ultrasound will be performed in the first trimester between 5 to 9 weeks to determine the vitality of the fetus. A second routine ultrasound will be performed around 10-12 weeks to estimate the due date. To minimalize the amount of extra site visits, we decided that this standard 'due date' ultrasound at 10-12 weeks of pregnancy is enough for determining the primary outcome (ongoing pregnancy). The ultrasound can be performed in the midwife practice. Information about the outcome of this ultrasound is reported in the 15-months questionnaire.

**Outcome measures**

This study investigates the effect of food supplement Impryl® on ongoing pregnancy rate when used by the male of an infertile couple.

Primary endpoint:
The number of ongoing pregnancies, conceived in the time window between randomization up to and including month 6 of intervention use.

Secondary endpoints:

- Number of pregnancies conceived in the optimal intervention time window, i.e. between start of month 4 till the end of month 6.
- Overall number of pregnancies
  - EM/IUI group: cumulative pregnancy number up to 9 months after start of intervention
  - IVF/ICSI group: cumulative pregnancy number up to 9 months after start of intervention, and the pregnancies conceived from cryopreserved embryos obtained from the IVF/ICSI cycle(s) within this period of 9 months.
- Time to pregnancy defined as

a) The time between randomization and reaching ongoing pregnancy (confirmed by ultrasound)

b) The time between start of fertility treatment during the study (EM, IUI, IVF/ICSI) and reaching ongoing pregnancy

- Change in semen parameters in IUI/IVF/ICSI group based on pre-wash total motile sperm count (TMSC), allocated to change in treatment category (EM, IUI, IVF, ICSI)
- Improvement between Impryl® and control group in fertilization rate and embryo-utilization rate in IVF/ICSI group. Fertilization rate is defined as the percentage of oocytes with 0 PN or >=2 PN after insemination (IVF) of injection (ICSI). Abnormal fertilization such as 3PN will be recorded, in case this percentage differs or increases in the study group. The embryo-utilization rate (EUR) is defined as the number of high quality embryos obtained, embryo's used at transfer plus the number of embryos frozen, divided by the number of zygotes obtained in a cycle. Due to the differences in embryo evaluation and embryo selection criteria for cryopreservation, we decided to measure the relative increase in fertilization and utilization rate observed for each clinic.
- Number of miscarriages (defined as non-vital intra-uterine pregnancy before 16 weeks of gestation)
- Live birth rate (beyond 24 weeks, defined as the birth of a living child) within study-period of 15 months
- Adverse effects

Male baseline parameters that are collected by online questionnaires are: age, length, weight, ethnicity, alcohol/drugs/nicotine use, use of vitamins/steroids or other supplements, diet specifics (options: normal, vegetarian, vegan, gluten-free, diary free, other), medication use, activities that cause increased scrotal temperature such as often visiting a sauna, taking a hot bath or race cycling, general health, operations or trauma in genital area, exposure to toxins in environment, conception of previous children, duration of infertility and type of diagnosis of infertility. Furthermore the age and parity of the female partner has to be documented, as well as the occurrence of endometriosis or an anovulatory cycle.

**Handling and storage of data and documents**

Data will be collected in an online registration system (Castor) by the coordinating investigator or research nurses. Data handling will be done anonymously, with the patient code only available to the local investigator and the research nurse working in the local centre. The results will be extracted from Castor. If there are missing data this will be mentioned along with the reason. The data will be preserved for the duration of 15 years. The handling of personal data complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP).

**Monitoring and Quality Assurance**

Monitoring will be performed in compliance with GCP and other rules and regulations in order to achieve high quality research and secure patient safety. Monitoring will be done by an independent party of the Radboud University Medical Center. The monitor is certified and has been approved by the local medical ethical review committee (METC).

**Statistical analysis**

We will analyze all data on an intention-to-treat basis. Data of patients who are lost to follow-up will be included in their randomized group as far as possible. In addition, a per-protocol analysis will be performed for the primary outcome and the secondary outcomes overall pregnancy number, time to pregnancy, number of miscarriages and live birth rate.

Descriptive statistics will be calculated to check for major dissimilarities between study groups with respect to baseline information. Baseline data will be described quantitatively. For continuous variables, we will examine the distribution of the observations, and if normally distributed we will summarize them as means with standard deviations (SDs). If they are not normally distributed, then medians and inter-quartile ranges (IQRs) will be reported. For dichotomous data, we will provide counts and proportions.

The primary outcome variable ‘ongoing pregnancy’ will be assessed by estimating the ongoing pregnancy percentages for both treatment arms, overall, and separately for the three strata (i.e. EM, IUI, IVF/ICSI). Differences in pregnancy percentages between the experimental and control group, and the corresponding 95% confidence intervals will be estimated using a fixed effects binomial model with an identity link, including intervention, fertility treatment, centre and female age (centered). If this model does not converge, a logit link will be used.

Time to ongoing pregnancy will be evaluated by the Kaplan-Meier approach; differences between the two arms will be tested with the log-rank test, adjusted for stratum (EM, IUI or IVF/ICSI). The other secondary outcomes (overall pregnancy number, number of miscarriages, live birth rate, fertilization rate, embryo-utilization rate) will be evaluated similarly to the primary outcome. Changes in semen analysis, leading to a change in allocated treatment category, will be calculated with descriptive statistics. Furthermore, adverse events will be summarized.

The per-protocol population will consist of all randomized patients without any major deviation from the protocol. A major protocol deviation is defined as

- Use of other nutritional supplements
- Intake of study medication of less than 75% of the prescribed amount

**AEs, SAEs and SUSARs**

Adverse events (AEs) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to Impryl®. All adverse events reported by the subject or observed by the investigator or his staff will be recorded.

A SAE 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;
- 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.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor and METC without undue delay after obtaining knowledge of the events.

SUSARs are all untoward and unintended responses to the food supplement or placebo related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:

- Summary of Product Characteristics (SPC) for an authorised medicinal product;
- Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;

- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

**Annual safety report**

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

**Patient and Public Involvement**

Patients were not involved in the development of this research. However, the overall results of the study will be communicated to the study participants by sending the end-product (article) to the provided email address.

## ETHICS, DISSEMINATION AND SAFETY MONITORING

The protocol and all protocol modifications are approved by the local medical ethical review committee at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects (CCMO) with protocol ID NL61414.091.17.

This study is registered in the American registry for clinical studies and trials (NCT03337360; https://clinicaltrials.gov) and the Dutch Trial registry (NTR6551; www.trialregister.nl). The investigator obtains written informed consent before study participation from all participants.

Due to the known safety of Impryl®, the study team and ethics committee decided the establishment of a Data Safety Monitoring Board (DSMB) is not necessary. However, the trial is monitored by an independent party according to the monitor plan. After the first 3 inclusions every centre is visited by the monitor, followed by a yearly visit. Given the low risk on adverse events of a nutritional supplement, an interim analysis or safety surveillance by a data safety monitoring board is not indicated. All participants are insured by the sponsor in case of harm due to trial participation.

The study is conducted according to the principles of the Declaration of Helsinki (latest version WMA General Assembly 2008, Seoul) and in accordance with the Medical Research Involving Human Subjects Act. Directly after study inclusion, we assign a random ID code to the participant, which will be used on all documents to ensure confidentiality.

A manuscript with the results of the primary study will be published in a peer-reviewed journal. The results of this study will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of care for infertility patients.

On completion of the trial, and after publication of the primary manuscript, data requests can be submitted to the researchers

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## AUTHOR'S CONTRIBUTION

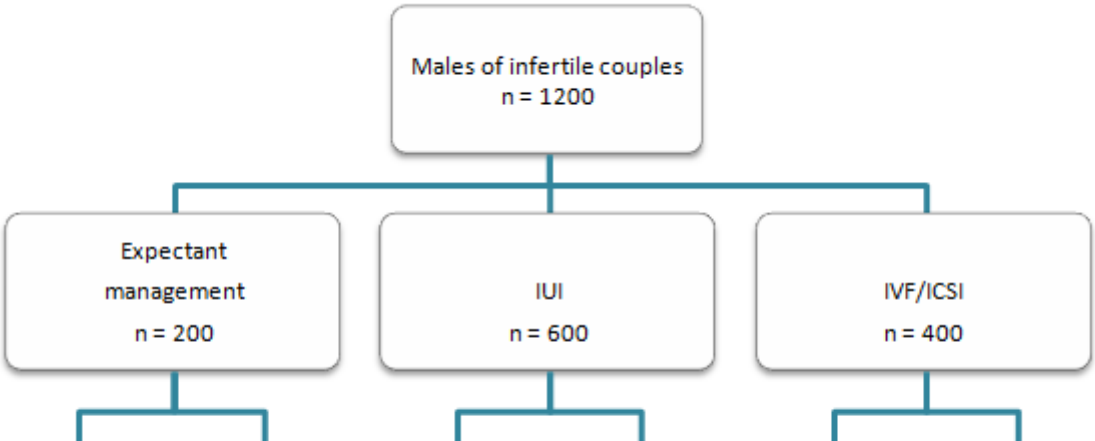
Study concept and design were conducted by authors RS and KF. Critical revision of concept and design and intellectual input in the study protocol was done by authors RS, KF, KDH and DB. Study supervision and coordination is conducted by authors RS and KF.

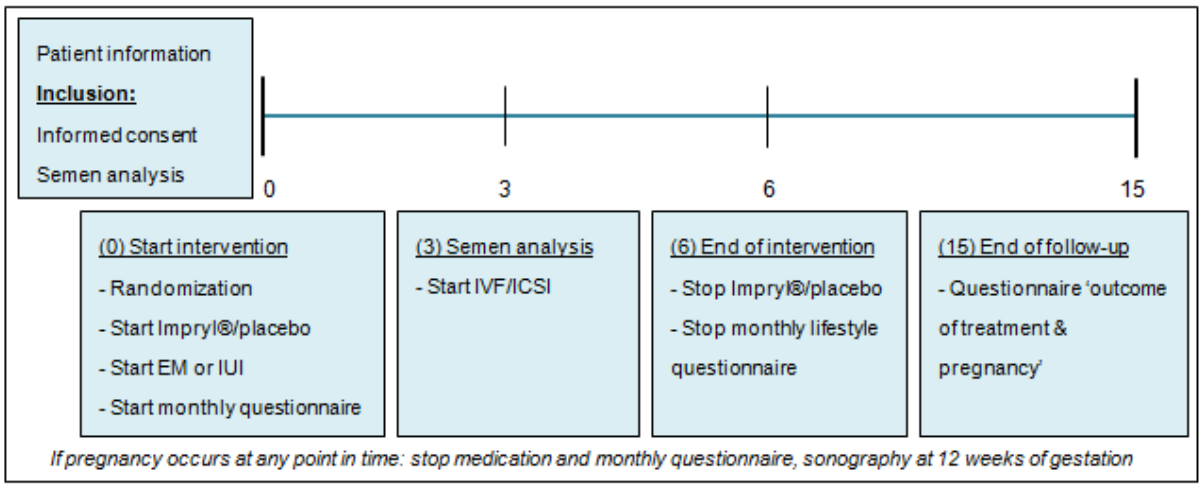
## FUNDING STATEMENT

The study is supported by an unrestricted grant from Goodlife Pharma B.V. This company has developed and marketed Impryl®. The grant is unrestricted in means that the funder did not have any role in the design of the study, collection, analysis, and/or interpretation of data. The investigators have full access to the data and have the right to publish this data separate and apart from any sponsor.

## COMPETING INTEREST STATEMENT

The study is conducted by the department of Gynaecology and Obstetrics of the Radboudumc. As stated above in the funding section, an unrestricted grant from Goodlife Pharma B.V. was provided for the conduct of the trial.





PARTHENOGEN

# Impryl®

Impryl® is a Food supplement with Betaine, Cystine, Zinc, Niacin, Folic Acid (in the form of 5MTHF-glucosamine), Vitamin B12 (in the form of Methylcobalamin), Vitamin B6 and Riboflavin. Vitamin B6, Folates and B12 all contribute to normal homocysteine metabolism and Vitamin E and B2 help protect cells from oxidative stresses. Zinc protects cellular structures against oxidative damage and contributes in DNA synthesis and to normal fertility and reproduction.

Nutritional substances contained in Impryl® are known to be part of the normal diet and metabolism and may be of benefit in case of absolute (low content in the diet) or relative (increased demand) deficiency. These deficiencies may associate to altered homocysteine metabolism and other disturbances to the defense against oxidative aggressions and are often observed in: men and women with reproductive problems; in women carrying a PCOS syndrome and at pre-menopausal and menopausal stage; in type 2 diabetes; in subjects developing neurodegenerative diseases; in subjects undergoing intensive activity and energy expenditure, e.g. athletes; and in vegans and vegetarians. Moreover, the same disturbances have also been linked to dyslipidemia and to the cardiovascular disease. Finally, due to the full coverage of all the needs for the folate/homocysteine pathway, Impryl® can be used for pre-conceptional supplementation for both partners and pregnancy supplementation for the pregnant ladies.

It is to be noted that disturbances of homocysteine metabolism and of oxidative defenses may occur more frequently in subjects carrying a defective genetic variant of one of the main enzymes of homocysteine metabolism including those denominated MTHFR, MTRR, BHMT and CBS. Impryl® has been formulated so to compensate these defects by providing an already activated substrate (i.e. methylfolate for MTHFR and methyl cobalamin for MTRR), or by providing an excess of substrate (i.e. betaine for BHMT) or by delivering a ready substrate downstream to the genetic blockade (i.e. cystine for CBS). Thus, Impryl® will deliver an effective dietary support to everybody independently of their genetic substrate for the mentioned enzymes.

**Ingredients**

Bulking agent: microcrystalline cellulose; betaine hydrochloride, L-cystine, anticaking agents: mono- and diglycerides of fatty acids, magnesium stearate, silicon dioxide; zinc bisglycinate, stabilizer: cross-linked sodium carboxy methyl cellulose; niacin (nicotinamide), vitamin B6 (pyridoxine hydrochloride), vitamin B2 (riboflavin), folic acid ((6S)5- methyltetrahydrofolic acid, glucosamine salt), vitamin B12 (methylcobalamin).

| Characterising Ingredients | Per daily dose (1 tab) | % NRV |
|---|---|---|
| Betaine | 200 mg | |
| L-cystine | 200 mg | |
| Niacin | 16 mg | 100% |
| Zinc | 10 mg | 100% |
| Vitamin B6 | 1.4 mg | 100% |
| Riboflavin (Vit. B2) | 1.4 mg | 100% |
| Folic acid (as methylfolate) | 400 µg | 200% |
| Vitamin B12 (as methylcobalamin) | 2.5 µg | 100% |

NRV: nutrient reference value according to Reg. (EU) n.1169/2011

**Instructions for use**

Impryl® is lactose free and gluten free. The recommended daily dose is 1 tablet per day to be swallowed with abundant water, possibly between meals. The assumption of a smaller amount does not guarantee full coverage of the daily need in all subjects.

Impryl® is a support to a balanced diet and there is no fixed duration for such support. However, males and females with reproductive problems should consider that the gametes maturation process takes about 4 months to be completed. In addition, those subjects with known issues of homocysteine metabolism should consider that the supportive effects will last only as long as the product is assumed.

**Warnings**

Do not exceed the recommended daily dose. Keep out of reach of children under three years of age. Food supplements should not be intended as a substitute to a varied diet and to a healthy life style. Do not take in case of known or suspected allergy to one or more ingredients.

**Storage**

Store in a cool dry place away from light, humidity and direct sources of heat. The best-before date refers to the undamaged and properly stored product.

**Presentation**

Cartoon box containing two PVC/Aluminium blisters of 15 tablets each for a total of 30 film coated tablets of 1.3 gr.

**Manufacturer**

Manufactured in Italy for Parthenogen SAGL, Via F. Pelli 1, 6900 Lugano, Switzerland, in the plant of via N. Sauro 35/D, 31036 Istrana (TV), Italy.

**Distributor**

Distributed in Turkey by Partogen Ilac San Tic Ltd Sti, Barbaros Bulvari, Cigdem Apt., Guzel Konutlar 52/7, Besiktas, Istanbul.


SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Reported on page No |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 2 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | - |
| Protocol version | 3 | Date and version identifier | 1 |
| Funding | 4 | Sources and types of financial, material, and other support | 17 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1 |
| | 5b | Name and contact information for the trial sponsor | 1 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 17 |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 17 |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 4 |
| | 6b | Explanation for choice of comparators | 5 |
| Objectives | 7 | Specific objectives or hypotheses | 5 |

| | | | |
|---|---|---|---|
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 6 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 11 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 8 |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 11-12 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 9 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 7-8 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 9-10 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 7 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 7 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 8-9 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8-9 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8-9 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 8-9 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 8-9 |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 9-11 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 10-11 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 10-11 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 12 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 12 |

| | | | |
|---|---|---|---|
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 15 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 15 |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 12-13 |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 15 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 15 |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 8 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NA |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 8-9, 11 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 18 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 18 |

| | | | |
|---|---|---|---|
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | NA |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 15 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 15 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 15 |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Only in Dutch, can be requested |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | NA |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

# The impact of a nutritional supplement (Impryl®) on male fertility – study protocol of a multicentre, randomized, double-blind, placebo-controlled clinical trial (SUMMER-trial)

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2019-035069.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 02-Jan-2020 |
| Complete List of Authors: | Smits, Roos; Radboudumc, Obstetrics and Gynaecology<br>D'Hauwers, Kathleen; Radboudumc, Urology<br>IntHout, Joanna; Radboud university medical center, Radboud Institute for Health Sciences<br>Braat, Didi<br>Fleischer, K.; Radboud Universiteit, |
| Primary Subject Heading: | Reproductive medicine |
| Secondary Subject Heading: | Obstetrics and gynaecology, Nutrition and metabolism |
| Keywords: | Nutritional support < GASTROENTEROLOGY, Subfertility < GYNAECOLOGY, Male infertility < UROLOGY, REPRODUCTIVE MEDICINE |
| | |

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# The impact of a nutritional supplement (Impryl®) on male fertility – study protocol of a multicentre, randomized, double-blind, placebo-controlled clinical trial (SUMMER-trial)

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Version 3, 23th of December 2019

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## ABSTRACT

**Introduction** Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children. In approximately 50% of infertile couples a male factor is involved. Most of the male infertile cases are characterized as “idiopathic”, except for a small percentage of cases which are causative by a genetic etiology. In the past decade, the role of oxidative stress related to sperm quality has been researched thoroughly and estimated to be the problem in 30% to 80% of male infertility cases. Impryl® is a nutritional supplement which works on the metabolic system and regulation of oxidative stress by activating the 1-Carbon cycle and therefore recycling of homocysteine.
We hypothesize that the nutritional supplement Impryl® in men of infertile couples might improve the ongoing pregnancy rate.

**Methods and analysis** We designed a multicentre, randomized, double-blind, placebo-controlled clinical trial. We aim to include 1200 male adults, age 18-50 years, part of a couple that is diagnosed with infertility. The couple will either start or has already been started with fertility treatment, i.e. expectative management (EM, duration 6 months), intra-uterine insemination (IUI) with or without mild ovarian stimulation (MOH) or ovulation induction (OI), either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment. Male participants will be randomized in either Impryl® or placebo group, with identical appearance of the tablets to be distributed (doses: one tablet each day), for a total duration of maximal 6 months. Patients can start directly with fertility treatment and/or natural conception. The primary outcome is the number of ongoing pregnancies confirmed by ultrasound at ≥ 10-12 weeks, and conceived in the time window between randomization up to and including month 6 of intervention use. Secondary outcomes are change in semen parameters between baseline and after 3 months intervention in IUI/IVF/ICSI group, based on (pre-wash) total motile sperm count (TMSC). Furthermore the number of pregnancies conceived in the optimal intervention time window (after full spermatogenesis of 72 days), overall number of pregnancies, time to pregnancy, embryo fertilization rate in IVF/ICSI, embryo-utilization rate in IVF/ICSI, number of miscarriages, live birth rate and adverse events are documented within the study period of 15 months.

**Ethics and dissemination** The protocol is approved by the local medical ethical review committee (METC) at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects (CCMO). Findings will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of medical care and quality of life for patients with infertility.

**Trial registration number** NCT03337360 / NTR6551


**Keywords** nutritional supplements, fertility, male infertility, oxidative stress, clinical pregnancy rate, live birth rate

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- This multicentre randomized double-blind, placebo-controlled trial will provide important information to patients with diagnosis of male infertility and clinicians about the efficacy of nutritional supplement Impryl® with combined antioxidants
- With a sample size of 1200 patients this is up to date the largest randomized study with the use of nutritional supplements in fertility patients
- Permuted block randomization ensures treatment group numbers are evenly balanced, with stratification for centre and type of infertility treatment

The optimal effect of Impryl® is expected after full spermatogenesis of 72 days.

