The impact of a nutritional supplement (Impryl®) on male fertility

SUMMER-trial

PROTOCOL TITLE 'The impact of a nutritional supplement (Impryl®) on male fertility'

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Pharmacy	***Not applicable***

PROTOCOL SIGNATURE SHEET

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
ART	**Assisted reproduction technology**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EM	**Expectative management**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
ICSI	**Intra-cytoplasmic sperm injection**
IUI	**Intra-uterine insemination**
IVF	**In-vitro fertilisation**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MOH	**Mild ovarian hyperstimulation**
OI	**Ovulation-induction**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**

SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: 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. In the past decade, the role of oxidative stress on sperm has been researched thoroughly and found 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.
Objective: To determine the effectiveness of nutritional supplement Impryl® in men of infertile couples on ongoing pregnancy rate, with or without assisted reproduction technology (ART).
Study design: Multicentre, randomised double blind placebo controlled clinical trial/superiority study.
Study population: All participants in this study are male adults, age 18-50 years, part of a couple that is diagnosed with infertility, regardless the outcome of semen analysis. The couple will either start or is already started with fertility treatment, i.e. expectative management (EM, duration 6 months), intra-uterine insemination (IUI) with or without ovarian stimulation (mild ovarian hyperstimulation (MOH) or ovulation induction (OI)), either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment.
Intervention: Impryl® or placebo, with identical appearance one tablet each day for a total duration of maximal 6 months. Patients can start directly with fertility treatment and/or to conceive.
Main study parameters/endpoints: 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 3 months intervention in IUI/IVF/ICSI group, based on (pre-wash) total motile sperm count (TMSC), leading to a different fertility treatment categorisation. Furthermore the number of pregnancies conceived in the optimal intervention time window, the overall number of pregnancies, time to pregnancy, embryo fertilization rate in IVF/ICSI, embryo-utilization rate in IVF/ICSI, number of miscarriages and live birth rate are documented within the study period of 15 months. The occurrence of adverse events will be reported.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Couples with infertility will receive standard fertility treatment, i.e. EM or ART. The risks and burden of participating in the trial are small. After a complete diagnostic work-up for infertility, the males will be randomised for use of either Impryl® or placebo. Impryl® is a food supplement already free available throughout Europe, online in the

Netherlands. Males need to take study medication (one tablet each day) for a maximum of 6 months in total. For this study, we want to measure improvement of semen parameters after at least 3 months use of study medication. Performing a pre-wash TMSC is standard procedure at most sites when semen is used for IUI or IVF/ICSI. However, in couples with EM performing a TMSC after 3 months is not standard care. We decided not to perform a semen analysis in the EM group due to the fact that improvement in fertility treatment from expectative management is not possible. Participants are required to collect study medication directly at their local hospital or at Radboudumc. At the start of taking study medication the couple is asked to fill in a questionnaire about their baseline characteristics. To asses lifestyle changes during intervention and amount of used study medication, every male will be asked each month (6 times in total) to fill in an online questionnaire, these monthly questionnaires stop when a pregnancy has been reached. Every couple will receive a final questionnaire, 15 months after inclusion, about the outcome of fertility treatment and occurrence of pregnancy. If a women is pregnant, standard care is an ultrasound around 10-12 weeks to estimate the due date. To limit the amount of extra site visits to zero, we decided that this ’due date’ ultrasound at 10-12 weeks of pregnancy is enough for determining the primary outcome. The ultrasound can be made in the midwife practice. Information about the outcome of this ultrasound is reported in the 15-months questionnaire. In conclusion, the burden and risks associated with participation in this trial can be considered negligible.

