BMJ Open

Impact of a nutritional supplement (Impryl) on male fertility: study protocol of a multicentre, randomised, double-blind, placebo-controlled clinical trial (SUppleMent Male fERtility, SUMMER trial)

Roos Smits ,[1] Kathleen D'Hauwers,[2] Joanna IntHout,[3] Didi Braat,[1] Kathrin Fleischer[1,4]

To cite: Smits R, D'Hauwers K, IntHout J, *et al*. Impact of a nutritional supplement (Impryl) on male fertility: study protocol of a multicentre, randomised, double-blind, placebo-controlled clinical trial (SUppleMent Male fERtility, SUMMER trial). *BMJ Open* 2020;**10**:e035069. doi:10.1136/bmjopen-2019-035069

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2019-035069).

Received 21 October 2019
Revised 23 January 2020
Accepted 13 May 2020

For numbered affiliations see end of article.

Correspondence to
Roos Smits;
roos.smits@radboudumc.nl

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 characterised 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 25%–87% of male infertility cases. Impryl is a nutritional supplement which works on the metabolic system and the regulation of oxidative stress by activating the 1-carbon cycle and therefore recycling of homocysteine. We hypothesise that the nutritional supplement Impryl in men of infertile couples might improve the ongoing pregnancy rate.

Methods and analysis We designed a multicentre, randomised, double-blind, placebo-controlled clinical trial. We aimed to include 1200 male adults aged 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, that is, expectative management (duration of 6 months), intrauterine insemination (IUI) with or without mild ovarian stimulation or ovulation induction, either in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatment. Male participants will be randomised in either the Impryl or the 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 to 12 weeks, and conceived in the time window between randomisation up to and including month 6 of intervention use. Secondary outcomes are change in semen parameters between baseline and after 3 months of intervention in the IUI/IVF/ICSI group, based on (prewash) total motile sperm count. 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 fertilisation rate in IVF/ICSI, embryo-utilisation 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 at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects. Findings will be shared with the academic and medical community, funding and patient organisations in order to contribute to optimisation of medical care and quality of life for patients with infertility.

Trial registration numbers NCT03337360 and NTR6551.

Strengths and limitations of this study

- This multicentre randomised double-blind, placebo-controlled trial will provide important information to patients with diagnosis of male infertility and to 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 randomised study with the use of nutritional supplements in fertility patients.
- Permuted block randomisation 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 on different patient groups. However, these tests are not used in this study due to their complexity and lack of standardisation 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%–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 leucocytes 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 with 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 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 (GSH), amino acids (taurine and hypotaurine), albumin, carnitine and carotenoids.[14] Despite the common association between male infertility and oxidative damage, there is no WHO recommendation about which test to use for detecting sperm DNA damage and there are no standardised protocols which could help global interpretation of the results. Men with sperm subject to increased oxidative stress may have normal seminal parameters but 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 US$200–300 per test), complex and lack standardisation 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 (single-cell gel electrophoresis) or sperm chromatin structure assay, and another option is measuring the by-product of DNA oxidation called 8-hydroxydeoxyguanosine.

A recent Cochrane review suggested that antioxidant supplementation in male infertility might improve the outcomes in assisted reproductive technologies.[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 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-randomised 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-randomised 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 could be more effective than other nutritional supplements. The cost of Impryl is €30 for each 30 days of use.

It is well known that assisted reproduction technologies are expensive, with the cost per in vitro fertilisation (IVF) treatment in the Netherlands estimated to be between €2885 and €5259 and the cost of an intrauterine 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 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 first/ second/third cycle of IUI (with or without mild ovarian stimulation) or first/ second/third cycle of IVF or intracytoplasmic sperm injection (ICSI).
- Male aged 18–50 years.
- Female partner aged 18–43 years.
- Both male and female are willing and able to give informed consent.

Exclusion criteria

- Planned or performed testicular sperm extraction or percutaneous epididymal sperm aspiration.
- Use of donor semen, cryopreserved semen or electro-ejaculated semen or donor oocytes.
- Ovulation induction (OI) without IUI.
- IVF for an absolute tubal factor.
- Embryo transfer after preimplantation genetic diagnosis.
- Known endocrine abnormalities related to male infertility, or use of fertility enhancing drugs such as clomiphene citrate or follicle-stimulating hormone.
- 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 reincluded in the study once they dropped out, and their identification number and treatment will not be reused.

Sample size calculation

We aimed 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 19.8% and 21.5%, respectively, 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%to 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 OR of 3.43 (95% CI 1.92 to 6.11) based on seven RCTs with a total of 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 with placebo. However, after randomisation, 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% (ie, 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 three 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)), which 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 online supplementary file 1), Certificates of Analysis, certificate of GMP and ISO 9001 form. Intervention will start directly after randomisation.