- The use of diagnostic tests to screen for oxidative stress in semen samples (like sperm DNA-fragmentation tests or 8-OH-dG levels) could be of use in predicting the effect of Impryl® in different patient groups. However, these tests are not used in this study due to their complexity and lack of standardization and validation

## INTRODUCTION

Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children.[1] In approximately 50% of infertile couples a male factor is involved.[1,2] In the past decade, the role of oxidative stress on sperm has been researched thoroughly and found to be the problem in 25% to 87% of male infertility cases.[3-9]

All cells in the human body use oxygen to survive. In this process, toxic radicals with high reactive activity, so called reactive oxygen species (ROS), are produced as a consequence.[10] To scavenge these toxins the body has developed a defense mechanism in which antioxidants play an important role. The production of ROS in sperm is a normal physiological process mostly regulated by cellular en extracellular antioxidative factors. However, when the ROS production overwhelms the natural antioxidant defense, increase of apoptosis is observed. Increased oxidative stress can occur due to environmental and lifestyle factors such as smoking, stress, obesity and nutrition.[10] In the reproductive tract, semen leukocytes and immature spermatozoa are major sources of ROS production.[11] However, a low production of ROS is also physiological and needed for adequate sperm functioning by supporting capacitation, maturation and hyperactivation.[12] Spermatozoa are more vulnerable and prone to oxidative stress compared to other body cells because of the lack of cytoplasm.[13] Cytoplasmic antioxidant enzymes are important for directly scavenging and repairing the damage of ROS. However, in the spermatozoa the cytoplasm is removed during the final stages of spermatogenesis, leaving them without these important enzymes to protect them from ROS altering the sperm deoxyribonucleic acid (DNA). Furthermore, the membrane of spermatozoa is rich in fatty acids, making them vulnerable for lipid peroxidation by ROS resulting in decreased flexibility of the sperm membrane and reduction of tail motion.[13] For these reasons, spermatozoa are dependent on seminal plasma which is rich in antioxidants. Examples of antioxidants already present within seminal plasma are ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione, amino acids (taurine, hypotaurine), albumin, carnitine and carotenoids.[14] Despite the common association between male infertility and oxidative damage, there is no World Health Organization (WHO) recommendation about which test to use for detecting sperm DNA damage and there are no standardized protocols which could help global interpretation of the results. Men with sperm subject to increased oxidative stress may have normal seminal parameters, however with DNA damage and therefore a lower chance of natural conception. [15,16] Sperm DNA fragmentation (SDF) tests are available, however most of them are expensive (roughly 200-300 USD per test), complex and lack standardization and validation.[17] Furthermore, a recent meta-analysis showed that an association does not imply an actual predictive value.[17] Examples of SDF test are deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), the COMET assay or sperm chromatin structure assay (SCSA) and another option is measuring the byproduct of DNA oxidation called 8-hydroxydeoxyguanosine (8-OHdG).

A recent Cochrane review suggested that antioxidant supplementation in male infertility might improve the outcomes in assisted reproductive technologies (ART).[18] However, the evidence was rated as low and clinical studies showed contradictory results with sometimes even a negative effect of high doses of antioxidants due to reductive stress as a rebound effect.[19] Alternatives were therefore explored, to support the natural antioxidant defenses that are predicted to act within the modulation of the natural cellular homeostasis without generating rebound effects. For example, the use of nutritional supplements supporting DNA methylation and the homocysteine pathway. Homocysteine is the end-product of the 1-Carbon cycle, feeding DNA methylation, and the starting substrate for the thiol glutathione (GSH, l-γ-glutamyl-l-cysteinyl-glycine) de novo biosynthesis. GSH is the most important endogenous antioxidant, involved in maintaining the antioxidant balance in human tissues and directly involved in the elimination of ROS.[20] Homocysteine is an inhibitor of the methylation process and a powerful pro-oxidant. It has a negative effect on spermatogenesis and its concentration in the ejaculate is inversely correlated with fertility outcome.[21,22] Dattilo et al stated that the ideal supplement should work by favoring homocysteine recycling by restoring the efficiency of the 1-Carbon cycle, therefore ensuring the availability of activated methyl groups for DNA methylation and feeding the intracellular antioxidant system by supporting GSH synthesis.[23] In non-randomized pilot studies with such a 1-Carbon cycle supporting nutritional supplement Condensyl®, the precursor of Impryl®, there was a significant decline of DNA fragmentation index leading to an improvement of the clinical pregnancy rate. However, the quality of these studies for the impact on pregnancy rate is rather low due to the non-randomized nature and having no control group.[21,24,25] Furthermore, the ideal parameters to measure DNA-damage might not be DNA-fragmentation, but sperm-oxidation level which indirectly affects DNA-damages.[26,27]

Impryl® is a nutritional supplement mainly consisting of vitamin B, which works on the metabolic system by activating the 1-Carbon cycle and recycling of homocysteine without the use of any direct strong antioxidant. Therefore it is could be more effective than other nutritional supplements. The cost of Impryl® is 30 euro for each 30 days of use.

It is well known that assisted reproduction technologies are expensive with the cost per in vitro fertilization (IVF) treatment in the Netherlands estimated to be between 2,885 and 5,259 euro (€) and the cost of an intra-uterine insemination (IUI) cycle between 497€ and 1123€, depending on the use and doses of medication during ovarian stimulation.[28-30] Therefore, substantial cost savings could be made if the use of relatively inexpensive nutritional supplements would lead to a shorter time to pregnancy with less treatment cycles necessary or even better, when the use of more expensive invasive reproductive techniques can be avoided at all.

## METHODS AND ANALYSIS

### Study design

This investigator-initiated, multicentre, placebo-controlled study has a double-blinded design, with blinding of participants and investigators. We conduct this study in 15 academic and non-academic hospitals in The Netherlands, at the Department of Gynaecology and/or Reproductive Medicine.

The study protocol was designed using the recommendations of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[31]

The study is currently ongoing; the first participant was included on 30 May 2018. We expect to include the last participant in June 2022 and study completion by December 2023.

### Participants

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

Inclusion criteria:
- Couples with failure to conceive for at least 12 months and starting with expectative management (EM) **or** couples starting with 1st/ 2nd/3rd cycle of IUI (with or without mild ovarian stimulation) **or** 1st/ 2nd/3rd cycle of IVF or intracytoplasmic sperm injection (ICSI) - Male with age 18-50 years
- Female partner with age 18-43 years
- Both male and female are willing and able to give informed consent

Exclusion criteria:
- Planned or performed TEsticular Sperm Extraction (TESE) or Percutaneous Epididymal Sperm Aspiration (PESA)
- Use of donor-, cryopreserved- or electro-ejaculated semen or donor oocytes
- Ovulation induction (OI) without IUI
- IVF for an absolute tubal factor
- Embryo-transfer after pre-implantation genetic diagnosis
- Known endocrine abnormalities related to male infertility, or use of fertility enhancing drugs such as clomiphene citrate or follicle-stimulating hormone (FSH)
- Known genetic abnormalities related to male infertility
- Known urological abnormality such as a varicocele or bilateral cryptorchism
- Use of other vitamins or nutritional supplements with antioxidant effect

Withdrawn or unblinded participants will not be replaced. They will not be re-included in the study once they dropped out, and their identification number and treatment will not be reused.

**Sample size calculation**

We aim to recruit 1200 male participants as a part of an infertile couple, based on the following data and power calculation.

Overall, the number of ongoing pregnancies in IVF and ICSI in the Netherlands was respectively 19.8% and 21.5% per started fresh cycle in 2015.[32] The number of ongoing pregnancies in IUI is estimated to be around 5 to 13% per cycle, around 18% after three cycles and 20-30% after six cycles.[33-35] Based on Radboudumc data, the ongoing pregnancy rate in EM is estimated around 20% after 6 months.

A Cochrane review estimated the increase in clinical pregnancy rates after use of antioxidants with an Odds Ratio (OR) of 3.43 (95% CI 1.92 to 6.11) based on 7 RCTs with in total 522 men.[36] However, this concerns low quality evidence, and the included population in this Cochrane review was couples with male factor subfertility, whereas in our study we also include couples with unexplained infertility. Therefore, we assumed a more conservative OR of 1.5. Assuming a 20% ongoing pregnancy rate in the placebo group, this reflects in an expected pregnancy rate of 27.3% in the Impryl® group.

The study is designed as a superiority trial. Based on the above mentioned data, we expect a 7.3% increase in ongoing pregnancy number when men are treated with Impryl® compared to placebo. However, after randomization patients can directly start with both intervention and achieving a pregnancy, either spontaneously or with fertility treatment. . In these first months the effect of the intervention is expected to be suboptimal due to the duration of the spermatogenesis (72 days before whole renewal). Therefore we adjusted the expected increase of 7.3% to a more realistic effect of 6.5%. We assume an equal increase in all fertility groups (meaning EM, IUI and IVF/ICSI) from 20% to 26.5%.

To test the effect of Impryl® on the probability of ongoing pregnancy, a sample size of 600 men per treatment group (1200 in total) is needed, assuming differences in ongoing pregnancy rates of 6.5% and an expected ongoing pregnancy rate of 20% in the placebo group, a two-sided alpha of 5% and a beta of 24% (i.e. 76% power). As we expect the number of participants lost to follow up to be minimal, we will allocate 600 patients to both the intervention and the placebo arm. Figure 1 shows the distribution of the number of participants for the 3 different types of fertility treatment (EM, IVF/ICSI and IUI).

**Investigational product/intervention**

Impryl® tablets will be produced by Labomar SRL (compliant with European Good Manufacturing Practice (GMP) who will also produce an identical looking placebo tablet. Impryl® is a food supplement and is therefore not considered an investigational medicinal product. To assess product quality, we have quality assessment documents of both the food supplement and placebo: Summary of Product Characteristics (SPC, see supplementary file 1), Certificates of Analysis (CoA), certificate of Good Manufacturing Practice (GMP) and ISO

9001 form. Intervention will either start directly after completing diagnostic work up in new couples (IUI) or after at least 3 months of using study medication before using semen for a new IVF/ICSI cycle. When EM is applied, patients start directly with study medication for a total duration of maximum 6 months.

All couples will receive our standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology. After diagnostic work up couples will either start with EM (6 months), IUI, IVF or ICSI. Participants will take study supplement for a maximum of 6 months, even if the fertility intervention (6 x IUI of 1 x IVF/ICSI cycles) is not completed.

Co-treatment with other vitamins or supplements is prohibited. If vitamins or supplements are already used by men, a wash-out period of 3 months is recommended. Patients using other supplements will be excluded or reported as protocol violation If after randomization it appears that a patient still uses other supplements (reported in the online questionnaire), they will be telephonically contacted and asked to stop use of other supplements. After this stop, there is a wash-out period of 72 days (duration of spermatogenesis). If no pregnancy occurs within this wash-out period the patient and data can still be included.

**Randomization and treatment allocation**

All couples visiting a fertility specialist for the first time or for evaluation of fertility treatment will be informed on this study. Prior to or at their actual appointment they will receive the patient information (PIF) from the fertility specialist (specialised nurse or doctor). Randomization will be performed, after signed informed consent has been obtained, using a web-based application (Castor). We will use permuted block-design, stratified for fertility treatment (1st) and recruiting centre (2nd). Block sizes are flexible. Participants will be randomized in a 1:1 ratio to study medication (Impryl®) or placebo. Assignment to medication, using the randomization list, will either performed at the local centre or at Radboudumc by an independent person. This person is neither participating in clinical treatment nor processing the study data. Randomization outcome is either A (Impryl®) or B (placebo).

Coding starts immediately after informed consent has been obtained. In Castor each patient will receive a combination of one letter and 3 numbers. The code will not provide any information about the received intervention (Impryl® or placebo). The list of codes corresponding with patient information and received medication will be saved in an separate file, locked (either digital with password or a locked cabinet), only accessible for the independent person who did the randomization.

The study is double blinded. All personnel, the researchers and patients will remain blinded to the intervention being received, except the personnel performing randomization and distributing the study medication. The indications for breaking the randomization code are: serious adverse event (SAE), serious adverse reaction (SAR) and Suspected unexpected

serious adverse reactions (SUSARs) SUSAR, as instructed by the local medical ethical review committee (METC), or in a dire emergency, as directed by the principal investigators or trial manager. Every site has access to a deblinding form in which patients details, reason for deblinding, statement of principal site investigator, date and time of intervention stop and randomization allocation will be reported. The principal site investigator will inform the coordinating investigator about the deblinding.

At all study sites the person distributing the study medication is an unblinded employee of the department of Reproductive medicine or Gynaecology who is not involved in the treatment of the patient nor involved in the data collecting of the research. Drug accountability will be performed in line with Good Clinical Practice (GCP) requirements. The investigator is responsible for drug accountability and these tasks will be delegated to the primary investigators at the sites. We will log all dispensing of the investigational product on a drug accountability log. On every distributing study site there will be a batch of study medication stored at room temperature, no special precautions. An unblinded authorized employee of the department of Reproductive medicine (or Gynaecology) will distribute the study medication after randomization has been performed by Castor. The flag label (identification A = Impryl® or B = placebo) will be removed from the box and the randomization number noted (handwritten) on the medication box. Thereafter, the box will be handed over to the patient. On the patient identification & drug accountability log (either digital or on paper) the following details will be reported: date of issue, study (randomization) number, batch number, expiration date, amount dispensed, current storage amount, randomizer/distributor initials, hospital number, patient name and initials, date of birth and monitoring check.

All unblinded personnel performing randomization will be trained by a site initiation to perform randomization in Castor EDC and distribution of medication. A log list of all personnel involved in the study, with blinding status, responsibilities and signature, will be saved.

Unblinding will be performed when the study has ended, database is locked and protocol violators have been defined.

Patients are not asked to return study medication because this would mean an extra burden for the patients: an extra visit for all EM patients and some IUI/IVF/ICSI patients. Furthermore, patients could also forget a strip at home that they did not collect or put in the box. Since it is a supplement and not medication there are no specials precautions for destruction or disposal.

**Participant timeline and intervention**

All couples will receive standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology (NVOG). After diagnostic work up couples will either start with EM (6 months), IUI, IVF or ICSI, according to the NVOG guidelines. Participants will take study medication (Impryl® or placebo) for a maximum of 6 months, even if the fertility intervention (6 x IUI or 1 x IVF/ICSI cycle) is not completed. Use of the food supplement or

placebo will be stopped earlier when a pregnancy is achieved. Participants will be followed for 15 months after randomization for detecting live births. Figure 2 shows the participant timeline.

During the study, participants are asked to report on baseline characteristics, lifestyle changes and pregnancy outcomes by short online questionnaires (see supplementary file 2).

- Baseline: directly after randomization, patients will receive an automatic email invitation to provide baseline information online in the Castor database system.
- Monthly: during use of study medication (six months), participants will be asked to report on occurrence of pregnancy (with date of positive test), changes in lifestyle, consumed total amount of study medication (used boxes and tablets) and occurrence of adverse events. They will receive an automatic email invitation.
- Follow-up: 15 months after randomization participants will receive an automatic email with invitation for the last short questionnaire. They will receive questions about fertility treatment and pregnancy outcome (ability to achieve a pregnancy, occurrence of miscarriage or childbirth, date of delivery, gestational age, mode of delivery, complications of delivery and birth weight, sex, congenital abnormalities and health of the neonate.

At least 2 semen analyses will be performed at two time points in this study: the first semen analysis will be performed during diagnostic work-up (standard care, analysis according to WHO criteria[37]) and the second one after approximately 3 months of using study medication for participants having the IUI or IVF/ICSI treatment. We decided not to perform a second semen analysis in the EM group due to the fact that they present with normal semen parameters at intake and we want to avoid the burden of an extra visit.

If a clinical pregnancy is achieved, a routine ultrasound will be performed in the first trimester between 5 to 9 weeks to determine the vitality of the fetus. A second routine ultrasound will be performed around 10-12 weeks to estimate the due date. To minimalize the amount of extra site visits, we decided that this standard 'due date' ultrasound at 10-12 weeks of pregnancy is enough for determining the primary outcome (ongoing pregnancy). The ultrasound can be performed in the midwife practice. Information about the outcome of this ultrasound is reported in the 15-months questionnaire.

**Outcome measures**

This study investigates the effect of food supplement Impryl® on ongoing pregnancy rate when used by the male of an infertile couple.

Primary endpoint:

The number of ongoing pregnancies, conceived in the time window between randomization up to and including month 6 of intervention use.

Secondary endpoints:

- Number of pregnancies conceived in the optimal intervention time window, i.e. between start of month 4 till the end of month 6.
- Overall number of pregnancies meaning the cumulative pregnancy number up to 9 months after start of intervention
- Time to pregnancy defined as

a) The time between randomization and reaching ongoing pregnancy (confirmed by ultrasound)

b) The time between start of fertility treatment during the study (EM, IUI, IVF/ICSI) and reaching ongoing pregnancy

- Change in semen parameters in IUI/IVF/ICSI group based on pre-wash total motile sperm count (TMSC), allocated to change in treatment category (EM, IUI, IVF, ICSI)
- Improvement between Impryl® and control group in fertilization rate and embryo-utilization rate in IVF/ICSI group. Fertilization rate is defined as the percentage of oocytes with 0 PN or >=2 PN after insemination (IVF) of injection (ICSI). Abnormal fertilization such as 3PN will be recorded, in case this percentage differs or increases in the study group. The embryo-utilization rate (EUR) is defined as the number of high quality embryos obtained, embryo's used at transfer plus the number of embryos frozen, divided by the number of zygotes obtained in a cycle. Due to the differences in embryo evaluation and embryo selection criteria for cryopreservation, we decided to measure the relative increase in fertilization and utilization rate observed for each clinic.
- Number of miscarriages (defined as non-vital intra-uterine pregnancy before 16 weeks of gestation)
- Live birth rate (beyond 24 weeks, defined as the birth of a living child) within study-period of 15 months
- Adverse effects

Male baseline parameters that are collected by online questionnaires are: age, length, weight, ethnicity, alcohol/drugs/nicotine use, use of vitamins/steroids or other supplements, diet specifics (options: normal, vegetarian, vegan, gluten-free, diary free, other), medication use, activities that cause increased scrotal temperature such as often visiting a sauna, taking a hot bath or race cycling, general health, operations or trauma in genital area, exposure to toxins in environment, conception of previous children, duration of infertility and type of diagnosis of infertility. Furthermore the age and parity of the female partner has to be documented, as well as the occurrence of endometriosis or an anovulatory cycle.

**Handling and storage of data and documents**

Data will be collected in an online registration system (Castor) by the coordinating investigator or research nurses. Data handling will be done anonymously, with the patient code only available to the local investigator and the research nurse working in the local centre. The results will be extracted from Castor. If there are missing data this will be mentioned along with the reason. The data will be preserved for the duration of 15 years. The handling of personal data complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP).

**Monitoring and Quality Assurance**

Monitoring will be performed in compliance with GCP and other rules and regulations in order to achieve high quality research and secure patient safety. Monitoring will be done by an independent party of the Radboud University Medical Center. The monitor is certified and has been approved by the local medical ethical review committee (METC).

**Statistical analysis**

We will analyze all data on an intention-to-treat basis. Data of patients who are lost to follow-up will be included in their randomized group:

- Patients who are lost to follow-up during the treatment period are considered not to have achieved a pregnancy.
- Patients, who are lost to follow-up right after achieving a pregnancy of less than 8 weeks of gestation, are considered not to have achieved an ongoing pregnancy
- Patients, who are lost to follow-up right after achieving a pregnancy of 8 or more weeks of gestation, are considered to have achieved an ongoing pregnancy.

In addition, a per-protocol analysis will be performed for the primary outcome and the secondary outcomes overall pregnancy number, time to pregnancy, number of miscarriages and live birth rate.

Descriptive statistics will be calculated to check for major dissimilarities between study groups with respect to baseline information. Baseline data will be described quantitatively. For continuous variables, we will examine the distribution of the observations, and if normally distributed we will summarize them as means with standard deviations (SDs). If they are not normally distributed, then medians and inter-quartile ranges (IQRs) will be reported. For dichotomous data, we will provide counts and proportions.

The primary outcome variable 'ongoing pregnancy' will be assessed by estimating the ongoing pregnancy percentages for both treatment arms, overall, and separately for the three strata (i.e. EM, IUI, IVF/ICSI). Differences in pregnancy percentages between the experimental and control group, and the corresponding 95% confidence intervals will be estimated using a fixed effects binomial model with an identity link, including intervention, fertility treatment, centre and female age (centered). If this model does not converge, a logit link will be used.

Time to ongoing pregnancy will be evaluated by the Kaplan-Meier approach; differences between the two arms will be tested with the log-rank test, adjusted for stratum (EM, IUI or IVF/ICSI). The other secondary outcomes (overall pregnancy number, number of miscarriages, live birth rate, fertilization rate, embryo-utilization rate) will be evaluated similarly to the primary outcome. Changes in semen analysis, leading to a change in allocated treatment category, will be calculated with descriptive statistics. Furthermore, adverse events will be summarized.

The per-protocol population will consist of all randomized patients without any major deviation from the protocol. A major protocol deviation is defined as

- Use of other nutritional supplements
- Intake of study medication of less than 75% of the prescribed amount

**AEs, SAEs and SUSARs**

Adverse events (AEs) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to Impryl®. All adverse events reported by the subject or observed by the investigator or his staff will be recorded.

A SAE 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;
- 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.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor and METC without undue delay after obtaining knowledge of the events.

SUSARs are all untoward and unintended responses to the food supplement or placebo related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:

- Summary of Product Characteristics (SPC) for an authorised medicinal product;
- Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

**Annual safety report**

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

**Patient and Public Involvement**

Patients were not involved in the development of this research. However, the overall results of the study will be communicated to the study participants by sending the end-product (article) to the provided email address.

## ETHICS, DISSEMINATION AND SAFETY MONITORING

The protocol and all protocol modifications are approved by the local medical ethical review committee at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects (CCMO) with protocol ID NL61414.091.17.

This study is registered in the American registry for clinical studies and trials (NCT03337360; https://clinicaltrials.gov) and the Dutch Trial registry (NTR6551; www.trialregister.nl). The investigator obtains written informed consent before study participation from all participants.

Due to the known safety of Impryl®, the study team and ethics committee decided the establishment of a Data Safety Monitoring Board (DSMB) is not necessary. However, the trial is monitored by an independent party according to the monitor plan. After the first 3 inclusions every centre is visited by the monitor, followed by a yearly visit. Given the low risk on adverse events of a nutritional supplement, an interim analysis or safety surveillance by a data safety monitoring board is not indicated. All participants are insured by the sponsor in case of harm due to trial participation.