INTRODUCTION AND RATIONALE

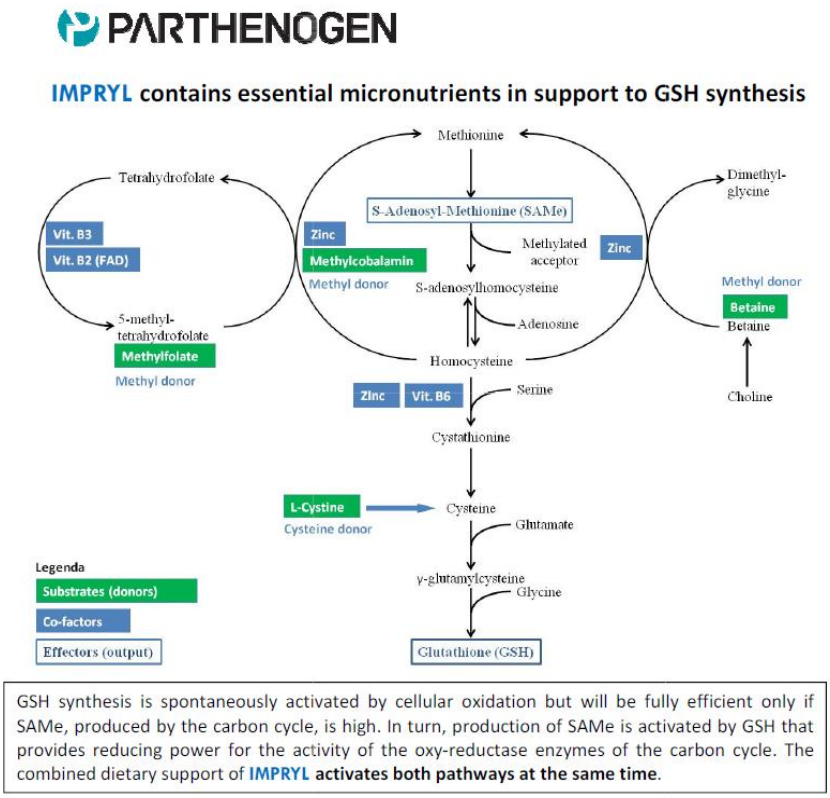
Infertility is a worldwide problem and about 10%-15% of all couples will be affected by the inability to have children.(1, 2) In approximately 50% of infertile couples a male factor is involved.(3) Male infertility is of multifactorial origin. In the past decade, the role of oxidative stress on sperm has been researched thoroughly and found to be the problem in 30% to 80% of male infertility cases. (4-8)

Reactive oxygen species (ROS) are products of normal cellular metabolism. However, oxidative stress occurs when the ROS production overwhelms the semen's natural antioxidant defenses and causes cellular damage.(9) Increased oxidative stress is due to environmental and lifestyle factors such as smoking, stress, obesity and nutrition. ROS act by directly altering the sperm DNA and by damaging the sperm membrane and therefore affecting the sperm motility and ability of the spermatozoa to break down the oocyte membrane during fertilization. Men with sperm DNA damage can still have normal seminal parameters, however having a poor chance of natural conception.(10) Antioxidants can provide protection against oxidative stress by neutralizing the free oxygen radicals. Examples of antioxidants already present within seminal plasma are ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione, amino acids (taurine, hypotaurine), albumin, carnitine, carotenoids, flavenoids, urate and prostasomes. Despite the common association between male infertility and oxidative damage, men are rarely treated or screened for oxidative stress. The latter because of the cost and complexity of testing for DNA damage. A recent Cochrane review suggested that antioxidant supplementation in male infertility might improve the outcomes in assisted reproductive technologies (ART). However, the evidence was rated as low and other clinical studies showed contradictory results with even a negative effect of high doses of antioxidants due to reductive stress as a rebound effect.(11)The clinical data on the effect of oral antioxidants in female infertility are even weaker, which is suggested to be the result of heterogeneity in study designs.(12) Alternatives to the straight oral antioxidants were therefore explored. A suitable alternative is to support the natural antioxidant defenses that are predicted to act within the modulation of the natural cellular homeostasis without generating rebound effects. Studies showed a positive effect of a nutritional supplement Condensyl®, the precursor of Impryl®, supporting DNA methylation patterns.(13, 14) Homocysteine is the end-product of the 1-Carbon cycle (feeding DNA methylation) as well the starting substrate for GSH (the tripeptide glutathione) de novo biosynthesis (feeding reducing power to the antioxidant defenses). Homocysteine is in turn an inhibitor of the methylation process and a powerful pro-oxidant. It has a negative effect on spermatogenesis quality and its concentration in the ejaculate is inversely correlated with fertility outcome.(15, 16) 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 by supporting GSH synthesis, feeding the intracellular antioxidant system. (17) In the non-randomized pilot studies with Condensyl® 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 in the study of Dattilo et al. performed in 2014 there was no control group.(13, 15)

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 antioxidants. Compared to Condensyl®, Impryl has the added advantage of the activated forms of folate (methylfolate) and B12 (methylcobalamine) to neutralize the effect of the enzyme variants and is therefore predicted to be more effective. The exact mechanism of action of Impryl® is shown in figure 1, Impryl® provides substrates and co-factors in the GSH synthesis to normalize the redox homeostasis.

