Investigation on the role of follicular homocysteine in assisted reproduction cycles of polycystic ovary syndrome (PCOS) patients

Protocol code: 2017-3/42 ***Version: February 2017***

Clinical study protocol

Summary

1. Promoter and location of the study

1.1 Principal investigator

Tansu Kucuk, MD, Professor of IVF at Acibadem Maslak Hospital

1.2 Study site

The study will be performed at Acibadem Fulya Hospital IVF Center, Hakki Yeten Cad Yesil Cimen Sokak 23, Fulya (Besiktas), Istanbul, Turkey.

2. Rationale

Polycystic ovary syndrome (PCOS) is a metabolic and endocrine disorder occurring in 6 to 20% of women in reproductive age, according to the diagnostic criteria used [1]. The clinical manifestations are variable, however the association with androgen excess, insulin resistance, obesity and metabolic syndrome points to a metabolic pathogenesis.

Among the metabolic findings, it is noted that women with PCOS exert an average increase of 23% of blood fasting homocysteine (Hcy) [2]. Increased Hcy in PCOS is not related to degree of insulin resistance (IR), as well as to obesity or androgen levels [3]. Moreover, lowering the insulin concentration in PCOS patients with metformin does not result in lower Hcy, rather a significant increase has been reported [4, 5], which points to the contribution of other genetic and/or dietary and/or metabolic factors besides the possible role of insulin.

Hyperhomocysteinemia (HHcy) has been linked to a variety of diseases including cardiovascular disease, neurodegeneration, diabetes and cancer [6]. HHcy has been recognized since long time also as a main marker of both female and male reproductive dysfunction [7], which generates further interest in the role of Hcy in reproductive issues of PCOS patients. Berker et al (2009) found a higher amount of Hcy in follicular fluid of PCOS women undergoing oocyte pick-up following FSH stimulation sharply marked lower oocyte quality, fertilization rates, embryo quality and pregnancy rates with no pregnancies occurring in ladies whose sampled follicles contained more than 8 µmolar Hcy [8]. Interestingly, the blood Hcy of the same patients was normal (11.7±2.9 µMol) suggesting the occurrence of a follicle-specific impairment of Hcy metabolism in PCOS and a main role of Hcy in PCOS subfertility also in women with normal Hcy blood values. These findings prompted follicular homocysteine as a possible quality marker for oocyte selection in assisted reproduction but the data from Berker et al (2009) could not address the question because there was no overlapping identity between the follicles sampled for follicular Hcy and the oocytes eventually used to perform the (multiple) embryos transfer.

Excess of circulating Hcy is removed by three alternative pathways: re-methylation to methionine by a methyl group donated by either 1) methyltetrahydrofolate or 2) betaine and 3) Hcy transulfuration to cysteine, which can be then used for the synthesis of the universal cellular antioxidant glutathione [14]. A combined micronutrient supplementation supporting both the re-methylation (folates, B2, B3, B12, betaine and zinc) and the transulfuration (B6, cysteines and zinc) of Hcy (Impryl, Parthenogen, Switzerland) had been already shown to be very efficient in decreasing blood fasting hcy in young PCOS women [9], although the effect of the supplementation on their reproductive competence had not been tested. The same supplementation has the potential to lower the hcy level also in the follicular fluid, which may result in improved fertility, but such a potential has not been tested so far.

The present pilot study is intended to preliminarily explore the role of follicular hcy in predicting the reproductive potential of oocytes in assisted reproduction cycles and the potential of a micronutrient support in decreasing the hcy concentration in the follicular fluid of PCOS ladies undergoing assisted reproduction treatments.

3. Study design

3.1 General description

This is a prospective, randomized, parallel-group, open-label, controlled versus no-intervention clinical study on an approved nutritional supplement.

Patients with PCOS referring for primary infertility and addressed to assisted reproduction by means of in vitro fertilization will be sampled for mono-follicular fluid at time of oocyte pick-up and all the collected fluids will be frozen. After in vitro fertilization of the collected oocytes and selection of the best embryo(s) to be transferred based on standard morphologic criteria, the follicular fluid relevant to the selected follicle/oocyte/embryo will be tested for hcy content.