All couples will receive our standard care for infertility according to the guidelines of the Dutch Society of Obstetrics and Gynaecology. After diagnostic workup, couples will either start with EM (6 months), IUI, IVF or ICSI. Participants will take study supplement for a maximum of

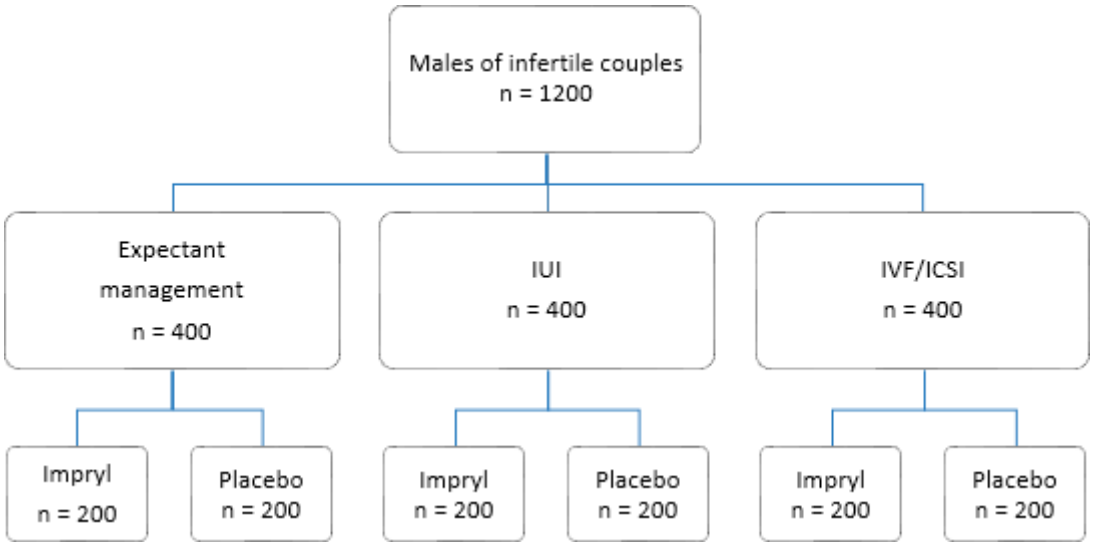

Figure 1 Flowchart study design. ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilisation.

6 months, even if the fertility intervention (6× IUI of 1× IVF/ICSI cycles) is not completed.

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

Randomisation 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). Randomisation 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 (first) and recruiting centre (second). Block sizes are flexible. Participants will be randomised in a 1:1 ratio to study medication (Impryl) or placebo. Assignment to medication, using the randomisation list, will be performed either at the local centre or at Radboudumc by an independent person. This person is neither participating in clinical treatment nor processing the study data. Randomisation 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 three numbers. The code will not provide any information about the received intervention (Impryl or placebo). The list of codes corresponding with PIF 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 randomisation.

The study is double blinded. All personnel, the researchers and patients will remain blinded to the intervention being received, except the personnel performing randomisation and distributing the study medication. The indications for breaking the randomisation code are serious adverse event (SAE), serious adverse reaction (SAR) and suspected unexpected serious adverse reactions (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 randomisation 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 authorised employee of the department of reproductive medicine (or gynaecology) will distribute the study medication after randomisation has been performed by Castor. The flag label (identification A=Impryl or B=placebo) will be removed from the box and the randomisation number noted (handwritten) on the medication box. Thereafter, the box will be

handed over to the patient. On the patient identification and drug accountability log (either digital or on paper), the following details will be reported: date of issue, study (randomisation) number, batch number, expiration date, amount dispensed, current storage amount, randomiser/distributor initials, hospital number, patient name and initials, date of birth and monitoring check.

All unblinded personnel performing randomisation will be trained by a site initiation to perform randomisation 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 three 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 workup, 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× IUI or 1× 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 randomisation 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 online supplementary file 2).

- Baseline: directly after randomisation, patients will receive an automatic email invitation to provide baseline information online in the Castor database system.
- Monthly: during use of study medication (6 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 (AEs). They will receive an automatic email invitation.
- Follow-up: 15 months after randomisation, 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).