Figure 1. Working mechanism of Impryl®



GSH synthesis is spontaneously activated by cellular oxidation but will be fully efficient only if SAMe, produced by the carbon cycle, is high. In turn, production of SAMe is activated by GSH that provides reducing power for the activity of the oxy-reductase enzymes of the carbon cycle. The combined dietary support of **IMPRYL activates both pathways at the same time**.

In conclusion, it is well known that assisted reproduction technologies are expensive and that male infertility is being responsible for approximately 30% of problems with conception and to be a contributory factor in 50%.(18, 19) The medical and non-medical cost per IVF treatment in the Netherlands is estimated to be between 2.885 and 5.259 euro (€) when being expressed in the year 2015 euro by means of the consumer price index (http://statline.cbs.nl).(20, 21) The medical and non-medical costs per non-stimulated and stimulated IUI cycle are respectively 497€ and 1123€ for the year 2015.(22) Therefore, substantial cost savings would be made if the use of relatively inexpensive nutritional supplements would lead to a higher ongoing pregnancy rate, a shorter time to pregnancy or to the use of less expensive, less invasive reproductive techniques.

OBJECTIVES

Primary Objective: To test the hypothesis that the number of ongoing pregnancies (i.e. ≥ 10-12 weeks of gestation) , conceived in the time window between randomization up to and including month 6 of intervention use, will be different in couples treated with Impryl® for infertility, compared to couples with a placebo.

Secondary Objectives:
Number of pregnancies conceived in the optimal intervention time window, i.e. between start of month 4 till the end of month 6. Overall pregnancy rate. Time to pregnancy defined as both the time between a) randomization and reaching ongoing pregnancy, and as b) start of fertility treatment during the study (EM, IUI, IVF/ICSI) and reaching ongoing pregnancy. Change in semen parameters leading to differentfertility treatment categorisation (for more details see page 26) between baseline and 3 months intervention, based on pre-wash total motile sperm count (TMSC) from the subpopulation from Radboudumc and sites that deliver a pre-wash TMSC before 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 a non-vital intra-uterine pregnancy before 16 weeks of gestation. Live birth rate defined as beyond 24 weeks of gestation, the birth of a living child. Live births (beyond 24 weeks, defined as the birth of a living child) will be reported within follow-up time of 15 months. Furthermore the following adverse events will be reported: gastro-intestinal problems such as reflux, obstipation, diarrhea, nausea or vomiting, furthermore loss of appetite, headache, dizziness, pruritus or skin rash.

STUDY DESIGN

A multicentre randomised, double blind placebo controlled, clinical trial for Impryl® administration will be performed in the Department of Gynaecology and/or Reproductive Medicine in both academic and non-academic hospitals in The Netherlands.

The 16 centers participating in the study:

Hospital	**Principal investigator**
Radboudumc, Nijmegen	K. Fleischer
Slingeland Ziekenhuis, Doetinchem	R.B. Donker
Canisius-Wilhelmina Ziekenhuis, Nijmegen	C.F. van Heteren
Jeroen Bosch Ziekenhuis, Den Bosch	J.P de Bruin
Elisabeth-TweeSteden, Tilburg	J.M.S. Smeenk
Maasziekenhuis Pantein, Boxmeer	E. Haagen
Bernhoven Ziekenhuis, Uden	M.P. Hoekstra
Maxima Medisch Centrum, Veldhoven	J.W.M. Maas
Maastricht UMC+, Maastricht	R.J.T. van Golde
Gelre ziekenhuizen, Apeldoorn	M.A.F. Traas
Nij Geertgen, Elsendorp	M. Schoonenberg
Rijnstate, Arnhem	A.W. Nap
Medisch Centrum Kinderwens, Leiderdorp	T. Cox
Nij Barrahûs	H. Mous
Fertiliteitscentrum Voorburg	J. Boxmeer
Catharina Ziekenhuis Eindhoven	M.M.E. van Rumste
Bravis Ziekenhuis locaties Roosendaal en Bergen op zoom	C.M. Boomsma and E. Timmermans

The duration of the study will be approximately 45 months. A run-in period of 6 months is needed for the study set up. The inclusion period will be 24 months. A period of 15 months is needed for follow-up data collection and report of results. We anticipate on a 50% participation rate; the study will be completed in 3.5 - 4 years. Our study design is summarized in figure 2 and a time line is shown in figure 3.