All patients giving consent to test their follicular Hcy will be randomized for an open label treatment with an already approved combination of micronutrients.

3.2 Study objectives

The primary objective of the study is to demonstrate that, in PCOS patients, the level of follicular Hcy tested at time of oocyte pick-up in assisted reproduction cycles is predictive of the treatment outcome from the same oocyte.

The secondary objective of the study is to demonstrate that a targeted micronutrient supplementation is effective in decreasing follicular hcy in PCOS patients.

The data obtained will be published in a peer-reviewed journal.

3.3 Study procedures

3.3.1 Enrolment, informed consent and randomization

Women referring due to infertility problems and diagnosed PCOS according to the Rotterdam criteria [10] and addressed to assisted reproduction procedures will be offered to participate in the study. After a preliminary check of the inclusion/exclusion criteria an informed consent will be obtained and a randomisation position will be assigned. The patients randomized for the treatment arm will also receive instructions on how to assume the treatment and how to report on adverse events.

3.3.2 Nutritional intervention and controlled ovarian hyperstimulation (COH)

The patients randomized for the nutritional intervention will assume the study product, 1 tab per day, during at least 2 months before the start of the COH and will continue up to the day of oocyte pick-up.

All patients will undergo COH according to the standard practices of the study site for the same patients without any study-intended adjustment. Briefly, supraphysiologic amounts of an FSH-containing drug will be injected daily to induce multi-follicular development and the FSH dose will be adjusted according to the patient's response. When the leading group of follicles reaches 13 mm, a GnRH antagonist will be commenced to inhibit premature LH surge. Once at least 3 follicles will reach 18 mm diameter, a bolus of hCG will be injected to induce final maturation.

3.3.3 Oocyte pick-up and follicular fluids collection

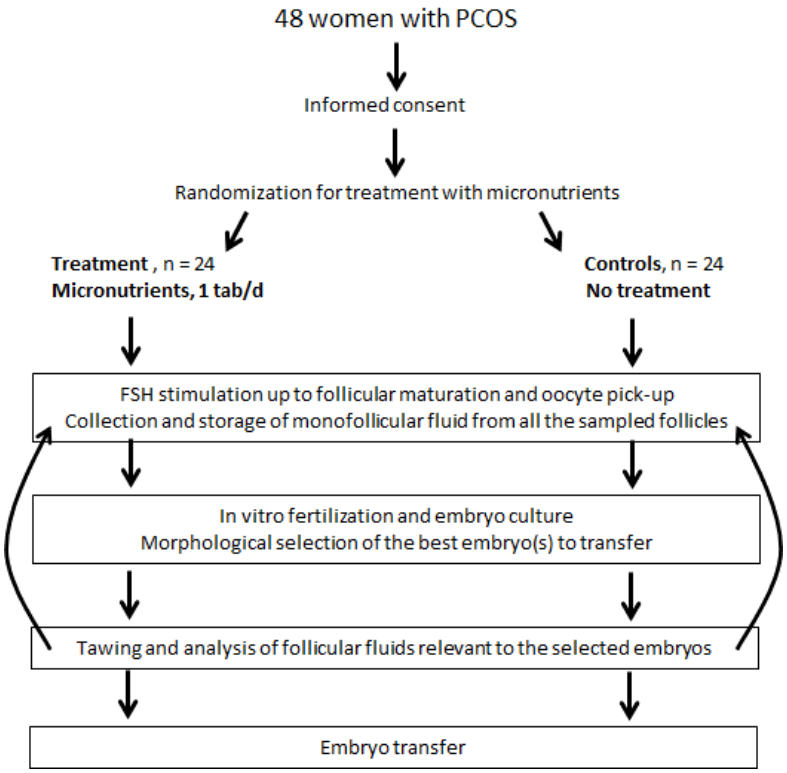
The oocyte pick-up will be performed 35-36 hours post hCG injection by US guided transvaginal needle aspiration. The fluid contained in each punctured follicle will be collected immediately after aspiration. The fluid samples will be placed on ice until centrifugation to eliminate any cells and freezing at -20.