The study is conducted according to the principles of the Declaration of Helsinki (latest version WMA General Assembly 2008, Seoul) and in accordance with the Medical Research Involving Human Subjects Act. Directly after study inclusion, we assign a random ID code to the participant, which will be used on all documents to ensure confidentiality.

A manuscript with the results of the primary study will be published in a peer-reviewed journal. The results of this study will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of care for infertility patients.

On completion of the trial, and after publication of the primary manuscript, data requests can be submitted to the researchers

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## AUTHOR'S CONTRIBUTION

Study concept and design were conducted by authors RS and KF. Advanced statistical input was given by author JIH. Critical revision of concept and design and intellectual input in the study protocol was done by authors RS, KF, KDH, JIH and DB. Study supervision and coordination is conducted by authors RS and KF.

## FUNDING STATEMENT

The study is supported by an unrestricted grant from Goodlife Pharma B.V. This company has developed and marketed Impryl®. The grant is unrestricted in means that the funder did not have any role in the design of the study, collection, analysis, and/or interpretation of data. The investigators have full access to the data and have the right to publish this data separate and apart from any sponsor.

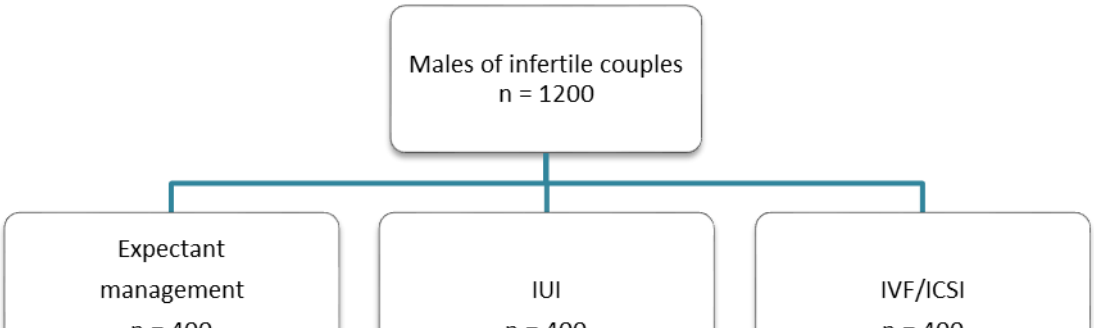
## COMPETING INTEREST STATEMENT

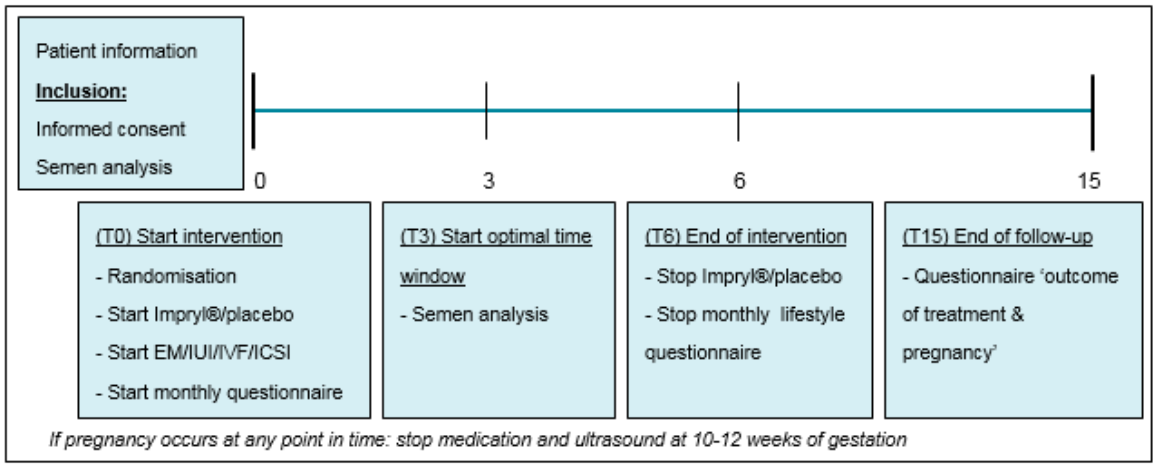
The study is conducted by the department of Gynaecology and Obstetrics of the Radboudumc. As stated above in the funding section, an unrestricted grant from Goodlife Pharma B.V. was provided for the conduct of the trial.

## FIGURE LEGENDS

Figure 1: Flow chart study design

Figure 2. Time line study (T in months)





PARTHENOGEN

# Supplementary file 1: Summary of Product Characteristics (SPC) of Impryl®

Impryl® is a Food supplement with Betaine, Cystine, Zinc, Niacin, Folic Acid (in the form of 5MTHF-glucosamine), Vitamin B12 (in the form of Methylcobalamin), Vitamin B6 and Riboflavin. Vitamin B6, Folates and B12 all contribute to normal homocysteine metabolism and Vitamin E and B2 help protect cells from oxidative stresses. Zinc protects cellular structures against oxidative damage and contributes in DNA synthesis and to normal fertility and reproduction.

Nutritional substances contained in Impryl® are known to be part of the normal diet and metabolism and may be of benefit in case of absolute (low content in the diet) or relative (increased demand) deficiency. These deficiencies may associate to altered homocysteine metabolism and other disturbances to the defense against oxidative aggressions and are often observed in: men and women with reproductive problems; in women carrying a PCOS syndrome and at pre-menopausal and menopausal stage; in type 2 diabetes; in subjects developing neurodegenerative diseases; in subjects undergoing intensive activity and energy expenditure, e.g. athletes; and in vegans and vegetarians. Moreover, the same disturbances have also been linked to dyslipidemia and to the cardiovascular disease. Finally, due to the full coverage of all the needs for the folate/homocysteine pathway, Impryl® can be used for pre-conceptional supplementation for both partners and pregnancy supplementation for the pregnant ladies.

It is to be noted that disturbances of homocysteine metabolism and of oxidative defenses may occur more frequently in subjects carrying a defective genetic variant of one of the main enzymes of homocysteine metabolism including those denominated MTHFR, MTRR, BHMT and CBS. Impryl® has been formulated so to compensate these defects by providing an already activated substrate (i.e. methylfolate for MTHFR and methyl cobalamin for MTRR), or by providing an excess of substrate (i.e. betaine for BHMT) or by delivering a ready substrate downstream to the genetic blockade (i.e. cystine for CBS). Thus, Impryl® will deliver an effective dietary support to everybody independently of their genetic substrate for the mentioned enzymes.

**Ingredients**

Bulking agent: microcrystalline cellulose; betaine hydrochloride, L-cystine, anticaking agents: mono- and diglycerides of fatty acids, magnesium stearate, silicon dioxide; zinc bisglycinate, stabilizer: cross-linked sodium carboxy methyl cellulose; niacin (nicotinamide), vitamin B6 (pyridoxine hydrochloride), vitamin B2 (riboflavin), folic acid ((6S)5- methyltetrahydrofolic acid, glucosamine salt), vitamin B12 (methylcobalamin).

| Characterising Ingredients | Per daily dose (1 tab) | % NRV |
|---|---|---|
| Betaine | 200 mg | |
| L-cystine | 200 mg | |
| Niacin | 16 mg | 100% |
| Zinc | 10 mg | 100% |
| Vitamin B6 | 1.4 mg | 100% |
| Riboflavin (Vit. B2) | 1.4 mg | 100% |
| Folic acid (as methylfolate) | 400 µg | 200% |
| Vitamin B12 (as methylcobalamin) | 2.5 µg | 100% |

NRV: nutrient reference value according to Reg. (EU) n.1169/2011

**Instructions for use**

Impryl® is lactose free and gluten free. The recommended daily dose is 1 tablet per day to be swallowed with abundant water, possibly between meals. The assumption of a smaller amount does not guarantee full coverage of the daily need in all subjects.

Impryl® is a support to a balanced diet and there is no fixed duration for such support. However, males and females with reproductive problems should consider that the gametes maturation process takes about 4 months to be completed. In addition, those subjects with known issues of homocysteine metabolism should consider that the supportive effects will last only as long as the product is assumed.

**Warnings**

Do not exceed the recommended daily dose. Keep out of reach of children under three years of age. Food supplements should not be intended as a substitute to a varied diet and to a healthy life style. Do not take in case of known or suspected allergy to one or more ingredients.

**Storage**

Store in a cool dry place away from light, humidity and direct sources of heat. The best-before date refers to the undamaged and properly stored product.

**Presentation**

Cartoon box containing two PVC/Aluminium blisters of 15 tablets each for a total of 30 film coated tablets of 1.3 gr.

**Manufacturer**

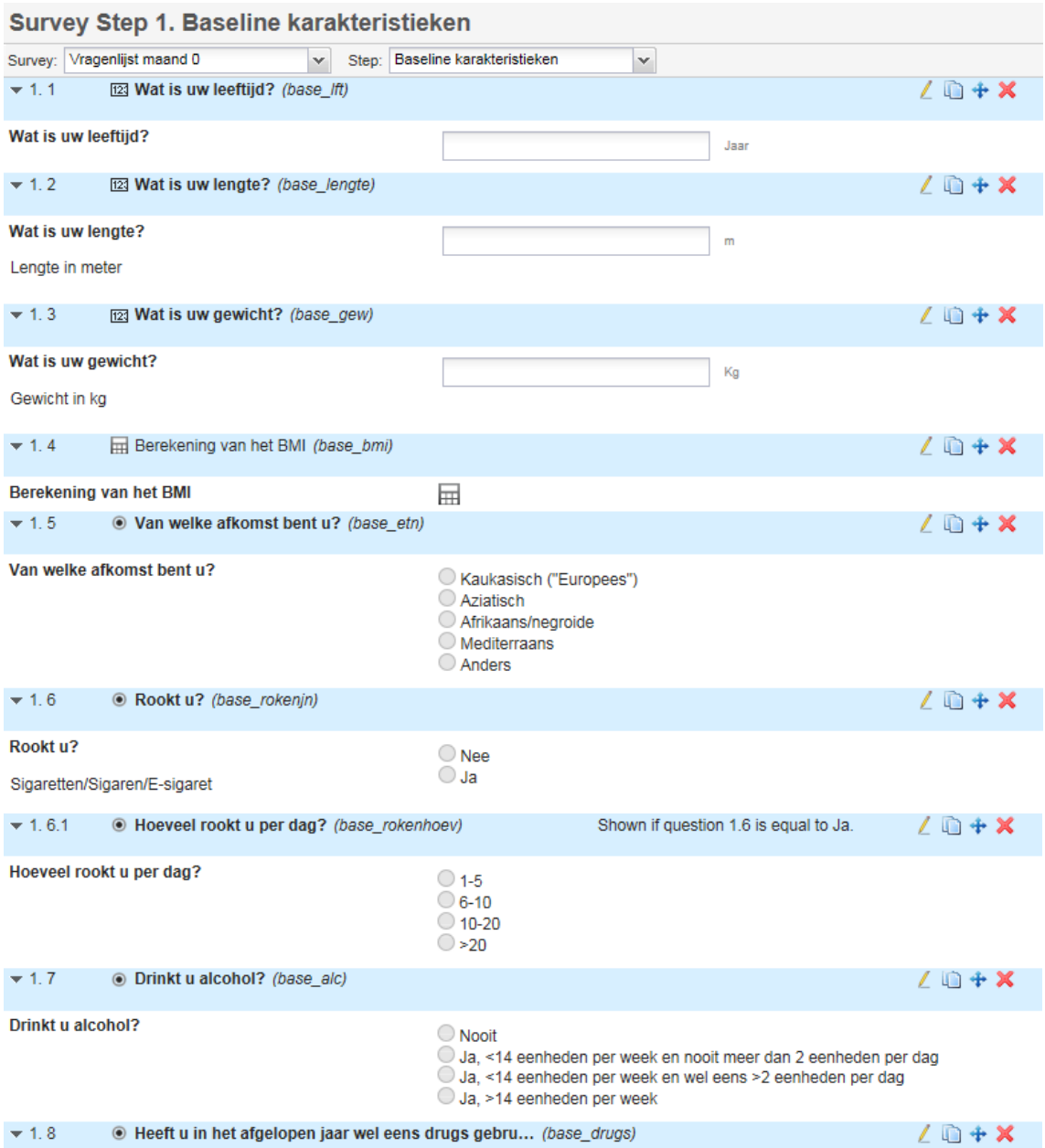
Manufactured in Italy for Parthenogen SAGL, Via F. Pelli 1, 6900 Lugano, Switzerland, in the plant of via N. Sauro 35/D, 31036 Istrana (TV), Italy.

**Distributor**

Distributed in Turkey by Partogen Ilac San Tic Ltd Sti, Barbaros Bulvari, Cigdem Apt., Guzel Konutlar 52/7, Besiktas, Istanbul.

**Supplementary file 2: patient questionnaires (in Dutch)**

**Vragenlijst maand 1 – Baseline karakteristieken, lifestyle en gebruik studiemedicatie**



## Survey Step 1. Baseline karakteristieken

Survey: Vragenlijst maand 0 Step: Baseline karakteristieken

**1. 1 Wat is uw leeftijd?** *(base_lft)*

**Wat is uw leeftijd?** Jaar

**1. 2 Wat is uw lengte?** *(base_lengte)*

**Wat is uw lengte?** m

Lengte in meter

**1. 3 Wat is uw gewicht?** *(base_gew)*

**Wat is uw gewicht?** Kg

Gewicht in kg

**1. 4** Berekening van het BMI *(base_bmi)*

**Berekening van het BMI**

**1. 5 Van welke afkomst bent u?** *(base_etn)*

**Van welke afkomst bent u?**

- Kaukasisch ("Europees")
- Aziatisch
- Afrikaans/negroide
- Mediterraans
- Anders

**1. 6 Rookt u?** *(base_rokenjn)*

**Rookt u?**

Sigaretten/Sigaren/E-sigaret

- Nee
- Ja

**1. 6.1 Hoeveel rookt u per dag?** *(base_rokenhoev)* Shown if question 1.6 is equal to Ja.

**Hoeveel rookt u per dag?**

- 1-5
- 6-10
- 10-20
- >20

**1. 7 Drinkt u alcohol?** *(base_alc)*

**Drinkt u alcohol?**

- Nooit
- Ja, <14 eenheden per week en nooit meer dan 2 eenheden per dag
- Ja, <14 eenheden per week en wel eens >2 eenheden per dag
- Ja, >14 eenheden per week

**1. 8 Heeft u in het afgelopen jaar wel eens drugs gebru...** *(base_drugs)*

**Heeft u in het afgelopen jaar wel eens drugs gebruikt?**

- Nee
- Marihuana
- XTC
- Paddo's
- Cocaine
- Ketamine
- Speed
- GHB
- LSD
- Heroine
- Anders
- Gecombineerd, meerdere middelen

**1. 9 Gebruikt u vitaminen/voedingssupplementen?** *(base_vit)*

**Gebruikt u vitaminen/voedingssupplementen?**

Let op: indien u dit gebruikt: neem contact op met uw behandelaar of de coördinerend onderzoeker! U moet namelijk eerst 3 maanden hiermee gestopt zijn voordat u het studiemiddel mag gaan gebruiken!

- Nee
- Ja, (multi) vitaminen
- Ja, ander voedingssupplement
- Ja, annabolen/steroiden

## Vervolg: Baseline karakteristieken, lifestyle en gebruik studiemedicatie

1. 10 **Heeft u een specifiek dieet?** *(base_dieet)*

**Heeft u een specifiek dieet?**

- Nee
- Ja, vegetarisch
- Ja, veganistisch
- Ja, lactose-vrij
- Ja, gluten of koolhydraatvrij
- Ja, anders

1. 11 **Gebruikt u medicijnen?** *(base_medjn)*

**Gebruikt u medicijnen?**

- Nee
- Ja

1. 11.1 **Welke medicatie gebruikt u?** *(base_medspec)* Shown if question 1.11 is equal to Ja.

**Welke medicatie gebruikt u?**

1. 12 **Heeft u de afgelopen 3 maanden koorts gehad?** *(base_koorts)*

**Heeft u de afgelopen 3 maanden koorts gehad?**

- Nee
- Ja

1. 13 **Bent u op dit moment gezond?** *(base_gez)*

**Bent u op dit moment gezond?**

- Nee
- Ja

1. 14 **Staat u onder controle bij een arts voor een aando...** *(base_arts)*

**Staat u onder controle bij een arts voor een aandoening/afwijking?**

- Nee
- Ja

1. 14.1 **Waarvoor staat u onder controle bij een arts? Wel...** *(base_ziekte)* Shown if question 1.14 is equal to Ja.

**Waarvoor staat u onder controle bij een arts? Welke aandoening?**

1. 15 **Bent u ooit op genitaal gebied geopereerd of heeft...** *(base_OKjn)*

**Bent u ooit op genitaal gebied geopereerd of heeft u ooit een genitaal trauma gehad?**

- Nee
- Ja

Genitaal trauma anders dan een "knietje krijgen"

1. 15.1 **Wat voor een operatie of trauma heeft u gehad?** *(base_OKspec)* Shown if question 1.15 is equal to Ja.

**Wat voor een operatie of trauma heeft u gehad?**

1. 16 **Staat u wel eens bloot aan gevaarlijke stoffen?** *(base_tox)*

**Staat u wel eens bloot aan gevaarlijke stoffen?**

- Nee
- Ja

1. 16.1 **Aan welke gevaarlijke/schadelijke stoffen?** *(base_toxspec)* Shown if question 1.16 is equal to Ja.

**Aan welke gevaarlijke/schadelijke stoffen?**

1. 17 **Doet u aan intensieve sportbeoefening zoals mara...** *(base_sport)*

**Doet u aan intensieve sportbeoefening zoals marathontraining of intensieve krachtsporttraining**

- Nee
- Ja

Intensief = meer dan 2x per week

## Vervolg: Baseline karakteristieken, lifestyle en gebruik studiemedicatie

1. 18 ☑ **Worden uw testikels regelmatig (wekelijks) blootg…** *(base_warmte)*

**Worden uw testikels regelmatig (wekelijks) blootgesteld aan veel warmte?**

- ☐ Nee
- ☐ Ja, sauna of warme baden
- ☐ Ja, strakke (onder)broeken
- ☐ Ja, wielrennen

1. 19 ◉ **Heeft u ooit bij iemand een zwangerschap tot stan…** *(base_manzw)*

**Heeft u ooit bij iemand een zwangerschap tot stand gebracht?**

Het gaat om alle zwangerschappen, niet alleen bij de huidige partner, en ook een miskraam of zwangerschapsafbreking ("abortus") telt mee

- ○ Nee
- ○ Ja

1. 20 **Sinds wanneer heeft u samen met uw huidige part…** *(base_kiwens)*

**Sinds wanneer heeft u samen met uw huidige partner een actieve kinderwens?**

(dd-mm-yyyy)

1. 21 **Wat is de leeftijd van uw partner?** *(base_lftvr)*

**Wat is de leeftijd van uw partner?**

Jaar

1. 22 ◉ **Is uw partner ooit zwanger geweest?** *(base_vrgrav)*

**Is uw partner ooit zwanger geweest?**

Een miskraam of zwangerschapsafbreking ("abortus") telt ook mee

- ○ Nee
- ○ Ja

1. 22.1 **Hoe vaak is uw partner zwanger geweest?** *(base_gravida)* Shown if question 1.22 is equal to Ja.

**Hoe vaak is uw partner zwanger geweest?**

Keer

1. 22.1 **Hoe vaak is uw partner zwanger geweest?** *(base_gravida)* Shown if question 1.22 is equal to Ja.

**Hoe vaak is uw partner zwanger geweest?**

Keer

1. 23 ◉ **Heeft uw partner de aandoening endometriose?** *(base_endom)*

**Heeft uw partner de aandoening endometriose?**

Endometriose is een aandoening die alleen door een gynaecoloog vastgesteld kan worden

- ○ Nee
- ○ Ja

1. 24 ◉ **Heeft uw partner een cyclusprobleem?** *(base_cyclus)*

**Heeft uw partner een cyclusprobleem?**

- ○ Nee
- ○ Ja, onregelmatige cyclus
- ○ Ja, helemaal geen cyclus

1. 25 ◉ **Heeft u partner recent een eileider foto gehad, of k…** *(Base_HSG)*

**Heeft u partner recent een eileider foto gehad, of krijgt zij dit binnenkort?**

Eileider foto = doorspuiten met contrast van de baarmoeder en eileider en tegelijk Röntgen opname maken, ook wel HSG genoemd

Recent = afgelopen 6 maanden

- ○ Nee
- ○ Ja, een eileiderfoto met contrast en een rontgenfoto/filmpje
- ○ Ja, een 'schuimecho'

1. 26 **Met welke (fertiliteits)behandeling gaat u starten?** *(base_behtype)*

**Met welke (fertiliteits)behandeling gaat u starten?**

Indien u niet zo goed weet of u IVF of ICSI gaat krijgen:

- het verschil tussen IVF en ICSI zit in het werk wat in het laboratorium wordt verricht, voor de man en de vrouw zelf veranderd de voorbehandeling niet
- indien er heel weinig zaadcellen beschikbaar zijn, of deze van matige kwaliteit zijn, wordt een ICSI verricht
- na een TESE/PESA procedure wordt altijd een ICSI gedaan, geen IVF

- ○ Geen, naar huis met 6 maanden spontane kans afwachten
- ○ Inseminatie (IUI), zonder hormonen
- ○ Inseminatie (IUI), met hormonen
- ○ IVF
- ○ ICSI

1. 27 **Wanneer verwacht u te gaan starten (of bent u ges...** *()*

**Wanneer verwacht u te gaan starten (of bent u gestart) met het onderzoeksmiddel?**

(dd-mm-yyyy)

1. 28 **Wat is uw telefoon nummmer waarop wij u kunnen...** *(base_telnr)*

**Wat is uw telefoon nummmer waarop wij u kunnen bereiken indien er vragen zijn?**

Bijvoorbeeld als een en ander niet duidelijk is binnen deze vragenlijst of toekomstige vragenlijsten. Uw telefoon nummer wordt niet voor andere doeleinden gebruikt.