Figure 2. Flow chart study design

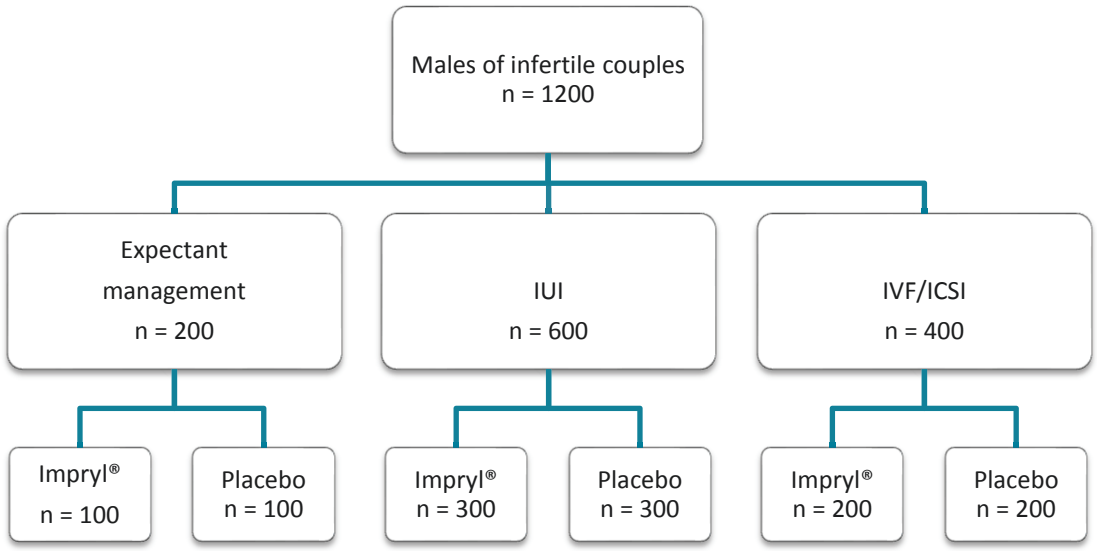
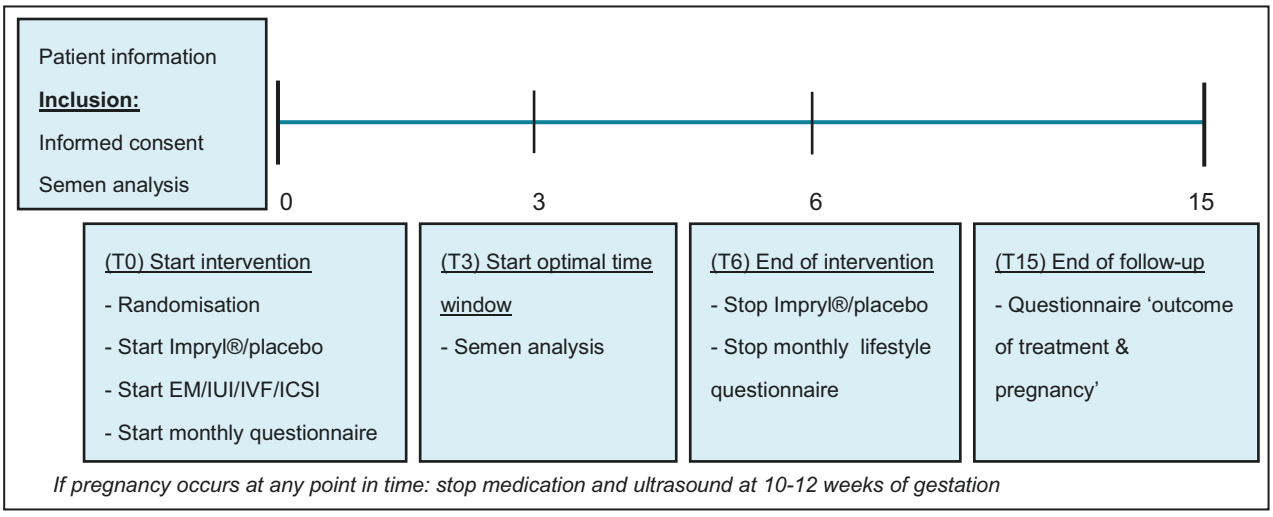


Figure 3. Time line study (T in months)



1. STUDY POPULATION

1.1 Population (base)

The research population will be recruited from couples either presenting for evaluation of started fertility treatment or newly presenting with infertility to a gynaecologist or after successful pregnancy. A recruitment of 1200 male patients is proposed. Participants will be recruited from both tertiary and secondary care hospitals. Expected inclusion period is 24 months, since we expect to enrol around 50 patients per month. With a participation rate of 50%, the recruitment of 1200 males in 24 months is feasible (see 2.3 sample size calculation). Due to the existence of the NVOG Consortium 2.0 structure (Dutch Consortium for Healthcare Evaluation in Obstetrics and Gynaecology) the experience of the participating hospitals in including patients is high, most clinics have an onsite research nurse.

