

Study protocol

Project title: Micronutrient Supplementation for Women with PCO-syndrome -
Influence of Nutrition and Physiology on the Development of PCOS-typical
Parameters

Participants

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Introduction and scientific background

In the Western world, malnutrition and subfertility have become increasingly common problems affecting both men and women. A recent study showed that conforming to nutritional recommendations significantly increased the probability of a clinical pregnancy following IVF/ICSI (1). They included fruit, vegetables, certain types of meat, fish, whole-grain products and specific fats (according to the Netherlands Nutrition Centre Foundation). Another study specifically analysed preconceptional consumption of unsaturated fatty acids by women before IVF/ICSI. It discovered a positive effect on the embryo's morphology (2).

The effect of micronutrients on female fertility is currently under investigation. A 2012 review on the subject included 13 studies with more than 90,000 female participants and provided no generalised recommendations, but allowed the authors to deduce a certain positive effect of supplementation with certain micronutrients on female fertility (3).

This current study will test the effect of a currently available micronutrient supplementation in women suffering from polycystic ovary (PCO)-syndrome and sterility. The product (PROfertil ® female, Lenus Pharma, Seeböckgasse 59, 1160 Vienna, Austria) is based on several studies that showed (i) an increased need for these micronutrients of women with a desire to have a child or currently pregnant and/or (ii) a positive effect on female fertility. They are collected in the attached PROfertil ® female study report (see attachment "PROfertil Studienreport final". These documents summarise the evidence for the contents of the product in detail. PROfertil ® female is a micronutrient supplement containing selenium, vitamin E, catechin, glycyrrhizin, co-enzyme Q10, folic acid and omega-3 fatty acids.

This study seems of impact from a scientific perspective. Clinical experience with PROfertil ® female suggests that patients suffering from PCO-syndrome experience an increase of anti-müllerian hormone and androgen levels. The reductive effect of typical PCO-seroparameters can be explained pathophysiologically: A chronic low-level pro-inflammatory state – both locally in the ovary and systemically – appears to be among the central pathophysiological correlates of PCO-syndrome (4). PROfertil ® female contains a mix of several antioxidative agents. A favourable effect on PCO syndrome is expected. Metformin is an anti-inflammatory substance commonly used to treat PCO-syndrome. The anti-inflammatory effect is seen as positive (5). This explains why women suffering from PCO-syndrome without insulin glucose balance problems react positively to treatment with Metformin (6).

Study aims

The primary aim of this study is to analyse the effects of three months of treatment with PROfertile[®] female compared to a supplementation with 400µg of folic acid for women suffering from PCO-syndrome, sterility, anovulation. The study will examine the parameters typical for PCO-syndrome (anti-Mullerian hormone, total testosterone, androstendione). The secondary goal is to document the individual course of further fertility treatment.

The **products** being administered to the patients for four weeks are:

- PROfertile[®] female (Lenus Pharma GesmbH, Seeböckgasse 59, 1160 Vienna; treatment group) – one soft capsule and one pill per day.
- 200µg folic acid (Folsäure Kapseln 400µg[®], OTC Produktion und Forschung GmbH, Fischergasse 17, 5020 Salzburg; control group) – two capsules per day.
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The study will be performed in a double-blinded manner. The medications are unlabeled for both groups and will be dispensed in unlabeled blisters. However, patients could search for the actual look of the PROfertile[®] female soft capsules and the pills (for example on the internet). Since they differ from the folic acid capsules, patients could identify the control medications. The study team is aware of the fact that this kind of blinding is not according to standards and could introduce some kind of bias.

PROfertile[®] female composition overview

	Amount per day
Contents	New composition
Soft capsule	
Omega-3 fatty acids	500 mg
Pill	
Folic acid	800 µg
Selenium	70 µg
Vitamin E	30 mg
Green-tea extract catechin	4 mg
Glycyrrhizin from licorice extract	12 mg
Coenzyme Q10	30 mg

Study hypothesis:

First hypothesis: A three-month treatment with PROfertile[®] female causes a drop of anti-müllerian hormone, total testosterone and androstenedione levels. This does not happen if treated with 400µg folic acid.