**Vragenlijst maand 2 t/m 6 – Levensstijl en studiemedicatie**

## Survey Step 1. Levensstijl en studiemedicatie

Survey: Vragenlijst maand 1 - maand 6 Step: Levensstijl en studiemedicatie

1. 1 **Gebruikt u de studiemedicatie nog?** *(int_gebruikjn)*

**Gebruikt u de studiemedicatie nog?**
- Nee
- Ja

1. 1.1 **Waarom bent u gestopt?** *(int_stop)* Shown if question 1.1 is equal to Nee.

**Waarom bent u gestopt?**

1. 2 **Is uw gewicht sinds start van het onderzoek veran…** *(int_gew)*

**Is uw gewicht sinds start van het onderzoek veranderd?**
- Nee
- Ja

1. 2.1 **Wat is uw huidige gewicht** *(int_gewkg)* Shown if question 1.2 is equal to Ja.

**Wat is uw huidige gewicht** Kg

1. 3 **Is er sinds start van de studie wat veranderd in ge…** *(int_intox)*

**Is er sinds start van de studie wat veranderd in gebruikt van alcohol/drugs/roken/steroiden gebruik**
- Nee
- Ja

1. 3.1 **Wat is er veranderd?** *(int_intoxspec)* Shown if question 1.3 is equal to Ja.

**Wat is er veranderd?**

1. 4 **Heeft u de afgelopen maand koorts gehad?** *(int_koorts)*

**Heeft u de afgelopen maand koorts gehad?**
- Nee
- Ja

Temperatuur > 38 graden Celsius

1. 5 **Zijn uw testikels de afgelopen maand blootgesteld…** *(int_warmte)*

**Zijn uw testikels de afgelopen maand blootgesteld aan veel warmte?**
- Nee
- Ja, sauna of warme baden
- Ja, strakke (onder)broeken
- Ja, wielrennen

1. 6 **Bent u sinds start van de studie meer of minder in…** *(int_sport)*

**Bent u sinds start van de studie meer of minder intensieve sport gaan uitoefenen? Sport zoals marathontraining of intensieve krachttraining**
- Nee (gelijk gebleven)
- Ja, meer
- Ja, minder

1. 7 **Heeft u iedere dag 1 tablet ingenomen?** *(int_inname)*

**Heeft u iedere dag 1 tablet ingenomen?**
- Nee
- Ja
- Ik weet het niet precies

1. 8 **Hoeveel tabletten heeft u momenteel over?** *(int_tabl)*

**Hoeveel tabletten heeft u momenteel over?** tabletten

Tel het aantal tabletten wat over is

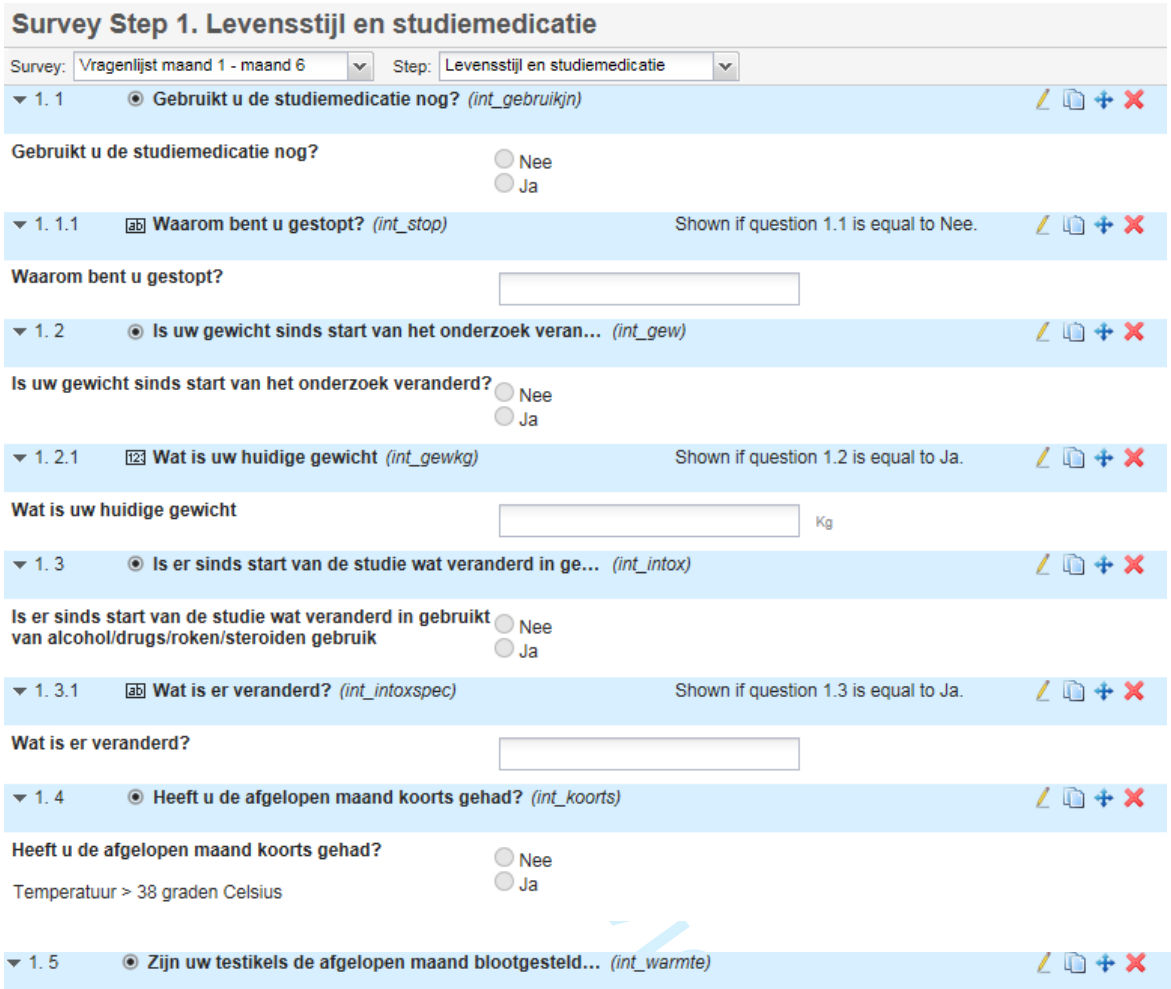
1. 9 **Is er sinds start van studiemedicatie een zwanger…** *(int_zwjn)*

**Is er sinds start van studiemedicatie een zwangerschap ontstaan?**
- Nee
- Ja

1. 9.1 **Wanneer was de eerste positieve zwangerschapst…** *(int_zwtest)* Shown if question 1.9 is equal to Ja.

**Wanneer was de eerste positieve zwangerschapstest?** (dd-mm-yyyy)

**Vragenlijst maand 12 – Uitkomst behandeling en optreden van zwangerschap**



## Survey Step 1. Levensstijl en studiemedicatie

Survey: Vragenlijst maand 1 - maand 6 Step: Levensstijl en studiemedicatie

1. 1 **Gebruikt u de studiemedicatie nog?** *(int_gebruikjn)*

**Gebruikt u de studiemedicatie nog?**
- Nee
- Ja

1. 1.1 **Waarom bent u gestopt?** *(int_stop)* Shown if question 1.1 is equal to Nee.

**Waarom bent u gestopt?**

1. 2 **Is uw gewicht sinds start van het onderzoek veran...** *(int_gew)*

**Is uw gewicht sinds start van het onderzoek veranderd?**
- Nee
- Ja

1. 2.1 **Wat is uw huidige gewicht** *(int_gewkg)* Shown if question 1.2 is equal to Ja.

**Wat is uw huidige gewicht** Kg

1. 3 **Is er sinds start van de studie wat veranderd in ge...** *(int_intox)*

**Is er sinds start van de studie wat veranderd in gebruikt van alcohol/drugs/roken/steroiden gebruik**
- Nee
- Ja

1. 3.1 **Wat is er veranderd?** *(int_intoxspec)* Shown if question 1.3 is equal to Ja.

**Wat is er veranderd?**

1. 4 **Heeft u de afgelopen maand koorts gehad?** *(int_koorts)*

**Heeft u de afgelopen maand koorts gehad?**

Temperatuur > 38 graden Celsius
- Nee
- Ja

1. 5 **Zijn uw testikels de afgelopen maand blootgesteld...** *(int_warmte)*

**Zijn uw testikels de afgelopen maand blootgesteld aan veel warmte?**
- Nee
- Ja, sauna of warme baden
- Ja, strakke (onder)broeken
- Ja, wielrennen

1. 6 **Bent u sinds start van de studie meer of minder in...** *(int_sport)*

**Bent u sinds start van de studie meer of minder intensieve sport gaan uitoefenen? Sport zoals marathontraining of intensieve krachttraining**
- Nee (gelijk gebleven)
- Ja, meer
- Ja, minder

1. 7 **Heeft u iedere dag 1 tablet ingenomen?** *(int_inname)*

**Heeft u iedere dag 1 tablet ingenomen?**
- Nee
- Ja
- Ik weet het niet precies

1. 8 **Hoeveel tabletten heeft u momenteel over?** *(int_tabl)*

**Hoeveel tabletten heeft u momenteel over?** tabletten

Tel het aantal tabletten wat over is

1. 9 **Is er sinds start van studiemedicatie een zwanger...** *(int_zwjn)*

**Is er sinds start van studiemedicatie een zwangerschap ontstaan?**
- Nee
- Ja

1. 9.1 **Wanneer was de eerste positieve zwangerschapst...** *(int_zwtest)* Shown if question 1.9 is equal to Ja.

**Wanneer was de eerste positieve zwangerschapstest?** (dd-mm-yyyy)

## Vervolg: Uitkomst behandeling en optreden van zwangerschap

### Survey Step 1. Uitkomst behandeling en zwangerschap

Survey: Vragenlijst maand 15 Step: Uitkomst behandeling en zwangersc

**1. 1 Welke behandeling heeft u tijdens de 6 maanden g... *(fu_behtype)***

**Welke behandeling heeft u tijdens de 6 maanden gebruik van het studiemiddel gehad?**

Indien u niet zo goed weet of u IVF of ICSI heeft gehad:

- het verschil tussen IVF en ICSI zit in het werk wat in het laboratorium wordt verricht, voor de man en de vrouw zelf veranderd de voorbehandeling niet
- indien er heel weinig zaadcellen beschikbaar zijn, of deze van matige kwaliteit zijn, wordt een ICSI verricht
- na een TESE/PESA procedure wordt altijd een ICSI gedaan, geen IVF

- ○ Geen, naar huis met 6 maanden spontane kans afwachten
- ○ Inseminatie (IUI), zonder hormonen
- ○ Inseminatie (IUI), met hormonen
- ○ IVF
- ○ ICSI

**1. 2 Hoeveel behandelrondes heeft u in totaal gehad? (... *(fu_cycli)***

**Hoeveel behandelrondes heeft u in totaal gehad? (Toelichting: zie grijze "i")**

Een behandelronde telt als er in die ronde een inseminatie (IUI) of eicel punctie (IVF/ICSI) heeft plaats gevonden. Indien u 6 maanden mocht afwachten dan vult u hier het aantal maanden in, namelijk: 6

**1. 3 Bent u na de 6 maanden van gebruik studiemiddel... *(fu_behswitch)***

**Bent u na de 6 maanden van gebruik studiemiddel overgestapt op een andere behandeling?**

- ○ Nee, door met dezelfde behandeling
- ○ Ja, begonnen met IUI zonder hormonen
- ○ Ja, begonnen met IUI met hormonen
- ○ Ja, begonnen met een IVF traject
- ○ Ja, begonnen met een ICSI traject
- ○ Gestopt met alle vruchtbaarheidsbehandelingen

**1. 4 Bent u zwanger geworden? *(fu_zwjn)***

**Bent u zwanger geworden?**

Iedere zwangerschap (vanaf start van deze studie tot vandaag) telt, ook een miskraam of buitenbaarmoederlijke zwangerschap telt mee.

- ○ Nee
- ○ Ja

**1. 4.1 Na welke behandeling bent u zwanger geworden? *(fu_zwbeh)*** Shown if question 1.4 is equal to Ja.

**Na welke behandeling bent u zwanger geworden?**

- ○ Geen, naar huis met 6 maanden spontane kans afwachten
- ○ Inseminatie (IUI), zonder hormonen
- ○ Inseminatie (IUI), met hormonen
- ○ IVF
- ○ ICSI

**1. 4.2 Welke datum had u een positieve zwangerschapst... *(fu_datpos)*** Shown if question 1.4 is equal to Ja.

**Welke datum had u een positieve zwangerschapstest?** (dd-mm-yyyy)

Indien niet precies bekend: ongeveer aangeven

**1. 4.3 Was er een kloppend hartje te zien bij de eerste ec... *(fu_1ehart)*** Shown if question 1.4 is equal to Ja.

**Was er een kloppend hartje te zien bij de eerste echo?**

- ○ Nee
- ○ Ja

**1. 4.3.1 Bij ongeveer hoeveel weken zwangerschap werd d... *(fu_1ehartnee)*** Shown if question 1.4.3 is equal to Nee.

**Bij ongeveer hoeveel weken zwangerschap werd deze eerste echo gemaakt?**

**1. 4.3.2 Was er een kloppend hartje te zien bij de termijnec... *(fu_12hart)*** Shown if question 1.4.3 is equal to Ja.

**Was er een kloppend hartje te zien bij de termijnecho rond 10-12 weken?**

- ○ Nee
- ○ Ja

**1. 4.3.3 Wie heeft de termijnecho gemaakt? (Vernoem naa... *()*** Shown if question 1.4.3 is equal to Ja.

**Wie heeft de termijnecho gemaakt? (Vernoem naam zorgverlener, praktijkgevens)**

Bv: verloskundige praktijk 'Cyclus', te Nijmegen (met evt telefoon nummer!)

1. 4.3.2.1 **Heeft de zwangerschap tot een geboorte geleid?** *(fu_gebjn)* Shown if question 1.4.3.2 is equal to Ja.

**Heeft de zwangerschap tot een geboorte geleid?**

- Nee, er is alsnog een (late) miskraam opgetreden
- Ja

1. 4.3.2.1.1 **Wat is de geboorte datum van uw kind?** *(fu_gebdatum)* Shown if question 1.4.3.2.1 is equal to Ja.

**Wat is de geboorte datum van uw kind?** (dd-mm-yyyy)

1. 4.3.2.1.2 **Bij hoeveel weken is uw kind geboren?** *(fu_gebAD)* Shown if question 1.4.3.2.1 is equal to Ja.

**Bij hoeveel weken is uw kind geboren?** aantal weken en aantal dagen

Bijvoorbeeld: 39 weken en 3 dagen

1. 4.3.2.1.3 **Is uw kind levendgeboren?** *(fu_gebpos)* Shown if question 1.4.3.2.1 is equal to Ja.

**Is uw kind levendgeboren?**

- Nee
- Ja

1. 4.3.2.1.3.1 **Kunt u ons vertellen wat de reden was dat uw kind...** *(fu_gebneg)* Shown if question 1.4.3.2.1.3 is equal to Nee.

**Kunt u ons vertellen wat de reden was dat uw kind niet levend geboren is?**

1. 4.3.2.1.4 **Op welke manier is uw kind geboren?** *(fu_gebmech)* Shown if question 1.4.3.2.1 is equal to Ja.

**Op welke manier is uw kind geboren?**

- Vaginaal
- Vacuumpomp of tang
- Keizersnede

1. 4.3.2.1.5 **Onder leiding van wie bent u bevallen? Graag type...** *()* Shown if question 1.4.3.2.1 is equal to Ja.

**Onder leiding van wie bent u bevallen? Graag type persoon en naam instelling vernoemen**

Voorbeelden: "verloskundige van praktijk Cyclus, thuis" "arts, in Radboudumc ziekenhuis" "poliklinisch met verloskundige, in het Jeroen Bosch ziekenhuis"

1. 4.3.2.1.6 **Wat is het geboortegewicht van uw kind?** *()* Shown if question 1.4.3.2.1 is equal to Ja.

**Wat is het geboortegewicht van uw kind?** gram

Gemiddeld rond de 40 weken is 3000 gram.

1. 4.3.2.1.3.2 **Is uw kind gezond geboren?** *(fu_gezneon)* Shown if question 1.4.3.2.1.3 is equal to Ja.

**Is uw kind gezond geboren?**

- Nee
- Ja

1. 4.3.2.1.3.2.1 **Wat is de reden dat uw kind niet gezond is?** *(fu_ziekneo)* Shown if question 1.4.3.2.1.3.2 is equal to Nee.

**Wat is de reden dat uw kind niet gezond is?**

1. 4.3.2.1.3.3 **Heeft uw kind aangeboren afwijkingen?** *()* Shown if question 1.4.3.2.1.3 is equal to Ja.

**Heeft uw kind aangeboren afwijkingen?**

- Nee
- Ja

Bijvoorbeeld aandoeningen zoals een syndroom van Down, hazenlip, een vinger/teen te veel of een open ruggetje.

**Reply to the reviewers**

**Editorial requests**

We note that you have stated that you are willing to "attach the example questionnaires to the manuscript" in response to one of Reviewer 5's previous concerns. If these questionnaires are not under copyright, please consider providing these as supplementary files.

**Authors:** thank you for this comment, the (Dutch) questionnaires are provided as a supplementary file.

**Reviewer: 1**
**Reviewer Name: Rik van Eekelen**
**Institution and Country: Amsterdam University Medical Centres, location Academic Medical Centre, Amsterdam, the Netherlands**

I commend the authors for tackling some of the concerns of us reviewers and ultimately aiming at conducting and reporting a more valid trial. I regret to say that some points seemed 'swept under the rug' and that the study still has some flaws that, in my opinion, weaken the design of such a grand undertaking to such a degree that needs to be handled in more detail before commencing with the trial.

The three main weaknesses that remain are:
- The inconsistency in treatment protocols i.e. wait for three months before commencing IVF/ICSI still applies, including the issue of couples conceiving naturally before IVF/ICSI, which is of course also possible in clinical practice but warrants a more thorough check of the authors' reasoning of the 72 day waiting period before an effect of Impryl can be expected and what that means for clinical protocols involving Impryl
- The interpretation issue on the three options EM, IUI and IVF/ICSI still applies i.e. that the primary ITT effect is a mixture of the EM/IUI and IVF/ICSI protocols and the fact that the IVF/ICSI result applies to couples in the future who follow the protocol of 'starting Impryl and waiting for three months to commence treatment'
- The issue of patients not wanting to be included in the study when receiving IVF/ICSI because they will have to wait for three months still applies

Major comments:

1. **Reviewer:** I still have concerns regarding the three month wait for IVF/ICSI and the mixture it yields when combined with EM/IUI protocols. Let us say that the trial shows promising results of Impryl, what would the clinical protocol be when using it? Use whenever for EM/IUI but use for a minimum of three months before IVF/ICSI? Is that a realistic clinical protocol? If not, it is probably best to not do this in the trial…

   **Authors:** thank you for reviewing our manuscript. We understand your objections and are very grateful for your valuable comments.

   Initially, we deliberately chose this design, so every group (EM, IUI and IVF/ICSI) has the same chance to achieve a pregnancy in the *optimal* time window: the spermatogenesis is estimated to take 72 days, which means that the sperm cells are optimally influenced by the intervention after this full period (rounded up to 3 months to be on the safe side).

   However, because we agree with the reviewer that answering the treatment policy question is more relevant: as most couples would rather not wait and directly start with their IVF/ICSI treatment, we decided to change this part of the protocol. Furthermore, spermatogenesis is a

gradual process; even though the optimal effect of the antioxidant effect is expected after 72 days, in the period up to this we can expect a gradual influence on the antioxidant scavenging effects of the intervention.

This decision leads to a change in the sample size calculation as can been seen in the manuscript: some IVF/ICSI couples can become pregnant within the suboptimal (for the intervention group) time frame between randomization up to 72 days and this has a direct effect on the expected pregnancy rate in the Impryl® group. We do not expect a different effect between the EM, IUI, IVF/ICSI groups separately and they all start directly with the intervention and achieving pregnancy (with or without ART) after randomization, therefore we expect a 6.5% increase in ongoing pregnancy rate in the Impryl® group within all treatment groups (EM, IUI, IVF/ICSI). According to the current inclusion rate, the ratio between this groups is 1:1:1 instead of the initially expected 1:3:2 ratio. We adjusted this.

Furthermore, we added the clinical implications of this design to the (strengths and) limitations of the study.

2. **Reviewer:** If I may present the authors with an alternative: have the protocol for IVF/ICSI to start when possible after randomization (which hopefully has some variability to it) and then conduct a landmark analysis after three months for all three groups i.e. EM/IUI/IVF for the time window in which Impryl is expected to be particularly effective. This provides clear protocols for all three options, avoids recruitment issues, and has clearer interpretability when combining results whilst still providing information on the time window after three months. The main downside to this approach is that there need to remain a sufficient number of couples in all three groups after three months and the possibility of selecting a particular subgroup who did not conceive for those first three months.

   **Authors:** thank you for this suggestion. As can be read in the section above, we decided to change the protocol: after randomization all groups (EM, IUI and IVF/ICSI) are allowed to start fertility treatment when possible. We decided not to perform a landmark analysis because we already differentiate between the suboptimal and optimal time window by conducting this secondary outcome: Number of pregnancies conceived in the optimal intervention time window, i.e. between start of month 4 till the end of month 6.