1.2 Inclusion criteria

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

Either: couples with failure to conceive for at least 12 months and starting with EM **or** couples starting with 1st/ 2nd/3rd cycle of IUI (with/without ovarian stimulation) **or** 1st/ 2nd/3rd cycle of IVF/ICSI
Male with age 18-50 years
Female partner with age 18-43 years
Willing and able to give informed consent

Exclusion criteria
A potential subject who meets any of the following criteria will be excluded from participation in this study:

Planned or performed diagnostic testicular biopsy (TESE) or percutaneous epididymal sperm aspiration (PESA)
Use of donor-, cryopreserved- or electro-ejaculated semen
Ovulation induction (OI) without IUI
IVF for an absolute tubal factor
Embryo-transfers after cryopreservation
Embryo-transfer after pre-implantation genetic diagnosis
Known endocrine abnormalities related to infertility, or use of fertility enhancing drugs
Known genetic abnormalities related to infertility
Known urological abnormality such as a varicocele or bilateral cryptorchism
Use of other vitamin supplements

1.3 Sample size calculation

The overall success rate (number of ongoing pregnancies, not specified per cycle number) in IVF and ICSI in the Netherlands was respectively 19.8% and 21.5% per cycle in 2015.(23) The cumulative 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. (24-26) Based on our Radboudumc data, the ongoing pregnancy rate in EM is estimated around 20% after 6 months.

A Cochrane review 'antioxidants for male subfertility' 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, P < 0.0001, 7 RCTs, 522 men, I2 = 0%, low quality evidence). (27) 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 study. 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%.

The ratio between the 3 fertility treatment groups (EM 1: IUI 3: IVF/ICSI 2) is based on the number of new patients treated at Radboudumc and the sites each year. 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, with 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 in both intervention and control arm. Figure 1 shows the distribution of the number of participants for the 3 different types of fertility treatment (EM, IUI and IVF/ICSI).

2. TREATMENT OF SUBJECTS

For the purpose of this study subjects will either be treated with a food supplement (Impryl®) 1 tablet once a day or with a placebo 1 tablet once a day, both for a duration of maximum 6 months. Use of the food supplement or placebo will be stopped earlier when pregnancy is achieved.

2.1 Investigational product/intervention

Impryl® tablets will be produced by Labomar SRL who will also produce an identical looking placebo tablet. Patients start directly with study medication for a total duration of maximum 6 months.

2.2 Use of co-intervention

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.

2.3 Escape medication

Not applicable

3. INVESTIGATIONAL PRODUCT

Impryl® is a food supplement and is therefore not considered an investigational medicinal product. To assess product quality, we have submitted following documents of both the food supplement and placebo: Summary of Product Characteristics (SPC), Certificates of Analysis (CoA), certificate of Good Manufacturing Practice (GMP) and ISO 9001 form (see documents D2 SPC, D2 CoA, D2 GMP Labomar SRL and D2 ISO 9001 Labomar SRL).

3.1 Name and description of investigational product(s)

Impryl®. Tablets of ovoid shape sized 20x8 mm, white color, smooth surface, weight 1300 milligram with active ingredients: microcrystalline cellulose, betaine hydrochloride, L-cysteine, mono and diglycerides of fatty acids, magnesium stearate, disilicio dioxide, zinc bisglycinate, croscarmellose sodium, niacin (nicotinamide), vitamin B6 (pyridoxine hydrochloride), vitamin B2 (riboflavin), folic acid [(6S) -5-methyltetrahydrofolate acid, glucosamine salt], vitamin B12 (cobalamin).