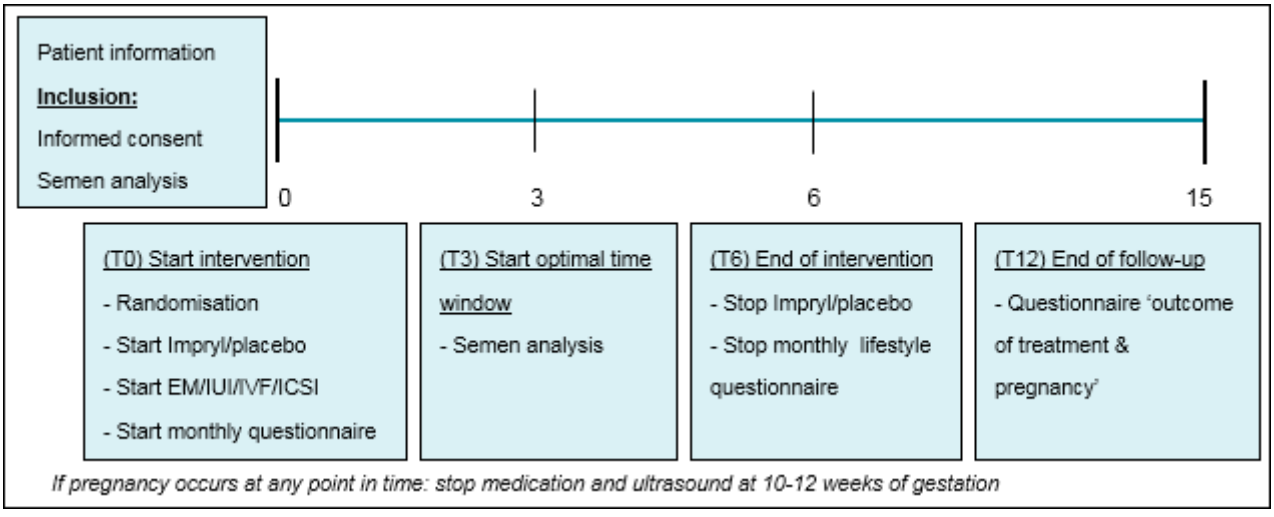

Figure 2 Timeline study (T in months). EM, expectative management; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilisation.

At least two semen analyses will be performed at two time points in this study: the first semen analysis will be performed during diagnostic workup (standard care and 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 and 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 minimalise 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 randomisation up to and including month 6 of intervention use.

Secondary endpoints

- Number of pregnancies conceived in the optimal intervention time window, that is, between start of month 4 until 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 (1) the time between randomisation and reaching ongoing pregnancy (confirmed by ultrasound) and (2) the time between start of fertility treatment during the study (EM, IUI and IVF/ICSI) and reaching ongoing pregnancy.
- Change in semen parameters in the IUI/IVF/ICSI group based on prewash total motile sperm count, allocated to change in treatment category (EM, IUI, IVF, ICSI).
- Improvement between Impryl and control group in fertilisation rate and embryo utilisation rate (EUR) in the IVF/ICSI group. Fertilisation rate is defined as the percentage of oocytes with 0 pronucleus (PN) or ≥2 PN after insemination (IVF) or injection (ICSI). Abnormal fertilisation such as 3 PN will be recorded, in case this percentage differs or increases in the study group. The 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 fertilisation and use rate observed for each clinic.
- Number of miscarriages, defined as non-vital intrauterine 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, others), 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 the 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).

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 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 randomised 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 summarise them as means with SDs. If they are not normally distributed, then medians and 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 (ie, EM, IUI and 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% CIs will be estimated using a fixed effects binomial model with an identity link, including intervention, fertility treatment, centre and female age (centred). 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, fertilisation rate and EUR) will be evaluated similarly to the primary outcome. Changes in semen analysis, leading to different fertility treatment categorisations, will be calculated with descriptive statistics. Furthermore, AEs will be summarised. Sensitivity analyses will be performed to evaluate possible effects in case of protocol amendments.

The per-protocol population will consist of all randomised 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

AEs are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to Impryl. All AEs 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.
- Any other important medical event that did not result in any of the outcomes listed previously due to medical or surgical intervention but could have been based on appropriate judgement by the investigator.

An elective hospital admission will not be considered as a SAE. 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
   - 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) SARs, along with an aggregated summary table of all reported SARs, 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 METC at the Radboud University Medical Centre and by the national Central Committee on Research Involving Human Subjects with protocol ID NL61414.091.17.

This study is registered in the American registry for clinical studies and trials (https://clinicaltrials.gov) and the Dutch Trial registry (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 is not necessary. However, the trial is monitored by an independent party according to the monitor plan. After the first three inclusions, every centre is visited by the monitor, followed by a yearly visit. Given the low risk on AEs 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 assigned 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 organisations in order to contribute to optimisation 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

Author affiliations
[1]Obstetrics and Gynaecology, Radboudumc, Nijmegen, The Netherlands
[2]Urology, Radboudumc, Nijmegen, The Netherlands
[3]Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands
[4]MVZ VivaNeo Kinderwunschzentrum, Dusseldorf, Germany

Acknowledgements We thank embryologist Liliana Ramos for her critical opinion and feedback during the development of this study.

Contributors 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 were done by authors RS, KF, KD'H, JIH and DB. Study supervision and coordination were conducted by authors RS and KF.

Funding The study is supported by an unrestricted grant from Goodlife Pharma BV. 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 these data separate and apart from any sponsor.

Competing interests 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.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD
Roos Smits http://orcid.org/0000-0002-9949-2687

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