Outcome parameters:

Primary outcome parameters: Primary outcome parameters: anti-müllerian hormone (AMH), total testosterone and androstenedione levels

Study design:

Monocentral, prospective randomized double-blinded trial

Participants:

The participants will be chosen or excluded using the following criteria:

Inclusion criteria:

- 1) The patient has been diagnosed with PCO-syndrome using revised 2004 Rotterdam-criteria (7).
- 2) The patient is sterile, defined as being unable to become pregnant within a year despite unprotected sexual intercourse.
- 3) The patient suffers either from oligomenorrhoea (defined as an interval of ≥ 60 days between the last three menstruations) or complete amenorrhoea for at least 90 days.
- 4) The patient has given her written informed consent after detailed information on the study by medical professionals at the Department of Obstetrics and Gynecology of the Medical University of Vienna.
- 5) Both partners are at least 19 years old and are younger than 35. The age limit was chosen to take physiologically reduced fertility beyond this age (8). This measure allowed the women to avoid a 100-day delay of fertility treatments due to this study.

Exclusion criteria:

- 1) No informed consent.
- 2) At least one partner is younger than 19 years or older than 35 years.
- 3) The patient has been subject to one of the following PCO-syndrome-related treatments within three months before inclusion: metformine, combined oral contraceptives, cortisol therapy, inositol, ovarian drilling, any kind of ovarian stimulation, in-vitro fertilisation. Promoting menstruation using gestagen products is acceptable.

The inclusion and exclusion criteria are checked at the start of the study and must be fulfilled at that point. All inclusion criteria have to be fulfilled to participate. A single exclusion criterion is sufficient to prevent participation.

Recruitment:

The participants are recruited by medical professionals at the Department of Obstetrics and Gynecology of the Medical University of Vienna using the above mentioned criteria during routine examinations before any treatment for PCO syndrome and/or sterility is initiated. Potential participants are informed about the procedure, clinical relevance and possible additional effort caused by study participation. The patients willing to participate then have to sign the written informed consent.

Sample size calculation:

Assuming a reduction of the mean AMH levels by 2 ng/ml at a standard deviation of 3 ng/ml and an alpha value of 0.05 and a power of 0.90, the paired t-test requires 26 patients per group. Due

to the fact that the participants wish to conceive a child and have been diagnosed with sterility, a low drop-out rate can be expected. There is no reliable data on this or similar treatments that allow prediction of the chance to conceive. Due to the favourable benefit-to-risk profile, a 15% drop-out rate is assumed. This corresponds to 4 patients per group. Thus, the final sample size is calculated as 30 patients per group, which leads to a total study population of 60.

Statistical analysis:

The bio-statistician will randomise the data using nQuery advisor™ Version 7.0. The information will then be packaged in an envelope by a person independent of the participating medical professionals and other scientific personnel. This will only be opened after the inclusion of the patient. Categorical variables will be presented as absolute numbers and percentages, numerical variables as median and interquartile range.

The following statistical analyses are planned: The result parameters and the patient characteristics of the two groups will be compared using the Welch test (for numerical variables) and the Chi-square-test or Fisher’s exact test (for categorical variables). A p-value of <0.05 is considered to be statistically significant. The analysis will be performed as an intention-to-treat analysis. The statistical analyses will be performed using SPSS 24.0 for Windows (SPSS Inc., 1989-2017).

Overview on parameters to be analysed:

<i>Parameter</i>	<i>Unit</i>	<i>Type</i>	<i>Planned statistical test</i>
Age	Years	Numerical	Welch test
Body mass index	Kg/m ²	Numerical	Welch test
Sterility: Secondary (versus primary)	n (%)	Categorical	Chi-square/Fisher’s exact test
Duration of infertility	Years	Numerical	Welch test
Pre-treatment FSH	mU/mL	Numerical	Welch test
Pre-treatment LH	mU/mL	Numerical	Welch test
Pre-treatment AMH	ng/mL	Numerical	Welch test
Pre-treatment testosterone	ng/mL	Numerical	Welch test
Pre-treatment androstenedione	ng/mL	Numerical	Welch test
Post-treatment AMH	ng/mL	Numerical	Welch test
Post-treatment testosterone	ng/mL	Numerical	Welch test
Post-treatment androstenedione	ng/mL	Numerical	Welch test

Abbreviations used: n (%) = number (frequency)

Further details on study design:

After recruiting the participants at the outpatient clinics for Gynecologic Endocrinology and Infertility Treatment, they are randomly assigned to one of the groups. Participants will receive either 2 unlabelled soft capsules containing 200µg folic acid each or 1 unlabelled soft capsule containing omega-3 fatty acids and 1 tablet containing folic acid, selenium, vitamin E, catechines, glyzyrrhizin, and co-enzyme Q10 (see Table above for details on doses).