Placebo tablet of identical appearance produced by Labomar SRL.
List of ingredients of placebo (composition):

E num.	Common name	Grams per dose
E 460	Cellulose	0,852
E 341	Calcium phosphates	0,300
E 172	Iron oxides	0,024
E 551	Silicon dioxide	0,012
E 470b	Magnesium salts of fatty acids	0,012

3.2 Summary of findings from (non-) clinical studies

To date no (non-)clinical research with a primary focus on increasing the ongoing pregnancy number by Impryl® has been performed. There is evidence that Impryl® reduces oxidative stress and DNA damage, this is based on research performed with the previous version of the supplement (Condensyl®) in humans.(13)

3.3 Summary of known and potential risks and benefits

There is substantial evidence that supplementation with the precursor of Impryl®, named Condensyl®, is safe and of beneficial in case of absolute (low content in the diet) or relative (increased demand) deficiency.(17) 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. 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 ethyltetrahydrofolate reductase (MTHFR), methionine transferase reductase (MTRR), Betaine Homocysteine Methyl Transferase (BHMT) and cystathionine beta synthase (CBS).(13) 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 for 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.

Impryl is safe by administering only essential micronutrients that are usually occurring in food, supplied according the recommended daily dose. There are no major warning, there are no reports on side effects or allergies, it is gluten- and lactose free.

3.4 Description and justification of route of administration and dosage

The route of administration of both Impryl® and placebo is by oral intake with a small amount of water, preferentially without food. The recommended daily dose is 1 tablet per day. The assumption of a smaller amount does not guarantee full coverage of the daily need in all subjects. The recommended daily dose should not be exceeded.

3.5 Dosages, dosage modifications and method of administration

All subjects will receive the same dosage of 1 tablet (1.3 gram) taken between meals once a day. No dosage modifications are possible. The medication will be taken orally.

3.6 Preparation and labelling of Investigational Medicinal Product

Manufacturing, preparation, labelling and packaging of both the active verum (Impryl®) and placebo tablets will be performed by Labomar SRL compliant with EU GMP. The labels will be in Dutch. A flaglabel (to be teared off) is used to identify placebo and Impryl®. Distribution of study medication will be organised by either the Radboudumc, department of Reproductive Medicine, or at the local department of Gynaecology at every site. Every centre was given the option to perform randomisation and distribute the study medication at their own hospital or asking their included patients to collect study medication after randomisation at Radboudumc at the above mentioned department.

3.7 Drug storage accountability

The coding and distribution to the trial will be organised by Labomar SRL in collaboration with the principal and coordinating investigators of the Radboudumc. The study medication has to be stored in a dry place at room temperature. No special precautions have to be taken into account. Patients are not asked to return study medication. Since it is a supplement and not medication there are no specials precautions for destruction or disposal.

Drug accountability will be performed in line with 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 . See paragraph 5.2 of this protocol for further details on the dispensing and logging.

4. NON-INVESTIGATIONAL PRODUCT

Not applicable

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

Number of ongoing pregnancies, conceived in the time window between randomization up to and including month 6 of intervention use. Ongoing pregnancy is defined as a visible embryonic heartbeat at ultrasound from 10-12 weeks of gestation onwards.

5.1.2 Secondary study parameters/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 start 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), leading to different fertility treatment categorisation (EM, IUI, IVF, ICSI), from a subpopulation of patients: Radboudumc patients and patients from other sites having pre-wash TMSC as standard procedure when semen is used for IUI or 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.

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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

5.1.3 Other study parameters

Baseline parameters are age, length, weight, ethnicity, alcohol/drugs/nicotine use, use of vitamins/steroids or other supplements, diet specifics (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 anovulation. Furthermore, when IVF/ICSI is performed, the amount of embryos transferred will be reported, either a single-embryo transfer (SET) or double-embryo transfer (DET).

5.2 Randomisation, blinding and treatment allocation

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). 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 (1st) and recruiting centre (2nd). 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 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. Randomisation outcome is either A (Impryl®) or B (placebo).
CODING: coding starts immediatly after informed consent has been obtained. In Castor each patient will receive a combination of one letter and 3 numbers. The code will be assigned as followed: Radboudumc (A), Slingeland (B), Canisius-Wilhelmina (C), Jeroen Bosch (D), Elisabeth-TweeSteden (E), Maasziekenhuis Pantein (F), Bernhoven (G), Maxima Medisch Centrum (H), Maastricht UMC+ (I), Gelre ziekenhuizen (J), Nij Geertgen (K) Rijnstate (L), Medisch Centrum Kinderwens (M), Nij Barrahûs (N),Fertiliteitscentrum Voorburg (O), Catharina Ziekenhuis Eindhoven (P) and Bravis Ziekenhuis (Q). For example, the first patient included in Radboudumc will receive the code A001. 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 randomisation.
BLINDING: 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.
DEBLINDING: The indications for breaking the randomisation code are: SAE, SAR and SUSAR, as instructed by METC, or in a dire emergency, as directed by the principal investigators or trial manager. Every site has access to a deblinding form ("Formulier verbreken randomisatie code") 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.
DISTRIBUTION: In Radboudumc and at the local 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. Every study site can decide to perform randomisation and distribution of medication at local site or at Radboudumc. 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 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 & 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, randomizer/distributor initials, hospital number, patient name and initials, date of birth and monitoring check.