3. **Reviewer:** I understand the authors' reasoning regarding the different time frames for secondary outcomes but this does lead to an ongoing pregnancy rate after IVF/ICSI that is defined differently and does not have a direct comparison with the EM/IUI groups. I do agree that as long as the time frames are equivalent in intervention and control groups, it could be informative to include all frozen/thawed cycles.

   **Authors:** We removed the different definitions for EM, IUI and IVF/ICSI in the secondary outcome of overall number of pregnancies. This is now defined as: the overall pregnancy rate meaning the cumulative pregnancy rate up to 9 months after start of intervention

4. **Reviewer:** Regarding the binomial identity link model: this model is not necessarily the best solution in all situations (as the reference provided by the authors also shows, including common convergence issues) and as there is not a large body of evidence from statistical literature to support this further, I am still surprised by the authors' choice.
   Not stratifying for center during randomization blocks and analyzing the data as simple proportions of binary outcomes in both groups seems reasonable with n=1200, might be more robust and relies on the strength of the study design rather than statistical modeling and identification of the correctly specified model.

**Authors:** Independent of the size of the study, not correcting for known (strong) prognostic factors may induce bias in the overall treatment effect estimate. Thus the primary analysis intends to correct for such known factors, while providing an overall treatment effect estimate in terms of a difference in proportion, which can be done directly in the binomial identity link model. The potential convergence problems are acknowledged, hence an alternative solution is also provided (logit link).

5. **Reviewer:** It has now become unclear to me from the Statistical Analysis section what will be the analysis regarding the primary outcome of ongoing pregnancy. What can readers expect in the final manuscript of the trial? Is it the two separate proportions based on the observed ongoing pregnancies, without modelling? Or is it only the absolute risk difference derived from the binomial identity link model? Is it both proportions estimated by that same model? Or is it either of these approaches but separate for EM/IUI/IVF?

   **Authors:** For the primary analysis it is the estimated overall risk difference in proportions based on the model. Additionally, as exploratory subgroup analyses will be presented as well.

6. **Reviewer:** Regarding conditioning on age, MAR and center, I think this should be built up: start with only center as your primary analysis, then as sensitivity analyses (rather than primary analyses) add MAR, then add age as the most important factor related to pregnancy. Due to conditioning and non-collapsibility, presenting only the adjusted/conditional estimates may be somewhat misleading.

   **Authors:** For the trial an unambiguous single primary analysis needs to be defined, otherwise control of the type 1 error at 5% two-sided is no longer assured. The reviewer proposes a stepwise analysis, which seems to suggest as primary analysis the one with only center as prognostic factor included, but in our view this lacks a clear rationale: all factors are known to be prognostic and there is no a priori reason to prefer one above the other as primary analysis. The choice for the binomial identity link model was made to allow a direct estimate of the effect on the risk difference scale, thus avoiding collapsibility issues related to non-linear transformations. Thus we propose to retain the original primary analysis.

7. **Reviewer:** On the Kaplan-Meier approach: do the authors mean presenting different log-rank tests for the effect of Impryl versus placebo separately for the groups EM, IUI and IVF/ICSI?

   **Authors:** The rationale followed is the same as with the primary analysis, where primarily an overall result of the adjusted logrank test will be presented, and subgroups will additionally be explored.

8. **Reviewer:** The authors claim that the EM group has nominal semen parameters and therefore the 3 month semen analysis is not of interest to them. Following that reasoning, none of the unexplained infertile patients should benefit from the intervention Impryl in the first place? Or do the authors mean that the mechanism in which Impryl improves sperm quality and thereby pregnancy outcomes is not captured in the regular semen analyses on motility and morphology?

   **Authors:** Indeed, the latter: semen analysis does not take into account the DNA damage due to oxidative stress. The mechanism of action of the nutritional supplement used in this study is based on the antioxidative effect. This can be read in the introduction in which we added the red part: “Men with sperm subject to increased oxidative stress may have normal seminal parameters, however with DNA damage and therefore a lower chance of natural conception

[15,16]. Sperm DNA fragmentation (SDF) tests are available, however most of them are expensive (roughly 200-300 USD per test), complex and lack standardization and validation.[17]"

9. **Reviewer:** How will the authors deal with loss to follow up in their study and analysis?

**Authors:** Thank you for pointing this out; in the first paragraph of the statistical analysis part we did mention dealing with the data of patients who are lost to follow-up. However, in accordance with the reviewer's comments we further specified this: "We will analyze all data on an intention-to-treat basis. Data of patients who are lost to follow-up will be included in their randomized group.

- Patients who are lost to follow-up during the treatment period are considered not to have achieved a pregnancy.
- Patients, who are lost to follow-up right after achieving a pregnancy of less than 8 weeks of gestation, are considered not to have achieved an ongoing pregnancy
- Patients, who are lost to follow-up right after achieving a pregnancy of 8 or more weeks of gestation, are considered to have achieved an ongoing pregnancy. "

**Reviewer: 2**
**Reviewer Name: Andrea Garolla**
**Institution and Country: University of Padova, Italy**

Please leave your comments for the authors below:
Authors did not address my concerns.

Major comments:

1. **Reviewer:** Including infertile and subfertile patients in the trial without understanding the reason for infertility (at least try to make appropriate assessments), can be a source of bias. I mean that administering a supplement indicated to reduce oxidative stress in patients without evaluation of the cause of this disorder, can be a source of error in the interpretation of the results. I remain of the idea that the cause of infertility should be investigated.

**Authors:** Thank you very much for reviewing our manuscript. As for the cause of oxidative stress: varicocele (which is a cause of oxidative stress) patients are excluded. Furthermore, lifestyle habits (great effects on the occurrence of oxidative stress) are reported, not only at baseline but also during every month of treatment.

As for the cause of infertility: in the Netherlands it is standard care to try to understand the reason for infertility of a couple. Standard fertility work-up for both, the female and male, consist of a comprehensive review of the medical history, including items relevant to the potential etiologies of infertility. Meaning: conducting further information on coital frequency and timing, any evidence of sexual dysfunction, including erectile or ejaculation issues, duration of infertility, childhood illness and developmental history, systemic medical illness (including inflammation), previous surgery (eg, cryptorchidism with or without surgery), medication use, including anabolic steroids and supplements (eg, testosterone), allergies, sexual history, sexually transmitted infections and exposure to gonadal trauma or toxins. A semen analysis is always part of standard fertility work-up. All male infertility patients with a sperm count <1 million are referred to a specialist with expertise in male reproductive medicine for further testing (scrotal ultrasound, hormonal blood panel, genetic testing).

Last but not least, it is well known that unexplained infertility is found in 30% of couples trying to conceive and consulting a fertility specialist. Unexplained infertility occurs when the definition of

infertility is met, the basic infertility evaluation is performed, and all the tests results are normal, meaning there is evidence of ovulation, tubal patency, and a normal semen analysis.

2. **Reviewer:** Many details in the protocol have not been clarified

   **Authors:** we are sorry to hear this.

3. **Reviewer:** If inflammatory conditions was even an exclusion criterion in many of the randomized trials included in the meta-analysis to which authors refer, they should adequately assess the included patients to avoid the risk to include subjects without hyperhomocysteinemia and/or oxidative stress

   **Authors:** we would like to refer to the answer above: "As for the cause of infertility: in the Netherlands it is standard care to try to understand the reason for infertility of a couple. Standard fertility work-up for both the female and male consist of a comprehensive review of the medical history, including items relevant to the potential etiologies of infertility. Meaning: conducting further information on coital frequency and timing, any evidence of sexual dysfunction, including erectile or ejaculation issues, duration of infertility, childhood illness and developmental history, systemic medical illness (including **inflammation!),** previous surgery (eg, cryptorchidism with or without surgery)**,** medication use, including anabolic steroids and supplements (eg, testosterone) and allergies**,** sexual history and sexually transmitted infections and exposure to gonadal trauma or toxins. A semen analysis is always part of standard fertility work-up. All male infertility patients with a sperm count <1 million are referred to a specialist with expertise in male reproductive medicine for further testing (scrotal ultrasound, hormonal blood panel, genetic testing)."

   Men with a known cause for male infertility are excluded as can be read in the exclusion criteria:
   "- Embryo-transfer after pre-implantation genetic diagnosis
   - Known endocrine abnormalities related to male infertility, or use of fertility enhancing drugs such as clomiphene citrate or follicle-stimulating hormone (FSH)
   - Known genetic abnormalities related to male infertility
   - Known urological abnormality such as a varicocele or bilateral cryptorchism"

   As for finding the direct cause of oxidative stress in our cohort by measuring inflammatory parameters in our patients: this is not the scope of our research. This is a clinical study on the effect of antioxidants; this research does not focus on finding the pathophysiological mechanism and etiology why men of an infertile couple are more likely to have higher levels of oxidative stress.

4. **Reviewer:** Cysteine and folates do not have a direct antioxidant role (such as glutathione and vitamin E) and not all group B vitamins are implicated in the homocysteine cycle (vitamin B1, B2, H, B5 etc are not implicated in this complex metabolism). However, the reduced literature that is available identifies as a functional dose of N-acetyl cysteine in 600mg / day. Regardless of the considerations on the other components of Impryl, cysteine is underdosed.

   **Authors:** in this study we use a nutritional supplement that is already available on the European market and is marketed as being specific for infertile patients. We did not develop this supplement and the scope of this research is not to establish the dose-efficacy.

However, from correspondence with the manufacturer we can report the following: the dosages are exactly those established by the European Safety Agency (EFSA) as the daily requirements. There is no fixed Nutrient Reference Values (NRV) for the ingredients betaine and L-cystine. In the case of Impryl® the amount is based on stoichiometric calculations.

5. **Reviewer:** Because the study is mainly based on the antioxidant effect of some substances on semen, authors must provide at least one test to evaluate this effect before and after treatment

   **Authors:** We do agree with the reviewer that measuring the antioxidative effect (by sperm DNA fragmentation (SDF) testing) strengthens the study.
   However, as said in the previous 'reply to the reviewers': "Adding DNA fragmentation tests would also mean an extra burden for all patients: the EM patients have to come back for an extra visit to deliver an extra semen sample, and all patients have an extra burden because performing a DNA fragmentation test would mean that we need an extra semen sample (next to the one necessary for the fertility treatment) because this material can't be used after the analysis for the IUI or ICSI/IVF procedure.

   Furthermore, there is no international consensus on which DNA fragmentation test should be used, most of them lack standardization and validation, and on top of it performing a DNA fragmentation test would mean tremendous extra costs: 1200 men, 2 DNA fragmentation tests for each patient (T0 en T3 months) = 2400 DNA fragmentation tests with an average cost of 200-300 USD per test = roughly half a million dollar extra on top of the initial study costs.."

   Currently, we do our utmost to get the financial resources to perform SDF testing in a subgroup of our study population in the future. However, at this point in time we are not able to make a promise.

6. **Reviewer:** Despite a right assessment of sperm DNA stability before and after treatment is expensive, the weight of the study would increase enormously. Moreover if the supplement has an indications as "contributing to the normal synthesis of DNA by offering a defence against oxidative stress" this test is mandatory beside the number of final pregnancies after treatment

   **Authors:** Thank you very much for your comment. This might be the point to agree to disagree: this is a clinical study, the outcome of most importance for daily practice, meaning clinicians and patients, is the number of pregnancies. Indeed, the mechanism of action of the nutritional supplement is believed to take place in the defense against oxidative stress. However, SDF testing would be fantastic and improve the weight of study, but is at this moment in time not feasible. Up to this moment, the clinical trials that did SDF testing were small studies.

   We would like to ask the reviewer to consider that this study is the largest study in the field so far; with the aim of including 1200 patients. The costs of SDF testing are not in comparison to studies done so far with a couple of hundred patients. Like we reported in the answer above: we do our utmost best to get the financial resources to perform SDF testing in a subgroup of patients.

**Reviewer: 3**
**Reviewer Name: Manuel Fernández-Sánchez**
**Institution and Country: IVI RMA Seville**

**Please leave your comments for the authors below:**
This article has improved significantly by considering the comments of the reviewers. Congratulations on this good job.

**Author:** thank you for the positive feedback and reviewing our manuscript!

**Reviewer: 4**
**Reviewer Name: Marco G. Alves**
**Institution and Country: Unit for Multidisciplinary Research in Biomedicine (UMIB) Institute of Biomedical Sciences Abel Salazar (ICBAS) University of Porto Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal**

**Please leave your comments for the authors below:**
The authors more or less answered the concerns though it is unclear how the supplement is metabolized and reaches the reproductive system

**Authors:** we would like to thank the reviewer for the feedback. We would like to refer to the study of M. Dattilo et al of 2016 in J Assist Reprod Genetic (Improvement of gamete quality by stimulating and feeding the endogenous antioxidant system: mechanisms, clinical results, insights on gene-environment interactions and the role of diet) and refer to the following paragraph: “The key to achieve an oxy-redox balance is the support to cellular homeostatic mechanisms: Full availability of micronutrients necessary to the 1CC favors the synthesis of GSH and the function of the endogenous antioxidant cascade resulting in measurable clinical gains;”

**FORMATTING AMENDMENTS (if any)**
Required amendments will be listed here; please include these changes in your revised version:

**Authors:** no required amendments were listed above. No action needed.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Reported on page No |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 2 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | - |
| Protocol version | 3 | Date and version identifier | 1 |
| Funding | 4 | Sources and types of financial, material, and other support | 17 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1 |
| | 5b | Name and contact information for the trial sponsor | 1 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 17 |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 17 |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 4 |
| | 6b | Explanation for choice of comparators | 5 |
| Objectives | 7 | Specific objectives or hypotheses | 5 |

| | | | |
|---|---|---|---|
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 6 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 11 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 8 |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 11-12 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 9 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 7-8 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 9-10 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 7 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 7 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 8-9 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8-9 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8-9 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 8-9 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 8-9 |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 9-11 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 10-11 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 10-11 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 12 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 12 |

| | | | |
|---|---|---|---|
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 15 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 15 |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 12-13 |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 15 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 15 |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 8 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NA |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 8-9, 11 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 18 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 18 |

| | | | |
|---|---|---|---|
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | NA |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 15 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 15 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 15 |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Only in Dutch, can be requested |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | NA |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

# The impact of a nutritional supplement (Impryl®) on male fertility – study protocol of a multicentre, randomized, double-blind, placebo-controlled clinical trial (SUMMER-trial)

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# The impact of a nutritional supplement (Impryl®) on male fertility – study protocol of a multicentre, randomized, double-blind, placebo-controlled clinical trial (SUMMER-trial)

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## ABSTRACT

**Introduction** Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children. In approximately 50% of infertile couples a male factor is involved. Most of the male infertile cases are characterized as “idiopathic”, except for a small percentage of cases which are causative by a genetic aetiology. In the past decade, the role of oxidative stress related to sperm quality has been researched thoroughly and estimated to be the problem in 30% to 80% of male infertility cases. Impryl® is a nutritional supplement which works on the metabolic system and regulation of oxidative stress by activating the 1-Carbon cycle and therefore recycling of homocysteine.
We hypothesize that the nutritional supplement Impryl® in men of infertile couples might improve the ongoing pregnancy rate.

**Methods and analysis** We designed a multicentre, randomized, double-blind, placebo-controlled clinical trial. We aim to include 1200 male adults, age 18-50 years, part of a couple that is diagnosed with infertility. The couple will either start or has already been started with fertility treatment, i.e. expectative management (EM, duration 6 months), intra-uterine insemination (IUI) with or without mild ovarian stimulation (MOH) or ovulation induction (OI), either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment. Male participants will be randomized in either Impryl® or placebo group, with identical appearance of the tablets to be distributed (doses: one tablet each day), for a total duration of maximal 6 months. Patients can start directly with fertility treatment and/or natural conception. The primary outcome is the number of ongoing pregnancies confirmed by ultrasound at ≥ 10-12 weeks, and conceived in the time window between randomization up to and including month 6 of intervention use. Secondary outcomes are change in semen parameters between baseline and after 3 months intervention in IUI/IVF/ICSI group, based on (pre-wash) total motile sperm count (TMSC). Furthermore the number of pregnancies conceived in the optimal intervention time window (after full spermatogenesis of 72 days), overall number of pregnancies, time to pregnancy, embryo fertilization rate in IVF/ICSI, embryo-utilization rate in IVF/ICSI, number of miscarriages, live birth rate and adverse events are documented within the study period of 15 months.

**Ethics and dissemination** The protocol is approved by the local medical ethical review committee (METC) at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects (CCMO). Findings will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of medical care and quality of life for patients with infertility.

**Trial registration number** NCT03337360 / NTR6551


**Keywords** nutritional supplements, fertility, male infertility, oxidative stress, clinical pregnancy rate, live birth rate

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- This multicentre randomized double-blind, placebo-controlled trial will provide important information to patients with diagnosis of male infertility and clinicians about the efficacy of nutritional supplement Impryl® with combined antioxidants
- With a sample size of 1200 patients this is up to date the largest randomized study with the use of nutritional supplements in fertility patients
- Permuted block randomization ensures treatment group numbers are evenly balanced, with stratification for centre and type of infertility treatment
- The optimal effect of Impryl® is expected after full spermatogenesis of 72 days
- The use of diagnostic tests to screen for oxidative stress in semen samples (like sperm DNA-fragmentation tests or 8-OH-dG levels) could be of use in predicting the effect of Impryl® in different patient groups. However, these tests are not used in this study due to their complexity and lack of standardization and validation

## INTRODUCTION

Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children.[1] In approximately 50% of infertile couples a male factor is involved.[1,2] In the past decade, the role of oxidative stress on sperm has been researched thoroughly and found to be the problem in 25% to 87% of male infertility cases.[3-9]

All cells in the human body use oxygen to survive. In this process, toxic radicals with high reactive activity, so called reactive oxygen species (ROS), are produced as a consequence.[10] To scavenge these toxins the body has developed a defence mechanism in which antioxidants play an important role. The production of ROS in sperm is a normal physiological process mostly regulated by cellular en extracellular antioxidative factors. However, when the ROS production overwhelms the natural antioxidant defence, increase of apoptosis is observed. Increased oxidative stress can occur due to environmental and lifestyle factors such as smoking, stress, obesity and nutrition.[10] In the reproductive tract, semen leukocytes and immature spermatozoa are major sources of ROS production.[11] However, a low production of ROS is also physiological and needed for adequate sperm functioning by supporting capacitation, maturation and hyperactivation.[12] Spermatozoa are more vulnerable and prone to oxidative stress compared to other body cells because of the lack of cytoplasm.[13] Cytoplasmic antioxidant enzymes are important for directly scavenging and repairing the damage of ROS. However, in the spermatozoa the cytoplasm is removed during the final stages of spermatogenesis, leaving them without these important enzymes to protect them from ROS altering the sperm deoxyribonucleic acid (DNA). Furthermore, the membrane of spermatozoa is rich in fatty acids, making them vulnerable for lipid peroxidation by ROS resulting in decreased flexibility of the sperm membrane and reduction of tail motion.[13] For these reasons, spermatozoa are dependent on seminal plasma which is rich in antioxidants. Examples of antioxidants already present within seminal plasma are ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione, amino acids (taurine, hypotaurine), albumin, carnitine and carotenoids.[14] Despite the common association between male infertility and oxidative damage, there is no World Health Organization (WHO) recommendation about which test to use for detecting sperm DNA damage and there are no standardized protocols which could help global interpretation of the results. Men with sperm subject to increased oxidative stress may have normal seminal parameters, however with DNA damage and therefore a lower chance of natural conception. [15,16] Sperm DNA fragmentation (SDF) tests are available, however most of them are expensive (roughly 200-300 USD per test), complex and lack standardization and validation.[17] Furthermore, a recent meta-analysis showed that an association does not imply an actual predictive value.[17] Examples of SDF test are deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL), the COMET assay or sperm chromatin structure assay (SCSA) and another option is measuring the by-product of DNA oxidation called 8-hydroxydeoxyguanosine (8-OHdG).

A recent Cochrane review suggested that antioxidant supplementation in male infertility might improve the outcomes in assisted reproductive technologies (ART).[18] However, the evidence was rated as low and clinical studies showed contradictory results with sometimes even a negative effect of high doses of antioxidants due to reductive stress as a rebound effect.[19] Alternatives were therefore explored, to support the natural antioxidant defences that are predicted to act within the modulation of the natural cellular homeostasis without generating rebound effects. For example, the use of nutritional supplements supporting DNA methylation and the homocysteine pathway. Homocysteine is the end-product of the 1-Carbon cycle, feeding DNA methylation, and the starting substrate for the thiol glutathione (GSH, l-γ-glutamyl-l-cysteinyl-glycine) de novo biosynthesis. GSH is the most important endogenous antioxidant, involved in maintaining the antioxidant balance in human tissues and directly involved in the elimination of ROS.[20] Homocysteine is an inhibitor of the methylation process and a powerful pro-oxidant. It has a negative effect on spermatogenesis and its concentration in the ejaculate is inversely correlated with fertility outcome.[21,22] Dattilo et al stated that the ideal supplement should work by favouring homocysteine recycling by restoring the efficiency of the 1-Carbon cycle, therefore ensuring the availability of activated methyl groups for DNA methylation and feeding the intracellular antioxidant system by supporting GSH synthesis.[23] In non-randomized pilot studies with such a 1-Carbon cycle supporting nutritional supplement Condensyl®, the precursor of Impryl®, there was a significant decline of DNA fragmentation index leading to an improvement of the clinical pregnancy rate. However, the quality of these studies for the impact on pregnancy rate is rather low due to the non-randomized nature and having no control group.[21,24,25] Furthermore, the ideal parameters to measure DNA-damage might not be DNA-fragmentation, but sperm-oxidation level which indirectly affects DNA-damages.[26,27]

Impryl® is a nutritional supplement mainly consisting of vitamin B, which works on the metabolic system by activating the 1-Carbon cycle and recycling of homocysteine without the use of any direct strong antioxidant. Therefore it is could be more effective than other nutritional supplements. The cost of Impryl® is 30 euro for each 30 days of use.