The planned examination of AMH, total testosterone and androstenedione levels will take place after a minimum of 90 days (and a maximum of 100 days) after beginning supplementation with PROfertil ® female or folic acid. The maximum duration of participation is thus 100 days. After taking the micronutrient supplementation (PROfertil ® female or folic acid) beyond the planned minimum treatment duration, an individualised fertility treatment can be performed.

There are two study-specific consultations: After including the patient (consultation 1: randomisation, distribution of drugs and start of study) and after 90 to 100 days (consultation 2). During consultation 2, unused study blisters are collected and patients are questioned on peculiarities and possible side effects of the micronutrient supplementation used.

It is expected that 30 minutes for consultation 1 and 15 minutes for consultation 2 will have to be spent by the patient. The consultations will be held during regular outpatient treatments and will be part of the planned routine examinations. They include discussions of examination results and recommendations for further treatment.

Study duration:

A total duration of about 12 months will not be exceeded.

Benefits-to-risk assessment and preventive measures:

Risks or complications due to the use of PROfertil ® female and additional venepuncture are not expected. A recent study using the same product showed no substance-related side effects (number of the ethics committee of the Medical University of: 1659/2013; accepted on 2013-09-24) (9). A direct benefit for the treatment group is possible. A positive effect on PCO syndrome can be assumed, which might result in a higher chance of pregnancy. The substance administered to the control group is state of the art for female fertility treatment.

- Fertility treatment can be postponed by a maximum of 100 days by this study. This information is communicated to the patients during informed consent. Should there be any unexpected complications or should the patient want to stop her participation for personal reasons, she will be excluded and will no longer be subject to further examinations.
- It is possible for the patients to benefit financially from this study since Lenus Pharma GesmbH provides them with the study medication (PROfertil ® female and Folsäure Kapseln 400µg). In addition, the company will provide the assigned medication free of charge for further 6 months after the end of the study, should they wish to continue treatment for PCO-syndrome and fertility.

Study-specific parameters:

None.

Parameters obtained during routine clinical examinations:

All parameters on outcome (anti-müllerian hormone, total testosterone and androstendion levels) will be obtained during routine clinical examinations.

Several additional parameters will also be collected and included in the database. They do not require additional effort on behalf of the patients. They include: general patient information (age, duration of infertility, height and body weight, additional medication); information pertaining to PCO-syndrome (menstruation history, Ferriman-Gallwey-score on hirsutism (if applicable), acne, scalp-hair effluvium); hormone parameters on days 2 to 5 of the menstrual cycle (androstendion, thyroid-stimulating hormone, luteal hormone, follicle-stimulating hormone, sex hormone binding globulin); as well as an oral glucose tolerance test, which is routinely performed by general practitioners.

In addition, the individual development of the fertility treatment will be documented for a maximum of 6 months following active study participation.

Approval of PROfertil ® female:

PROfertil ® female is approved in Austria by the Austrian Ministry of Health. A copy of the corresponding Free Sales Certificates can be found in the attachment.

Approval of 400µg Folsäure 400:

Folsäure Kapseln 200µg (OTC Produktion und Forschung GmbH ®) is a nutritional supplement. Contrary to drugs, these products do not require approval, registration or declaration in Austria.

Study financing:

Study medication and ongoing medication were provided by Lenus Pharma GesmbH (Seeböckgasse 59, 1160 Vienna, Austria). Lenus Pharma GesmbH supports clinical trial expenditures with a total sum of EUR 9,000.00 for 60 participants. Lenus Pharma GesmbH is not a direct sponsor of the study. The data and usage rights remain with the Medical University of Vienna.

Data access agreement:

Assoc.Prof. Priv.Do. Dr. Johannes Ott can access all data and statistics at any time.

Literature:

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