All unblinded personel performing randomisation will be trained by a site intiation to perform randomisation in Castor EDC and distribution of medication. A log list of all personel 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.

5.3 Study procedures

Couples matching the inclusion criteria will be asked to participate in the trial. If after diagnostic work up the diagnosis infertility for the couple remains, both are asked for informed consent. After signing informed consent, the male patient will be randomised and allocated to Impryl® or placebo (double blinded).

Medication
Patients can directly start using the food supplement or placebo. Impryl® or placebo will be taken for a total duration of 6 months and will be directly stopped after having a positive pregnancy test.

Baseline characteristics
Patients are asked to report on these characteristics by a short questionnaire. Patients will receive an automatic email invitation directly after randomisation and are asked to provide this information online in the Castor database system

Lifestyle and outcome characteristics
Patients will be asked once every month to report on occurrence of pregnancy (with date of positive test), changes in weight and diet, changes in habit of smoking and drug or alcohol use, occurrence of fever and change in activities increasing scrotal temperature (hot baths, sauna, race cycling). Furthermore patients are instructed about taking note of the total amount of consumed study medication (used boxes and tablets). There is a question about the occurrence of adverse events. Patients will receive an automatic email and are asked to provide this information online in the Castor database system. When a pregnancy has been reached during the 6 months use of study medication the monthly questionnaires will be stopped, because there is no more necessity to record on lifestyle changes and treatment compliance.

Semen analysis
Semen analysis will be performed two times: once during diagnostic work-up (standard care, analysis according to WHO criteria(28) and once more after approximately 3 months of using study medication (ie. after three months of IUI treatment or IVF/ICSI). At several sites, a pre-wash TMSC is standard care when performing an insemination (IUI group) and will be performed on the semen produced for IVF/ICSI as part of routine measurement before procedure. Because semen analysis performed at the time of treatment cannot be fully performed according to the WHO criteria, we will categorize the TMSC quality according to the allocated treatment category. This means, that the TMSC (evaluated at the start of treatment and after 3 months) will be analyzed according to changes in allocated treatment category based on the NVOG guideline(29).The criteria for treatment are given in the table below. A change in treatment category means an improvement in sperm quality that would allow a lower treatment category (e.g, from ICSI to IVF)

TMSC outcome (x10^6)	**WHO Diagnostic category**	**NVOG Treatment category**
> 10	Normospermia	EM
3 -10	Mild OAT	IUI
1-3	OAT	IVF/ICSI (if after wash VCM is <0,5)
<1	Extreme OAT/Azoospermia	ICSI

OAT = oligo-astheno-teratozoospermia.
* Or IVF depending on the prediction model of Hunault and other clinical characteristics

We decided not to perform a semen analysis in the EM group due to the fact that they already have normal semen parameters at intake and the burden of an extra visit is high.

Fertilization and embryo-utilization rate
In IVF/ICSI patients fertilization rate and embryo-utilization rate are standard reported by every IVF-laboratory. Fertilization rate is the percentage of oocytes with >=2 PN after insemination (IVF) of injection (ICSI). 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.

Transvaginal ultrasonography
If there is a clinical pregnancy in fertility patients, a routine (standard care) ultrasound will be performed in the first trimester between 5 to 9 weeks to determine the viability. Also routine (standard care) is an ultrasound around 10-12 weeks to estimate the due date. To limit the amount of extra site visits to zero, we decided that this 'due date' ultrasound at 10-12 weeks of pregnancy is enough for determining the primary outcome. The ultrasound can be made in the midwife practice. Information about the outcome of this ultrasound is reported in the 15-months questionnaire.

Questionnaire pregnancy/treatment outcome
To allow follow-up for treatment and pregnancy outcome, all couples will receive an automatic email with invitation for a short questionnaire 15 months after inclusion. 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, birth weight and sex of neonate). An email will be sent if the questionnaire is not returned after one month. If there is no reaction to the reminder email, either the patient or his general practitioner will be contacted by telephone to answer follow-up questions.

5.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. There are no specific criteria for withdrawal.