It is well known that assisted reproduction technologies are expensive with the cost per in vitro fertilization (IVF) treatment in the Netherlands estimated to be between 2,885 and 5,259 euro (€) and the cost of an intra-uterine insemination (IUI) cycle between 497€ and 1123€, depending on the use and doses of medication during ovarian stimulation.[28-30] Therefore, substantial cost savings could be made if the use of relatively inexpensive nutritional supplements would lead to a shorter time to pregnancy with less treatment cycles necessary or even better, when the use of more expensive invasive reproductive techniques can be avoided at all.

## METHODS AND ANALYSIS

### Study design

This investigator-initiated, multicentre, placebo-controlled study has a double-blinded design, with blinding of participants and investigators. We conduct this study in 15 academic and non-academic hospitals in The Netherlands, at the Department of Gynaecology and/or Reproductive Medicine.

The study protocol was designed using the recommendations of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[31]

The study is currently ongoing; the first participant was included on 30 May 2018. We expect to include the last participant in June 2022 and study completion by December 2023.

### Participants

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

Inclusion criteria:
- Couples with failure to conceive for at least 12 months and starting with expectative management (EM) **or** couples starting with 1st/ 2nd/3rd cycle of IUI (with or without mild ovarian stimulation) **or** 1st/ 2nd/3rd cycle of IVF or intracytoplasmic sperm injection (ICSI) - Male with age 18-50 years
- Female partner with age 18-43 years
- Both male and female are willing and able to give informed consent

Exclusion criteria:
- Planned or performed TEsticular Sperm Extraction (TESE) or Percutaneous Epididymal Sperm Aspiration (PESA)
- Use of donor-, cryopreserved- or electro-ejaculated semen or donor oocytes
- Ovulation induction (OI) without IUI
- IVF for an absolute tubal factor
- Embryo-transfer after pre-implantation genetic diagnosis
- Known endocrine abnormalities related to male infertility, or use of fertility enhancing drugs such as clomiphene citrate or follicle-stimulating hormone (FSH)
- Known genetic abnormalities related to male infertility
- Known urological abnormality such as a varicocele or bilateral cryptorchidism
- Use of other vitamins or nutritional supplements with antioxidant effect

Withdrawn or unblinded participants will not be replaced. They will not be re-included in the study once they dropped out, and their identification number and treatment will not be reused.

**Sample size calculation**

We aim to recruit 1200 male participants as a part of an infertile couple, based on the following data and power calculation.

Overall, the number of ongoing pregnancies in IVF and ICSI in the Netherlands was respectively 19.8% and 21.5% per started fresh cycle in 2015.[32] The number of ongoing pregnancies in IUI is estimated to be around 5 to 13% per cycle, around 18% after three cycles and 20-30% after six cycles.[33-35] Based on Radboudumc data, the ongoing pregnancy rate in EM is estimated around 20% after 6 months.

A Cochrane review estimated the increase in clinical pregnancy rates after use of antioxidants with an Odds Ratio (OR) of 3.43 (95% CI 1.92 to 6.11) based on 7 RCTs with in total 522 men.[36] However, this concerns low quality evidence, and the included population in this Cochrane review was couples with male factor subfertility, whereas in our study we also include couples with unexplained infertility. Therefore, we assumed a more conservative OR of 1.5. Assuming a 20% ongoing pregnancy rate in the placebo group, this reflects in an expected pregnancy rate of 27.3% in the Impryl® group.

The study is designed as a superiority trial. Based on the above mentioned data, we expect a 7.3% increase in ongoing pregnancy number when men are treated with Impryl® compared to placebo. However, after randomization patients can directly start with both intervention and achieving a pregnancy, either spontaneously or with fertility treatment. . In these first months the effect of the intervention is expected to be suboptimal due to the duration of the spermatogenesis (72 days before whole renewal). Therefore we adjusted the expected increase of 7.3% to a more realistic effect of 6.5%. We assume an equal increase in all fertility groups (meaning EM, IUI and IVF/ICSI) from 20% to 26.5%.

To test the effect of Impryl® on the probability of ongoing pregnancy, a sample size of 600 men per treatment group (1200 in total) is needed, assuming differences in ongoing pregnancy rates of 6.5% and an expected ongoing pregnancy rate of 20% in the placebo group, a two-sided alpha of 5% and a beta of 24% (i.e. 76% power). As we expect the number of participants lost to follow up to be minimal, we will allocate 600 patients to both the intervention and the placebo arm. Figure 1 shows the distribution of the number of participants for the 3 different types of fertility treatment (EM, IVF/ICSI and IUI).

**Investigational product/intervention**

Impryl® tablets will be produced by Labomar SRL (compliant with European Good Manufacturing Practice (GMP) who will also produce an identical looking placebo tablet. Impryl® is a food supplement and is therefore not considered an investigational medicinal product. To assess product quality, we have quality assessment documents of both the food supplement and placebo: Summary of Product Characteristics (SPC, see supplementary file 1), Certificates of Analysis (CoA), certificate of Good Manufacturing Practice (GMP) and ISO 9001 form. Intervention will start directly after randomization.

All couples will receive our standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology. After diagnostic work up couples will either start with EM (6 months), IUI, IVF or ICSI. Participants will take study supplement for a maximum of 6 months, even if the fertility intervention (6 x IUI of 1 x IVF/ICSI cycles) is not completed.

Co-treatment with other vitamins or supplements is prohibited. If vitamins or supplements are already used by men, a wash-out period of 3 months is recommended. Patients using other supplements will be excluded or reported as protocol violation If after randomization it appears that a patient still uses other supplements (reported in the online questionnaire), they will be telephonically contacted and asked to stop use of other supplements. After this stop, there is a wash-out period of 72 days (duration of spermatogenesis). If no pregnancy occurs within this wash-out period the patient and data can still be included.

**Randomization and treatment allocation**

All couples visiting a fertility specialist for the first time or for evaluation of fertility treatment will be informed on this study. Prior to or at their actual appointment they will receive the patient information (PIF) from the fertility specialist (specialised nurse or doctor). Randomization will be performed, after signed informed consent has been obtained, using a web-based application (Castor). We will use permuted block-design, stratified for fertility treatment (1st) and recruiting centre (2nd). Block sizes are flexible. Participants will be randomized in a 1:1 ratio to study medication (Impryl®) or placebo. Assignment to medication, using the randomization list, will either performed at the local centre or at Radboudumc by an independent person. This person is neither participating in clinical treatment nor processing the study data. Randomization outcome is either A (Impryl®) or B (placebo).

Coding starts immediately after informed consent has been obtained. In Castor each patient will receive a combination of one letter and 3 numbers. The code will not provide any information about the received intervention (Impryl® or placebo). The list of codes corresponding with patient information and received medication will be saved in an separate file, locked (either digital with password or a locked cabinet), only accessible for the independent person who did the randomization.

The study is double blinded. All personnel, the researchers and patients will remain blinded to the intervention being received, except the personnel performing randomization and distributing the study medication. The indications for breaking the randomization code are: serious adverse event (SAE), serious adverse reaction (SAR) and Suspected unexpected serious adverse reactions (SUSARs) SUSAR, as instructed by the local medical ethical review committee (METC), or in a dire emergency, as directed by the principal investigators or trial manager. Every site has access to a deblinding form in which patients details, reason for deblinding, statement of principal site investigator, date and time of intervention stop and

randomization allocation will be reported. The principal site investigator will inform the coordinating investigator about the deblinding.

At all study sites the person distributing the study medication is an unblinded employee of the department of Reproductive medicine or Gynaecology who is not involved in the treatment of the patient nor involved in the data collecting of the research. Drug accountability will be performed in line with Good Clinical Practice (GCP) requirements. The investigator is responsible for drug accountability and these tasks will be delegated to the primary investigators at the sites. We will log all dispensing of the investigational product on a drug accountability log. On every distributing study site there will be a batch of study medication stored at room temperature, no special precautions. An unblinded authorized employee of the department of Reproductive medicine (or Gynaecology) will distribute the study medication after randomization has been performed by Castor. The flag label (identification A = Impryl® or B = placebo) will be removed from the box and the randomization number noted (handwritten) on the medication box. Thereafter, the box will be handed over to the patient. On the patient identification & drug accountability log (either digital or on paper) the following details will be reported: date of issue, study (randomization) number, batch number, expiration date, amount dispensed, current storage amount, randomizer/distributor initials, hospital number, patient name and initials, date of birth and monitoring check.

All unblinded personnel performing randomization will be trained by a site initiation to perform randomization in Castor EDC and distribution of medication. A log list of all personnel involved in the study, with blinding status, responsibilities and signature, will be saved.

Unblinding will be performed when the study has ended, database is locked and protocol violators have been defined.

Patients are not asked to return study medication because this would mean an extra burden for the patients: an extra visit for all EM patients and some IUI/IVF/ICSI patients. Furthermore, patients could also forget a strip at home that they did not collect or put in the box. Since it is a supplement and not medication there are no specials precautions for destruction or disposal.

**Participant timeline and intervention**

All couples will receive standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology (NVOG). Initially, from the active start of the study and first inclusion in June 2018, couples in the EM or IUI group could start directly with both the intervention (study medication) and starting to conceive. However, in the IVF or ICSI treatment group participants had to use study medication at least 3 consecutive months before using semen for the actual IVF or ICSI to be sure of an optimal treatment effect of the intervention. However, in April 2020 there was a protocol change, which allowed all participants from each treatment category (EM, IUI and IVF or ICSI) to start directly with both the intervention (study medication) and fertility treatment and/or natural conception at the

same time. This amendment was made to make the situation more realistic and more in line with daily practice, with taking into account the gradual process of spermatogenesis rather than only an effect after 72 days, and was approved by the local ethics committee. After diagnostic work up couples will either start with EM (6 months), IUI, IVF or ICSI, according to the NVOG guidelines. Participants will take study medication (Impryl® or placebo) for a maximum of 6 months, even if the fertility intervention (6 x IUI or 1 x IVF/ICSI cycle) is not completed. Use of the food supplement or placebo will be stopped earlier when a pregnancy is achieved. Participants will be followed for 15 months after randomization for detecting live births. Figure 2 shows the participant timeline.

During the study, participants are asked to report on baseline characteristics, lifestyle changes and pregnancy outcomes by short online questionnaires (see supplementary file 2).

- Baseline: directly after randomization, patients will receive an automatic email invitation to provide baseline information online in the Castor database system.
- Monthly: during use of study medication (six months), participants will be asked to report on occurrence of pregnancy (with date of positive test), changes in lifestyle, consumed total amount of study medication (used boxes and tablets) and occurrence of adverse events. They will receive an automatic email invitation.
- Follow-up: 15 months after randomization participants will receive an automatic email with invitation for the last short questionnaire. They will receive questions about fertility treatment and pregnancy outcome (ability to achieve a pregnancy, occurrence of miscarriage or childbirth, date of delivery, gestational age, mode of delivery, complications of delivery and birth weight, sex, congenital abnormalities and health of the neonate.

At least 2 semen analyses will be performed at two time points in this study: the first semen analysis will be performed during diagnostic work-up (standard care, analysis according to WHO criteria[37]) and the second one after approximately 3 months of using study medication for participants having the IUI or IVF/ICSI treatment. We decided not to perform a second semen analysis in the EM group due to the fact that they present with normal semen parameters at intake and we want to avoid the burden of an extra visit.

If a clinical pregnancy is achieved, a routine ultrasound will be performed in the first trimester between 5 to 9 weeks to determine the vitality of the foetus. A second routine ultrasound will be performed around 10-12 weeks to estimate the due date. To minimalize the amount of extra site visits, we decided that this standard 'due date' ultrasound at 10-12 weeks of pregnancy is enough for determining the primary outcome (ongoing pregnancy). The ultrasound can be performed in the midwife practice. Information about the outcome of this ultrasound is reported in the 15-months questionnaire.

**Outcome measures**

This study investigates the effect of food supplement Impryl® on ongoing pregnancy rate when used by the male of an infertile couple.

Primary endpoint:
The number of ongoing pregnancies, conceived in the time window between randomization up to and including month 6 of intervention use.

Secondary endpoints:

- Number of pregnancies conceived in the optimal intervention time window, i.e. between start of month 4 till the end of month 6.
- Overall number of pregnancies meaning the cumulative pregnancy number up to 9 months after start of intervention
- Time to pregnancy defined as

a) The time between randomization and reaching ongoing pregnancy (confirmed by ultrasound)

b) The time between start of fertility treatment during the study (EM, IUI, IVF/ICSI) and reaching ongoing pregnancy

- Change in semen parameters in IUI/IVF/ICSI group based on pre-wash total motile sperm count (TMSC), allocated to change in treatment category (EM, IUI, IVF, ICSI)
- Improvement between Impryl® and control group in fertilization rate and embryo-utilization rate in IVF/ICSI group. Fertilization rate is defined as the percentage of oocytes with 0 PN or >=2 PN after insemination (IVF) of injection (ICSI). Abnormal fertilization such as 3PN will be recorded, in case this percentage differs or increases in the study group. The embryo-utilization rate (EUR) is defined as the number of high quality embryos obtained, embryo's used at transfer plus the number of embryos frozen, divided by the number of zygotes obtained in a cycle. Due to the differences in embryo evaluation and embryo selection criteria for cryopreservation, we decided to measure the relative increase in fertilization and utilization rate observed for each clinic.
- Number of miscarriages (defined as non-vital intra-uterine pregnancy before 16 weeks of gestation)
- Live birth rate (beyond 24 weeks, defined as the birth of a living child) within study-period of 15 months
- Adverse effects

Male baseline parameters that are collected by online questionnaires are: age, length, weight, ethnicity, alcohol/drugs/nicotine use, use of vitamins/steroids or other supplements, diet specifics (options: normal, vegetarian, vegan, gluten-free, diary free, other), medication use, activities that cause increased scrotal temperature such as often visiting a sauna, taking a hot bath or race cycling, general health, operations or trauma in genital area, exposure to toxins in environment, conception of previous children, duration of infertility and type of diagnosis of infertility. Furthermore the age and parity of the female partner has to be documented, as well as the occurrence of endometriosis or an anovulatory cycle.

**Handling and storage of data and documents**

Data will be collected in an online registration system (Castor) by the coordinating investigator or research nurses. Data handling will be done anonymously, with the patient code only available to the local investigator and the research nurse working in the local

centre. The results will be extracted from Castor. If there are missing data this will be mentioned along with the reason. The data will be preserved for the duration of 15 years. The handling of personal data complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP).

**Monitoring and Quality Assurance**

Monitoring will be performed in compliance with GCP and other rules and regulations in order to achieve high quality research and secure patient safety. Monitoring will be done by an independent party of the Radboud University Medical Centre. The monitor is certified and has been approved by the local medical ethical review committee (METC).

**Statistical analysis**

We will analyse all data on an intention-to-treat basis. Data of patients who are lost to follow-up will be included in their randomized group:

- Patients who are lost to follow-up during the treatment period are considered not to have achieved a pregnancy.
- Patients, who are lost to follow-up right after achieving a pregnancy of less than 8 weeks of gestation, are considered not to have achieved an ongoing pregnancy
- Patients, who are lost to follow-up right after achieving a pregnancy of 8 or more weeks of gestation, are considered to have achieved an ongoing pregnancy.

In addition, a per-protocol analysis will be performed for the primary outcome and the secondary outcomes overall pregnancy number, time to pregnancy, number of miscarriages and live birth rate.

Descriptive statistics will be calculated to check for major dissimilarities between study groups with respect to baseline information. Baseline data will be described quantitatively. For continuous variables, we will examine the distribution of the observations, and if normally distributed we will summarize them as means with standard deviations (SDs). If they are not normally distributed, then medians and inter-quartile ranges (IQRs) will be reported. For dichotomous data, we will provide counts and proportions.

The primary outcome variable ‘ongoing pregnancy’ will be assessed as follows. The ongoing pregnancy percentages as observed in the trial will be presented for both treatment arms, overall, and separately for the three strata (i.e. EM, IUI, IVF/ICSI). In order to adjust for possible imbalances between the treatment groups, the pregnancy percentages and differences in pregnancy percentages between the experimental and control group, and the corresponding 95% confidence intervals will be estimated using a fixed effects binomial model with an identity link, including intervention, fertility treatment, centre and female age (centered). If this model does not converge, a logit link will be used.

Time to ongoing pregnancy will be evaluated by the Kaplan-Meier approach; differences between the two arms will be tested with the log-rank test. Similar analyses will be conducted

per stratum (EM, IUI or IVF/ICSI). The other secondary outcomes (overall pregnancy number, number of miscarriages, live birth rate, fertilization rate, embryo-utilization rate) will be evaluated similarly to the primary outcome. Changes in semen analysis, leading to different fertility treatment categorisation, will be calculated with descriptive statistics. Furthermore, adverse events will be summarized. Sensitivity analyses will be performed to evaluate possible effects in case of protocol amendments.

The per-protocol population will consist of all randomized patients without any major deviation from the protocol. A major protocol deviation is defined as

- Use of other nutritional supplements
- Intake of study medication of less than 75% of the prescribed amount

**AEs, SAEs and SUSARs**

Adverse events (AEs) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to Impryl®. All adverse events reported by the subject or observed by the investigator or his staff will be recorded.

A SAE 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;
- 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.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor and METC without undue delay after obtaining knowledge of the events.

SUSARs are all untoward and unintended responses to the food supplement or placebo related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:

- Summary of Product Characteristics (SPC) for an authorised medicinal product;
- Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:
- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

**Annual safety report**

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:
- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

**Patient and Public Involvement**

Patients were not involved in the development of this research. However, the overall results of the study will be communicated to the study participants by sending the end-product (article) to the provided email address.

## ETHICS, DISSEMINATION AND SAFETY MONITORING

The protocol and all protocol modifications are approved by the local medical ethical review committee at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects (CCMO) with protocol ID NL61414.091.17.

This study is registered in the American registry for clinical studies and trials (NCT03337360; https://clinicaltrials.gov) and the Dutch Trial registry (NTR6551; www.trialregister.nl). The investigator obtains written informed consent before study participation from all participants.

Due to the known safety of Impryl®, the study team and ethics committee decided the establishment of a Data Safety Monitoring Board (DSMB) is not necessary. However, the trial is monitored by an independent party according to the monitor plan. After the first 3 inclusions every centre is visited by the monitor, followed by a yearly visit. Given the low risk on adverse events of a nutritional supplement, an interim analysis or safety surveillance by a data safety monitoring board is not indicated. All participants are insured by the sponsor in case of harm due to trial participation.

The study is conducted according to the principles of the Declaration of Helsinki (latest version WMA General Assembly 2008, Seoul) and in accordance with the Medical Research Involving Human Subjects Act. Directly after study inclusion, we assign a random ID code to the participant, which will be used on all documents to ensure confidentiality.

A manuscript with the results of the primary study will be published in a peer-reviewed journal. The results of this study will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of care for infertility patients.

On completion of the trial, and after publication of the primary manuscript, data requests can be submitted to the researchers

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## AUTHOR'S CONTRIBUTION

Study concept and design were conducted by authors RS and KF. Advanced statistical input was given by author JIH. Critical revision of concept and design and intellectual input in the study protocol was done by authors RS, KF, KDH, JIH and DB. Study supervision and coordination is conducted by authors RS and KF.

## FUNDING STATEMENT

The study is supported by an unrestricted grant from Goodlife Pharma B.V. This company has developed and marketed Impryl®. The grant is unrestricted in means that the funder did not have any role in the design of the study, collection, analysis, and/or interpretation of data. The investigators have full access to the data and have the right to publish this data separate and apart from any sponsor.

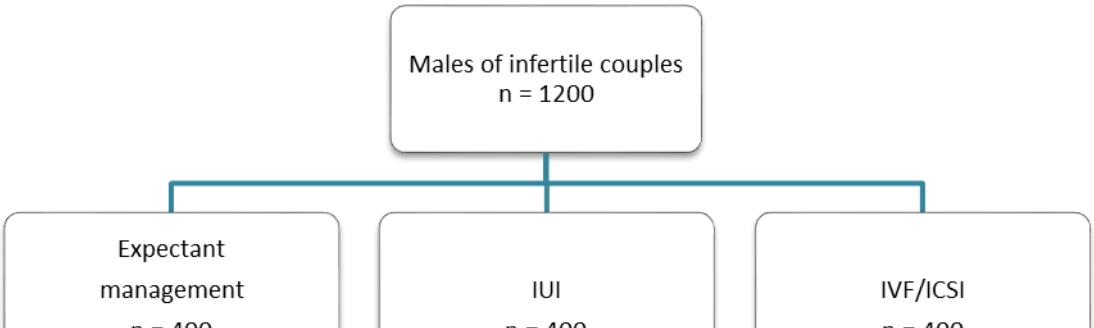
## COMPETING INTEREST STATEMENT

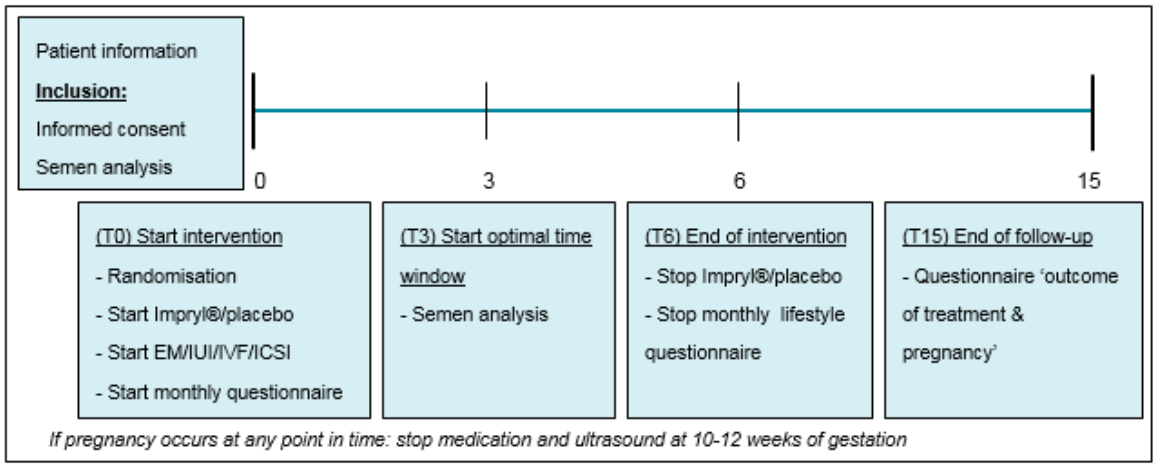
The study is conducted by the department of Gynaecology and Obstetrics of the Radboudumc. As stated above in the funding section, an unrestricted grant from Goodlife Pharma B.V. was provided for the conduct of the trial.