3.3.4 In vitro fertilization, embryo culture and embryo selection

Mature oocytes will be injected with the partner sperm and fertilization will be confirmed by the appearance of two pronuclei. The zygotes will be cultured up to blastocyst stage. The best embryo based on morphological score will be selected for transfer. All patients will receive luteal support with micronized progesterone.

The follicular fluid samples corresponding to the oocytes that generated the blastocysts selected for transfer will be thawed and analysed for hcy content. The concentration of HCY in follicular fluids will be tested by a commercially available ELISA kit (Sunred Bio, China).

3.4 Schematic synopsis of the study



4. Study population

The study population consists of infertile women referring for primary infertility and meeting the inclusion criteria listed below.

4.1 Inclusion criteria

- Female sex, age above 18;
- Primary infertility during at least 1 year
- Clinical diagnosis of PCOS according to Rotterdam criteria [10];
- Written informed consent

4.2 Exclusion criteria

- Ongoing pregnancy;
- Any known reasons for infertility but PCOS
- Ongoing treatment with; sugar lowering agents, anti-hypertensives, hormones;
- Systemic or endocrine diseases;
- Assumption of any dietary supplement in the week before enrolment or need to assume supplements during the study period;
- Known or suspected hypersensitivity to any ingredient of the supplement;
- Any condition that may compromise the subject's ability to issue informed consent.

4.3 Participant withdrawal

The possible reasons for the withdrawal of a participant from the study are the following:

- Judgement by the investigator at any point during the study that the participant’s safety was jeopardized or potentially compromised;

- Withdrawal of participant consent for any reasons

The participant will be clearly informed that the withdrawal of the consent to the study may occur at any time even without explaining the reason and that such withdrawal will not decrease by any means the level of clinical assistance received.

5. Treatment

5.1 Nutritional supplement

The nutritional supplement chosen for the treatment is called Impryl and is produced in Italy by Labomar SPA under GMP conditions. Impryl contains the following nutrients:

Ingredients	Per day (1 tab)	% NRV
Betaine	200 mg	
L-cystine	200 mg	
Niacine	16 mg	100%
Zinc	10 mg	100%
Vitamin B6	1.4 mg	100%
Riboflavin (Vit. B2)	1.4 mg	100%
Methyl folate	400 μg	200%
Vitamin B12 (methylcobalamin)	2.5 μg	100%

NRV: nutrient reference value as per Reg. (EU) n.1169/2011

5.2 Mode of assumption

The prescribed dose is one tablet a day to be swallowed with water, preferably away from meals.

5.3 Adverse events

An Adverse Event (AE) is defined as any event occurred during a clinical trial, including modifications of the concomitant illnesses, or any accident, which could impair the health status of the subject.

A causal relationship with the study treatment does not have to be necessarily implied. Any AE will be duly reported on the relevant Case Report Form.

AEs will be classified and recorded according to seriousness, intensity and causality as here below described.

Seriousness

- Definition of serious Adverse Events (SAE)

A “Serious Adverse Event” is defined as an adverse experience that fulfils at least one of the following criteria: results in death, is life threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, overdose, cancer.

- Definition of Non-Serious Adverse Events

Any adverse events, which do not fall into the above-described categories, are defined as Non-Serious. The evaluation of the AE as serious or not-serious will be made up independently of any attribution of causality.

Intensity

Any AE must be judged for intensity and will be graded on the following:

- MILD: causing no limitation of usual activities; the subject may experience mild discomfort;
- MODERATE: causing some limitation of usual activities; the subject may experience annoying discomfort;
- SEVERE: causing inability to carry out usual activities; the subject may experience intolerable discomfort or pain.