5.5 Follow-up of subjects withdrawn from treatment

As the statistical analysis is planned according to intention to treat principle, patients that violate the study protocol will be analysed in the group that they were allocated to. All patients will receive full follow-up.

5.6 Premature termination of the study

The principal investigator at the initiating centre Radboudumc, may temporarily or permanently discontinue the trial at a single site or at all sites for safety, ethical, compliance or other reasons. If this is necessary, the principal investigator will endeavour to provide advance notification to the site. If the site or trial is suspended or discontinued, the principal investigator will be responsible for ensuring prompt notification to the METC (CMO Arnhem Nijmegen). Where required by local regulations, the principal investigator will be responsible for informing the METC of trial or site discontinuation. In such cases, all trial data must be returned to the principlal investigator at the Radboudumc.

6. SAFETY REPORTING

6.1 Temporary halt for reasons of subject safety

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

6.2 AEs, SAEs and SUSARs

6.2.1 Adverse events (AEs)

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

6.2.2 Serious adverse events (SAEs)

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

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- 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 without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

6.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions 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 (see chapter 9.2.2);
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 sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

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.

6.3 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.

6.4 Follow-up of adverse events

There will be no follow-up of adverse events. There are no adverse events reported in previous use of Impryl. Therefore these are not expected. However, all SAEs and SUSARs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

6.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Due to the known safety of Impryl®, we do have the opinion that establishing a DSMB is not necessary.

7. STATISTICAL ANALYSIS

All data will be analyzed on an intention-to-treat basis. Data of patients who are lost to follow-up, will be included in their randomised group
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 summarise 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 first calculated with descriptive statistics, with the following codes:

- 0: if there is no change in treatment category
- 1: if there is 1 category change (i.e. from IUI to EM), or -1 if it goes from less severe to severe (i.e. IUI to IVF)
- 2: if there are 2 category changes (i.e. from IVF to EM)

The changes will be summarized with descriptive statistics (mean, SD, median, range) and frequency tables per treatment and stratum. Further, the difference between the experimental and control group will be assessed with a fixed effects linear model, adjusted for centre and stratum.
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 randomised patients without any major deviation from the protocol. A major protocol deviation is defined as

Use of other nutritional supplements without a wash-out period
Intake of study medication of less than 75% of the prescribed amount

Participants will be asked by automatic email to report in Castor their actual compliance to the intervention by taking note of total amount of consumed study medication (used boxes and tablets). Patients will also be asked to report about major changes of their lifestyle occurring during the study period (see page 22).

The results will be extracted from Castor. If there are missing data this will be mentioned along with the reason.

7.1 Interim analysis

Given the low risk on adverse events of a nutritional supplement, there are no planned interim analyses.

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki as amended most recently by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013 and in accordance with the Medical Research Involving Human Subjects Act (WMO).

8.2 Recruitment and consent

Potential subjects will be informed by the treating physician about the study. All subjects will be handed an PIF at their intake consultation. The information letter also contains contact information of the coordinating investigator. If requested the (potential) subject could also receive additional information from the coordinating investigator (R.M Smits) or they will get a telephonic consultation with an onsite research nurse. Only after counselling and reading the additional information the (potential) subject will be asked for their informed consent. A consent form must be signed by both the couple as the physician or research nurse who provided the information. Couples will receive the information preferably at or before (together with appointment letter) their first consultation. The following consultation the subject will be asked whether he will or will not participate. If a patient needs more time or only has one evaluation consult in between IVF/ICSI treatment, they will be telephonically contacted by the onsite research nurse or research coordinator to ask for participation. The information letter and informed consent are attached.

8.3 Objection by minors or incapacitated subjects

Not applicable.

8.4 Benefits and risks assessment, group relatedness

As this study compares a food supplement with placebo, it will not impose extra risk on participants. Infertility is a common problem which affects many couples. Therefore research in order to optimize treatment is imperative.

8.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

8.6 Incentives

Subject will receive no special incentives or compensation.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 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 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).

9.2 Monitoring and Quality Assurance

Monitoring will be performed in compliance with Good Clinical Practice (GCP) and other rules and regulations in order to achieve high quality research and secure patient safety.

9.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

9.4 Annual progress report

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

9.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit, in case of not achieving a pregnancy this will be a telephonic consultation.

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

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

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

9.6 Public disclosure and publication policy

The results will be disclosed unreservedly. The sponsor did not provide the investigators with any restrictions.

10. STRUCTURED RISK ANALYSIS

Not applicable.

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