## FIGURE LEGENDS

Figure 1: Flow chart study design

Figure 2. Time line study (T in months)





PARTHENOGEN

# Supplementary file 1: Summary of Product Characteristics (SPC) of Impryl®

Impryl® is a Food supplement with Betaine, Cystine, Zinc, Niacin, Folic Acid (in the form of 5MTHF-glucosamine), Vitamin B12 (in the form of Methylcobalamin), Vitamin B6 and Riboflavin. Vitamin B6, Folates and B12 all contribute to normal homocysteine metabolism and Vitamin E and B2 help protect cells from oxidative stresses. Zinc protects cellular structures against oxidative damage and contributes in DNA synthesis and to normal fertility and reproduction.

Nutritional substances contained in Impryl® are known to be part of the normal diet and metabolism and may be of benefit in case of absolute (low content in the diet) or relative (increased demand) deficiency. These deficiencies may associate to altered homocysteine metabolism and other disturbances to the defense against oxidative aggressions and are often observed in: men and women with reproductive problems; in women carrying a PCOS syndrome and at pre-menopausal and menopausal stage; in type 2 diabetes; in subjects developing neurodegenerative diseases; in subjects undergoing intensive activity and energy expenditure, e.g. athletes; and in vegans and vegetarians. Moreover, the same disturbances have also been linked to dyslipidemia and to the cardiovascular disease. Finally, due to the full coverage of all the needs for the folate/homocysteine pathway, Impryl® can be used for pre-conceptional supplementation for both partners and pregnancy supplementation for the pregnant ladies.

It is to be noted that disturbances of homocysteine metabolism and of oxidative defenses may occur more frequently in subjects carrying a defective genetic variant of one of the main enzymes of homocysteine metabolism including those denominated MTHFR, MTRR, BHMT and CBS. Impryl® has been formulated so to compensate these defects by providing an already activated substrate (i.e. methylfolate for MTHFR and methyl cobalamin for MTRR), or by providing an excess of substrate (i.e. betaine for BHMT) or by delivering a ready substrate downstream to the genetic blockade (i.e. cystine for CBS). Thus, Impryl® will deliver an effective dietary support to everybody independently of their genetic substrate for the mentioned enzymes.

## Ingredients

Bulking agent: microcrystalline cellulose; betaine hydrochloride, L-cystine, anticaking agents: mono- and diglycerides of fatty acids, magnesium stearate, silicon dioxide; zinc bisglycinate, stabilizer: cross-linked sodium carboxy methyl cellulose; niacin (nicotinamide), vitamin B6 (pyridoxine hydrochloride), vitamin B2 (riboflavin), folic acid ((6S)5- methyltetrahydrofolic acid, glucosamine salt), vitamin B12 (methylcobalamin).

| Characterising Ingredients | Per daily dose (1 tab) | % NRV |
|---|---|---|
| Betaine | 200 mg | |
| L-cystine | 200 mg | |
| Niacin | 16 mg | 100% |
| Zinc | 10 mg | 100% |
| Vitamin B6 | 1.4 mg | 100% |
| Riboflavin (Vit. B2) | 1.4 mg | 100% |
| Folic acid (as methylfolate) | 400 µg | 200% |
| Vitamin B12 (as methylcobalamin) | 2.5 µg | 100% |

NRV: nutrient reference value according to Reg. (EU) n.1169/2011

## Instructions for use

Impryl® is lactose free and gluten free. The recommended daily dose is 1 tablet per day to be swallowed with abundant water, possibly between meals. The assumption of a smaller amount does not guarantee full coverage of the daily need in all subjects.

Impryl® is a support to a balanced diet and there is no fixed duration for such support. However, males and females with reproductive problems should consider that the gametes maturation process takes about 4 months to be completed. In addition, those subjects with known issues of homocysteine metabolism should consider that the supportive effects will last only as long as the product is assumed.

## Warnings

Do not exceed the recommended daily dose. Keep out of reach of children under three years of age. Food supplements should not be intended as a substitute to a varied diet and to a healthy life style. Do not take in case of known or suspected allergy to one or more ingredients.

## Storage

Store in a cool dry place away from light, humidity and direct sources of heat. The best-before date refers to the undamaged and properly stored product.

## Presentation

Cartoon box containing two PVC/Aluminium blisters of 15 tablets each for a total of 30 film coated tablets of 1.3 gr.

## Manufacturer

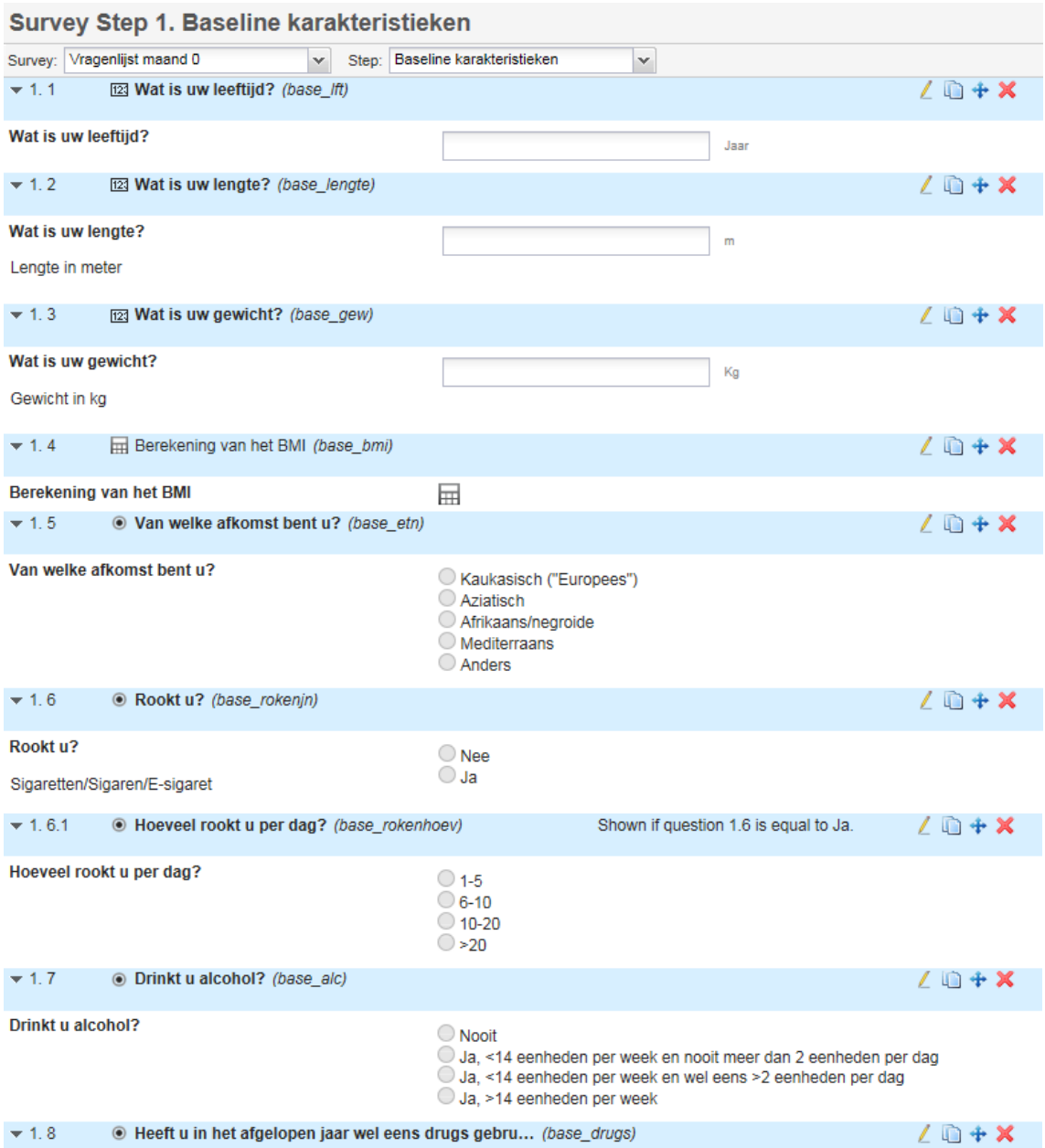
Manufactured in Italy for Parthenogen SAGL, Via F. Pelli 1, 6900 Lugano, Switzerland, in the plant of via N. Sauro 35/D, 31036 Istrana (TV), Italy.

## Distributor

Distributed in Turkey by Partogen Ilac San Tic Ltd Sti, Barbaros Bulvari, Cigdem Apt., Guzel Konutlar 52/7, Besiktas, Istanbul.

**Supplementary file 2: patient questionnaires (in Dutch)**

**Vragenlijst maand 1 – Baseline karakteristieken, lifestyle en gebruik studiemedicatie**



## Survey Step 1. Baseline karakteristieken

Survey: Vragenlijst maand 0 Step: Baseline karakteristieken

**1. 1 Wat is uw leeftijd?** *(base_lft)*

**Wat is uw leeftijd?** Jaar

**1. 2 Wat is uw lengte?** *(base_lengte)*

**Wat is uw lengte?** m

Lengte in meter

**1. 3 Wat is uw gewicht?** *(base_gew)*

**Wat is uw gewicht?** Kg

Gewicht in kg

**1. 4** Berekening van het BMI *(base_bmi)*

**Berekening van het BMI**

**1. 5 Van welke afkomst bent u?** *(base_etn)*

**Van welke afkomst bent u?**

- Kaukasisch ("Europees")
- Aziatisch
- Afrikaans/negroide
- Mediterraans
- Anders

**1. 6 Rookt u?** *(base_rokenjn)*

**Rookt u?**

Sigaretten/Sigaren/E-sigaret

- Nee
- Ja

**1. 6.1 Hoeveel rookt u per dag?** *(base_rokenhoev)* Shown if question 1.6 is equal to Ja.

**Hoeveel rookt u per dag?**

- 1-5
- 6-10
- 10-20
- >20

**1. 7 Drinkt u alcohol?** *(base_alc)*

**Drinkt u alcohol?**

- Nooit
- Ja, <14 eenheden per week en nooit meer dan 2 eenheden per dag
- Ja, <14 eenheden per week en wel eens >2 eenheden per dag
- Ja, >14 eenheden per week

**1. 8 Heeft u in het afgelopen jaar wel eens drugs gebru...** *(base_drugs)*

**Heeft u in het afgelopen jaar wel eens drugs gebruikt?**

- Nee
- Marihuana
- XTC
- Paddo's
- Cocaine
- Ketamine
- Speed
- GHB
- LSD
- Heroine
- Anders
- Gecombineerd, meerdere middelen

**1. 9 Gebruikt u vitaminen/voedingssupplementen?** *(base_vit)*

**Gebruikt u vitaminen/voedingssupplementen?**

Let op: indien u dit gebruikt: neem contact op met uw behandelaar of de coördinerend onderzoeker! U moet namelijk eerst 3 maanden hiermee gestopt zijn voordat u het studiemiddel mag gaan gebruiken!

- Nee
- Ja, (multi) vitaminen
- Ja, ander voedingssupplement
- Ja, annabolen/steroiden

## Vervolg: Baseline karakteristieken, lifestyle en gebruik studiemedicatie

1. 10 **Heeft u een specifiek dieet?** *(base_dieet)*

**Heeft u een specifiek dieet?**

- Nee
- Ja, vegetarisch
- Ja, veganistisch
- Ja, lactose-vrij
- Ja, gluten of koolhydraatvrij
- Ja, anders

1. 11 **Gebruikt u medicijnen?** *(base_medjn)*

**Gebruikt u medicijnen?**

- Nee
- Ja

1. 11.1 **Welke medicatie gebruikt u?** *(base_medspec)* Shown if question 1.11 is equal to Ja.

**Welke medicatie gebruikt u?**

1. 12 **Heeft u de afgelopen 3 maanden koorts gehad?** *(base_koorts)*

**Heeft u de afgelopen 3 maanden koorts gehad?**

- Nee
- Ja

1. 13 **Bent u op dit moment gezond?** *(base_gez)*

**Bent u op dit moment gezond?**

- Nee
- Ja

1. 14 **Staat u onder controle bij een arts voor een aando...** *(base_arts)*

**Staat u onder controle bij een arts voor een aandoening/afwijking?**

- Nee
- Ja

1. 14.1 **Waarvoor staat u onder controle bij een arts? Wel...** *(base_ziekte)* Shown if question 1.14 is equal to Ja.

**Waarvoor staat u onder controle bij een arts? Welke aandoening?**

1. 15 **Bent u ooit op genitaal gebied geopereerd of heeft...** *(base_OKjn)*

**Bent u ooit op genitaal gebied geopereerd of heeft u ooit een genitaal trauma gehad?**

- Nee
- Ja

Genitaal trauma anders dan een "knietje krijgen"

1. 15.1 **Wat voor een operatie of trauma heeft u gehad?** *(base_OKspec)* Shown if question 1.15 is equal to Ja.

**Wat voor een operatie of trauma heeft u gehad?**

1. 16 **Staat u wel eens bloot aan gevaarlijke stoffen?** *(base_tox)*

**Staat u wel eens bloot aan gevaarlijke stoffen?**

- Nee
- Ja

1. 16.1 **Aan welke gevaarlijke/schadelijke stoffen?** *(base_toxspec)* Shown if question 1.16 is equal to Ja.

**Aan welke gevaarlijke/schadelijke stoffen?**

1. 17 **Doet u aan intensieve sportbeoefening zoals mara...** *(base_sport)*

**Doet u aan intensieve sportbeoefening zoals marathontraining of intensieve krachtsporttraining**

- Nee
- Ja

Intensief = meer dan 2x per week

## Vervolg: Baseline karakteristieken, lifestyle en gebruik studiemedicatie

1. 18 ☑ **Worden uw testikels regelmatig (wekelijks) blootg…** *(base_warmte)*

**Worden uw testikels regelmatig (wekelijks) blootgesteld aan veel warmte?**

- ☐ Nee
- ☐ Ja, sauna of warme baden
- ☐ Ja, strakke (onder)broeken
- ☐ Ja, wielrennen

1. 19 ◉ **Heeft u ooit bij iemand een zwangerschap tot stan…** *(base_manzw)*

**Heeft u ooit bij iemand een zwangerschap tot stand gebracht?**

Het gaat om alle zwangerschappen, niet alleen bij de huidige partner, en ook een miskraam of zwangerschapsafbreking ("abortus") telt mee

- ○ Nee
- ○ Ja

1. 20 **Sinds wanneer heeft u samen met uw huidige part…** *(base_kiwens)*

**Sinds wanneer heeft u samen met uw huidige partner een actieve kinderwens?**

(dd-mm-yyyy)

1. 21 **Wat is de leeftijd van uw partner?** *(base_lftvr)*

**Wat is de leeftijd van uw partner?**

Jaar

1. 22 ◉ **Is uw partner ooit zwanger geweest?** *(base_vrgrav)*

**Is uw partner ooit zwanger geweest?**

Een miskraam of zwangerschapsafbreking ("abortus") telt ook mee

- ○ Nee
- ○ Ja

1. 22.1 **Hoe vaak is uw partner zwanger geweest?** *(base_gravida)* Shown if question 1.22 is equal to Ja.

**Hoe vaak is uw partner zwanger geweest?**

Keer

1. 22.1 **Hoe vaak is uw partner zwanger geweest?** *(base_gravida)* Shown if question 1.22 is equal to Ja.

**Hoe vaak is uw partner zwanger geweest?**

Keer

1. 23 ◉ **Heeft uw partner de aandoening endometriose?** *(base_endom)*

**Heeft uw partner de aandoening endometriose?**

Endometriose is een aandoening die alleen door een gynaecoloog vastgesteld kan worden

- ○ Nee
- ○ Ja

1. 24 ◉ **Heeft uw partner een cyclusprobleem?** *(base_cyclus)*

**Heeft uw partner een cyclusprobleem?**

- ○ Nee
- ○ Ja, onregelmatige cyclus
- ○ Ja, helemaal geen cyclus

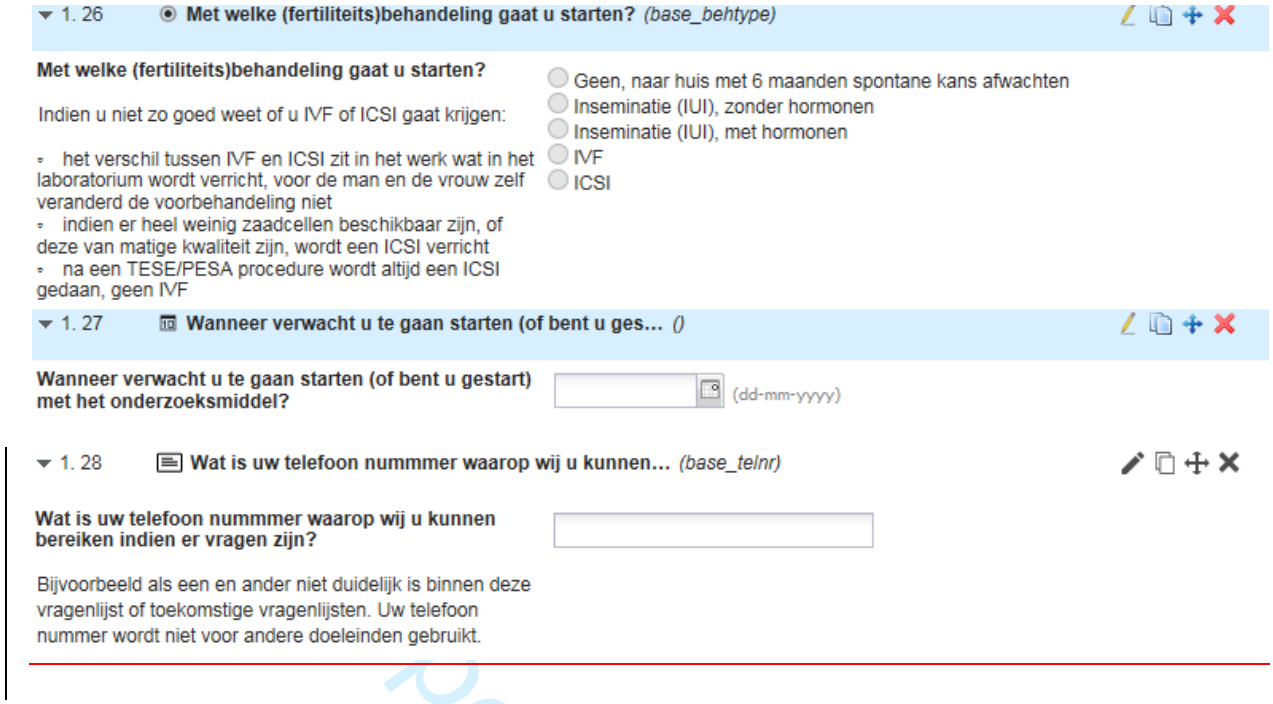
1. 25 ◉ **Heeft u partner recent een eileider foto gehad, of k…** *(Base_HSG)*

**Heeft u partner recent een eileider foto gehad, of krijgt zij dit binnenkort?**

Eileider foto = doorspuiten met contrast van de baarmoeder en eileider en tegelijk Röntgen opname maken, ook wel HSG genoemd

Recent = afgelopen 6 maanden

- ○ Nee
- ○ Ja, een eileiderfoto met contrast en een rontgenfoto/filmpje
- ○ Ja, een 'schuimecho'



1. 26 **Met welke (fertiliteits)behandeling gaat u starten?** *(base_behtype)*

**Met welke (fertiliteits)behandeling gaat u starten?**

Indien u niet zo goed weet of u IVF of ICSI gaat krijgen:

- het verschil tussen IVF en ICSI zit in het werk wat in het laboratorium wordt verricht, voor de man en de vrouw zelf veranderd de voorbehandeling niet
- indien er heel weinig zaadcellen beschikbaar zijn, of deze van matige kwaliteit zijn, wordt een ICSI verricht
- na een TESE/PESA procedure wordt altijd een ICSI gedaan, geen IVF

- ○ Geen, naar huis met 6 maanden spontane kans afwachten
- ○ Inseminatie (IUI), zonder hormonen
- ○ Inseminatie (IUI), met hormonen
- ○ IVF
- ○ ICSI

1. 27 **Wanneer verwacht u te gaan starten (of bent u ges...** *()*

**Wanneer verwacht u te gaan starten (of bent u gestart) met het onderzoeksmiddel?**

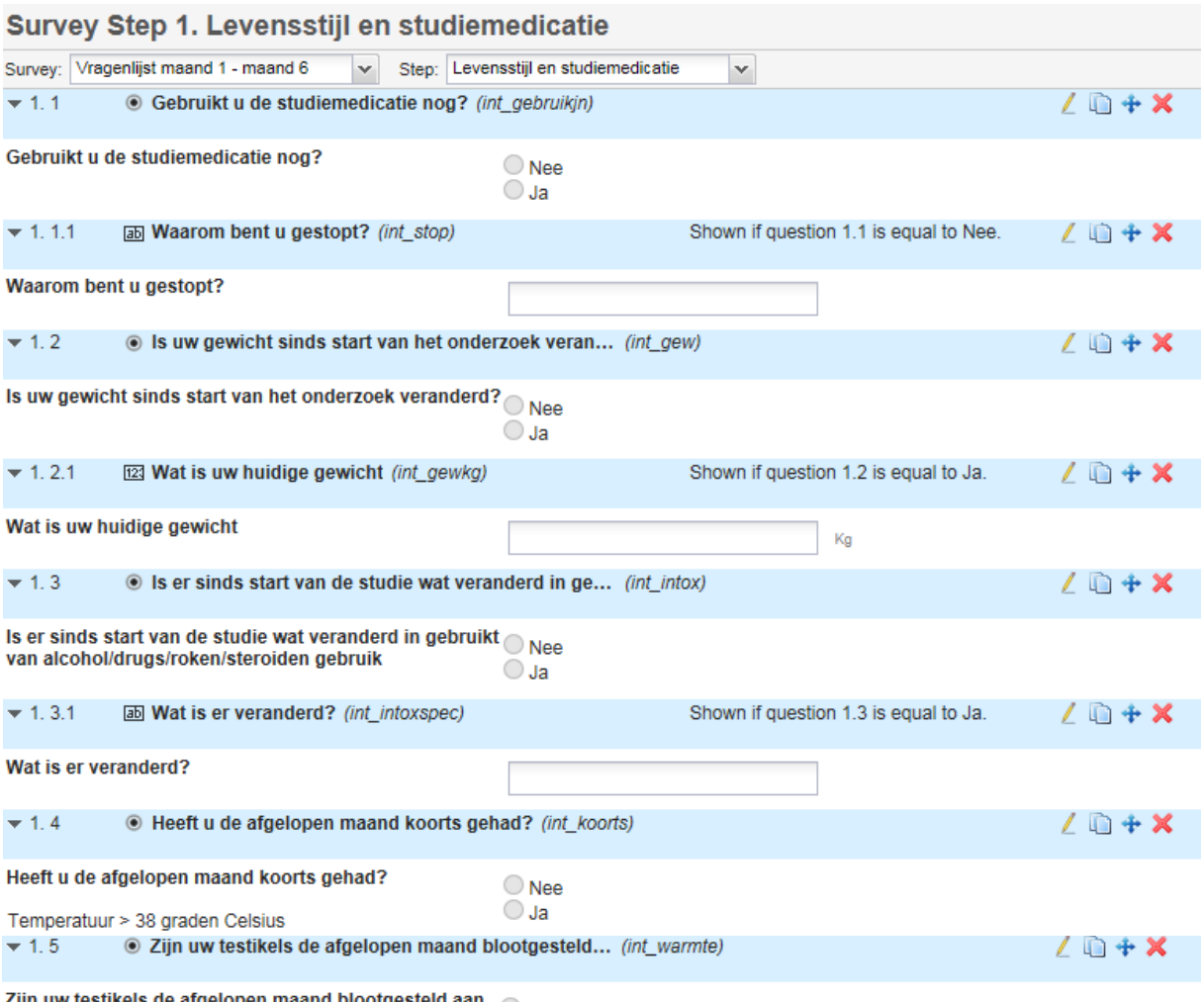
(dd-mm-yyyy)

1. 28 **Wat is uw telefoon nummmer waarop wij u kunnen...** *(base_telnr)*

**Wat is uw telefoon nummmer waarop wij u kunnen bereiken indien er vragen zijn?**

Bijvoorbeeld als een en ander niet duidelijk is binnen deze vragenlijst of toekomstige vragenlijsten. Uw telefoon nummer wordt niet voor andere doeleinden gebruikt.

**Vragenlijst maand 2 t/m 6 – Levensstijl en studiemedicatie**



Survey Step 1. Levensstijl en studiemedicatie

Survey: Vragenlijst maand 1 - maand 6 Step: Levensstijl en studiemedicatie

1. 1 Gebruikt u de studiemedicatie nog? *(int_gebruikjn)*

Gebruikt u de studiemedicatie nog?
- Nee
- Ja

1. 1.1 Waarom bent u gestopt? *(int_stop)* Shown if question 1.1 is equal to Nee.

Waarom bent u gestopt?

1. 2 Is uw gewicht sinds start van het onderzoek veran... *(int_gew)*

Is uw gewicht sinds start van het onderzoek veranderd?
- Nee
- Ja

1. 2.1 Wat is uw huidige gewicht *(int_gewkg)* Shown if question 1.2 is equal to Ja.

Wat is uw huidige gewicht Kg

1. 3 Is er sinds start van de studie wat veranderd in ge... *(int_intox)*

Is er sinds start van de studie wat veranderd in gebruikt van alcohol/drugs/roken/steroiden gebruik
- Nee
- Ja

1. 3.1 Wat is er veranderd? *(int_intoxspec)* Shown if question 1.3 is equal to Ja.

Wat is er veranderd?

1. 4 Heeft u de afgelopen maand koorts gehad? *(int_koorts)*

Heeft u de afgelopen maand koorts gehad?
- Nee
- Ja

Temperatuur > 38 graden Celsius

1. 5 Zijn uw testikels de afgelopen maand blootgesteld... *(int_warmte)*

Zijn uw testikels de afgelopen maand blootgesteld aan veel warmte?
- Nee
- Ja, sauna of warme baden
- Ja, strakke (onder)broeken
- Ja, wielrennen

1. 6 Bent u sinds start van de studie meer of minder in... *(int_sport)*

Bent u sinds start van de studie meer of minder intensieve sport gaan uitoefenen? Sport zoals marathontraining of intensieve krachttraining
- Nee (gelijk gebleven)
- Ja, meer
- Ja, minder

1. 7 Heeft u iedere dag 1 tablet ingenomen? *(int_inname)*

Heeft u iedere dag 1 tablet ingenomen?
- Nee
- Ja
- Ik weet het niet precies

1. 8 Hoeveel tabletten heeft u momenteel over? *(int_tabl)*

Hoeveel tabletten heeft u momenteel over? tabletten

Tel het aantal tabletten wat over is

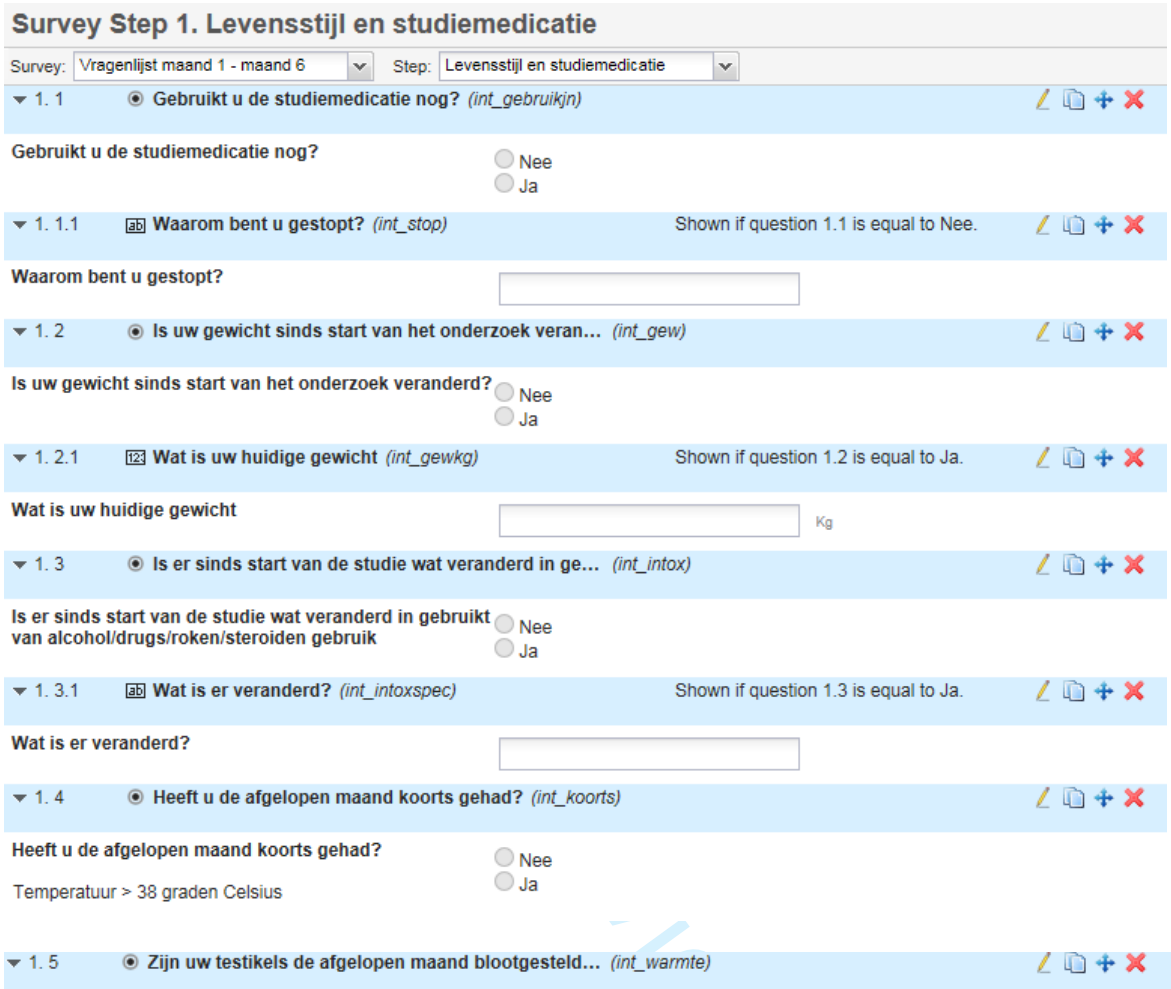
1. 9 Is er sinds start van studiemedicatie een zwanger... *(int_zwjn)*

Is er sinds start van studiemedicatie een zwangerschap ontstaan?
- Nee
- Ja

1. 9.1 Wanneer was de eerste positieve zwangerschapst... *(int_zwtest)* Shown if question 1.9 is equal to Ja.

Wanneer was de eerste positieve zwangerschapstest? (dd-mm-yyyy)

**Vragenlijst maand 12 – Uitkomst behandeling en optreden van zwangerschap**



## Survey Step 1. Levensstijl en studiemedicatie

Survey: Vragenlijst maand 1 - maand 6 Step: Levensstijl en studiemedicatie

1. 1 **Gebruikt u de studiemedicatie nog?** *(int_gebruikjn)*

**Gebruikt u de studiemedicatie nog?**
- Nee
- Ja

1. 1.1 **Waarom bent u gestopt?** *(int_stop)* Shown if question 1.1 is equal to Nee.

**Waarom bent u gestopt?**

1. 2 **Is uw gewicht sinds start van het onderzoek veran...** *(int_gew)*

**Is uw gewicht sinds start van het onderzoek veranderd?**
- Nee
- Ja

1. 2.1 **Wat is uw huidige gewicht** *(int_gewkg)* Shown if question 1.2 is equal to Ja.

**Wat is uw huidige gewicht** Kg

1. 3 **Is er sinds start van de studie wat veranderd in ge...** *(int_intox)*

**Is er sinds start van de studie wat veranderd in gebruikt van alcohol/drugs/roken/steroiden gebruik**
- Nee
- Ja

1. 3.1 **Wat is er veranderd?** *(int_intoxspec)* Shown if question 1.3 is equal to Ja.

**Wat is er veranderd?**

1. 4 **Heeft u de afgelopen maand koorts gehad?** *(int_koorts)*

**Heeft u de afgelopen maand koorts gehad?**

Temperatuur > 38 graden Celsius
- Nee
- Ja

1. 5 **Zijn uw testikels de afgelopen maand blootgesteld...** *(int_warmte)*

**Zijn uw testikels de afgelopen maand blootgesteld aan veel warmte?**
- Nee
- Ja, sauna of warme baden
- Ja, strakke (onder)broeken
- Ja, wielrennen

1. 6 **Bent u sinds start van de studie meer of minder in...** *(int_sport)*

**Bent u sinds start van de studie meer of minder intensieve sport gaan uitoefenen? Sport zoals marathontraining of intensieve krachttraining**
- Nee (gelijk gebleven)
- Ja, meer
- Ja, minder

1. 7 **Heeft u iedere dag 1 tablet ingenomen?** *(int_inname)*

**Heeft u iedere dag 1 tablet ingenomen?**
- Nee
- Ja
- Ik weet het niet precies

1. 8 **Hoeveel tabletten heeft u momenteel over?** *(int_tabl)*

**Hoeveel tabletten heeft u momenteel over?** tabletten

Tel het aantal tabletten wat over is

1. 9 **Is er sinds start van studiemedicatie een zwanger...** *(int_zwjn)*

**Is er sinds start van studiemedicatie een zwangerschap ontstaan?**
- Nee
- Ja

1. 9.1 **Wanneer was de eerste positieve zwangerschapst...** *(int_zwtest)* Shown if question 1.9 is equal to Ja.

**Wanneer was de eerste positieve zwangerschapstest?** (dd-mm-yyyy)

## Vervolg: Uitkomst behandeling en optreden van zwangerschap

### Survey Step 1. Uitkomst behandeling en zwangerschap

Survey: Vragenlijst maand 15 Step: Uitkomst behandeling en zwangersc

**1. 1 Welke behandeling heeft u tijdens de 6 maanden g... *(fu_behtype)***

**Welke behandeling heeft u tijdens de 6 maanden gebruik van het studiemiddel gehad?**

Indien u niet zo goed weet of u IVF of ICSI heeft gehad:

- het verschil tussen IVF en ICSI zit in het werk wat in het laboratorium wordt verricht, voor de man en de vrouw zelf veranderd de voorbehandeling niet
- indien er heel weinig zaadcellen beschikbaar zijn, of deze van matige kwaliteit zijn, wordt een ICSI verricht
- na een TESE/PESA procedure wordt altijd een ICSI gedaan, geen IVF

- ○ Geen, naar huis met 6 maanden spontane kans afwachten
- ○ Inseminatie (IUI), zonder hormonen
- ○ Inseminatie (IUI), met hormonen
- ○ IVF
- ○ ICSI

**1. 2 Hoeveel behandelrondes heeft u in totaal gehad? (... *(fu_cycli)***

**Hoeveel behandelrondes heeft u in totaal gehad? (Toelichting: zie grijze "i")**

Een behandelronde telt als er in die ronde een inseminatie (IUI) of eicel punctie (IVF/ICSI) heeft plaats gevonden. Indien u 6 maanden mocht afwachten dan vult u hier het aantal maanden in, namelijk: 6

**1. 3 Bent u na de 6 maanden van gebruik studiemiddel... *(fu_behswitch)***

**Bent u na de 6 maanden van gebruik studiemiddel overgestapt op een andere behandeling?**

- ○ Nee, door met dezelfde behandeling
- ○ Ja, begonnen met IUI zonder hormonen
- ○ Ja, begonnen met IUI met hormonen
- ○ Ja, begonnen met een IVF traject
- ○ Ja, begonnen met een ICSI traject
- ○ Gestopt met alle vruchtbaarheidsbehandelingen

**1. 4 Bent u zwanger geworden? *(fu_zwjn)***

**Bent u zwanger geworden?**

Iedere zwangerschap (vanaf start van deze studie tot vandaag) telt, ook een miskraam of buitenbaarmoederlijke zwangerschap telt mee.

- ○ Nee
- ○ Ja

**1. 4.1 Na welke behandeling bent u zwanger geworden? *(fu_zwbeh)*** Shown if question 1.4 is equal to Ja.

**Na welke behandeling bent u zwanger geworden?**

- ○ Geen, naar huis met 6 maanden spontane kans afwachten
- ○ Inseminatie (IUI), zonder hormonen
- ○ Inseminatie (IUI), met hormonen
- ○ IVF
- ○ ICSI

**1. 4.2 Welke datum had u een positieve zwangerschapst... *(fu_datpos)*** Shown if question 1.4 is equal to Ja.

**Welke datum had u een positieve zwangerschapstest?** (dd-mm-yyyy)

Indien niet precies bekend: ongeveer aangeven

**1. 4.3 Was er een kloppend hartje te zien bij de eerste ec... *(fu_1ehart)*** Shown if question 1.4 is equal to Ja.

**Was er een kloppend hartje te zien bij de eerste echo?**

- ○ Nee
- ○ Ja

**1. 4.3.1 Bij ongeveer hoeveel weken zwangerschap werd d... *(fu_1ehartnee)*** Shown if question 1.4.3 is equal to Nee.

**Bij ongeveer hoeveel weken zwangerschap werd deze eerste echo gemaakt?**

**1. 4.3.2 Was er een kloppend hartje te zien bij de termijnec... *(fu_12hart)*** Shown if question 1.4.3 is equal to Ja.

**Was er een kloppend hartje te zien bij de termijnecho rond 10-12 weken?**

- ○ Nee
- ○ Ja

**1. 4.3.3 Wie heeft de termijnecho gemaakt? (Vernoem naa... *()*** Shown if question 1.4.3 is equal to Ja.

**Wie heeft de termijnecho gemaakt? (Vernoem naam zorgverlener, praktijkgevens)**

Bv: verloskundige praktijk 'Cyclus', te Nijmegen (met evt telefoon nummer!)

1. 4.3.2.1 **Heeft de zwangerschap tot een geboorte geleid?** *(fu_gebjn)* Shown if question 1.4.3.2 is equal to Ja.

**Heeft de zwangerschap tot een geboorte geleid?**

- Nee, er is alsnog een (late) miskraam opgetreden
- Ja

1. 4.3.2.1.1 **Wat is de geboorte datum van uw kind?** *(fu_gebdatum)* Shown if question 1.4.3.2.1 is equal to Ja.

**Wat is de geboorte datum van uw kind?** (dd-mm-yyyy)

1. 4.3.2.1.2 **Bij hoeveel weken is uw kind geboren?** *(fu_gebAD)* Shown if question 1.4.3.2.1 is equal to Ja.

**Bij hoeveel weken is uw kind geboren?** aantal weken en aantal dagen

Bijvoorbeeld: 39 weken en 3 dagen

1. 4.3.2.1.3 **Is uw kind levendgeboren?** *(fu_gebpos)* Shown if question 1.4.3.2.1 is equal to Ja.

**Is uw kind levendgeboren?**

- Nee
- Ja

1. 4.3.2.1.3.1 **Kunt u ons vertellen wat de reden was dat uw kind...** *(fu_gebneg)* Shown if question 1.4.3.2.1.3 is equal to Nee.

**Kunt u ons vertellen wat de reden was dat uw kind niet levend geboren is?**

1. 4.3.2.1.4 **Op welke manier is uw kind geboren?** *(fu_gebmech)* Shown if question 1.4.3.2.1 is equal to Ja.

**Op welke manier is uw kind geboren?**

- Vaginaal
- Vacuumpomp of tang
- Keizersnede

1. 4.3.2.1.5 **Onder leiding van wie bent u bevallen? Graag type...** *()* Shown if question 1.4.3.2.1 is equal to Ja.

**Onder leiding van wie bent u bevallen? Graag type persoon en naam instelling vernoemen**

Voorbeelden: "verloskundige van praktijk Cyclus, thuis" "arts, in Radboudumc ziekenhuis" "poliklinisch met verloskundige, in het Jeroen Bosch ziekenhuis"

1. 4.3.2.1.6 **Wat is het geboortegewicht van uw kind?** *()* Shown if question 1.4.3.2.1 is equal to Ja.

**Wat is het geboortegewicht van uw kind?** gram

Gemiddeld rond de 40 weken is 3000 gram.

1. 4.3.2.1.3.2 **Is uw kind gezond geboren?** *(fu_gezneon)* Shown if question 1.4.3.2.1.3 is equal to Ja.

**Is uw kind gezond geboren?**

- Nee
- Ja

1. 4.3.2.1.3.2.1 **Wat is de reden dat uw kind niet gezond is?** *(fu_ziekneo)* Shown if question 1.4.3.2.1.3.2 is equal to Nee.

**Wat is de reden dat uw kind niet gezond is?**

1. 4.3.2.1.3.3 **Heeft uw kind aangeboren afwijkingen?** *()* Shown if question 1.4.3.2.1.3 is equal to Ja.

**Heeft uw kind aangeboren afwijkingen?**

- Nee
- Ja

Bijvoorbeeld aandoeningen zoals een syndroom van Down, hazenlip, een vinger/teen te veel of een open ruggetje.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Reported on page No |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 2 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | - |
| Protocol version | 3 | Date and version identifier | 1 |
| Funding | 4 | Sources and types of financial, material, and other support | 17 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1 |
| | 5b | Name and contact information for the trial sponsor | 1 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 17 |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 17 |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 4 |
| | 6b | Explanation for choice of comparators | 5 |
| Objectives | 7 | Specific objectives or hypotheses | 5 |

| | | | |
|---|---|---|---|
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 6 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 11 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 8 |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 11-12 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 9 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 7-8 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 9-10 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 7 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 7 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 8-9 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8-9 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8-9 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 8-9 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 8-9 |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 9-11 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 10-11 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 10-11 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 12 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 12 |

| | | | |
|---|---|---|---|
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 15 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 15 |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 12-13 |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 15 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 15 |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 8 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NA |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 8-9, 11 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 18 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 18 |

| | | | |
|---|---|---|---|
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | NA |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 15 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 15 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 15 |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Only in Dutch, can be requested |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | NA |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.