Causality

Any AE must be judged for causality. The relationship of an AE to the treatment will be graded on the following:

- RELATED: an AE is to be considered as definitely related to the test treatment if the following criteria are met:

1. The AE follows a reasonable temporal sequence from administration of the drug;

2. The AE follows a known response pattern to the suspected drug;

3. The AE is confirmed by improving on stopping the drug (dechallenge) and reappearance of the reaction on repeated exposure (rechallenge).

- POSSIBLE: an AE is to be considered as possible test treatment-related if the following two conditions are met:

1. There is a reasonable temporal relationship between the administration of the product and the AE; but

2. None of the conditions of RELATED is met or an alternative explanation for the AE is more likely. An AE will also be classified as possible test treatment-related if there is sufficient information to know that an AE occurs during test treatment but the temporal relationship is unknown.

- UNRELATED: An AE is to be considered as unrelated if any of the following tests are met:

1. There is no reasonable temporal relationship, or

2. A causal relationship between the test treatment and the AE is biologically implausible (e.g. a subject in a clinical trial is injured as a passenger in a car accident), or

3. A clearly more likely alternative explanation is present.

- UNCLEAR: a reported AE is to be classified as not clear if the available information does not permit the assessment of a causal relationship in one of the above categories.

Immediately reportable adverse events

During the study, if any serious or unexpected AE occurs, the Investigator will immediately inform the competent health authorities. In addition, the Investigator will perform appropriate diagnostic and therapeutic measures, and will interrupt the study treatment, if appropriate.

6. Data management

6.1 Source Data

All clinical records, laboratory reports as reported into the study centre archive are considered source data. Upon request of the competent authorities, the investigator/institution will permit inspection providing direct access to source documents.

6.2 Retention of Documentation

All study related documentation shall be retained for a period of minimal 5 years after the study completion or longer if deemed necessary. It is the investigator's responsibility to ensure these are filed in a secure place.

6.3 Data collection and processing

Clinical data will be copied from the source records and entered into a duly formatted Case Record Form (CRF). The data transcription will occur in real time, i.e. as soon as possible. At the end of the study the data from the CRFs will be entered into a database suitable for statistical analyses.

7. Statistics

7.1 Sample size

This is a pilot study and, in the absence of previous data to build a statistical hypothesis, the sample is not statistically sized. The inclusion of 40 patients, 20 per group, is believed to be a clinically significant sample allowing to calculate the sample size for any future larger studies. Assuming a drop-out rate post-randomization of 20%, a total of 48 patients (24 per group) will be randomized.

6.2 Randomization

The randomisation list is generated using a random number generator and organized into balanced blocks of 4 positions each, 2 for active intervention and 2 for non-intervention. The randomization positions will be assigned to patients following a strict chronologic order.

6.3 Statistical methods

The differences in quantitative variables will be assessed by the Student's t test. Pearson correlation coefficient will be used to determine the relationship between variables and paired categorical data will be tested by McNemar test. The analyses will be performed with IBM-SPSS® version 25.0 (IBM Corp., Armonk, NY, USA, 2017). In all analyses, a two-sided p value < 0.05 will be assumed as significant.

8. References

1 Sirmans SM, Pate KA (2014) Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clinical Epidemiology, 6: 1–13.

2 Murri M, Luque-Ramırez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale H (2013) Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update, 19(3): 268–288.

3 Meng Y, Chen X, Peng Z, Liu X, Sun Y, Dai S (2016) Association between High Serum Homocysteine Levels and Biochemical Characteristics in Women with Polycystic Ovarian Syndrome: A Systematic Review and Meta-Analysis. PLoS ONE 11(6): e0157389

4 Palomba S, Falbo A, Giallauria F, Russo T, Tolino A, Zullo F, Colao A, Iorio F (2010) Effects of Metformin With or Without Supplementation With Folate on Homocysteine Levels and Vascular Endothelium of Women With Polycystic Ovary Syndrome. Diabetes Care 33(2): 246-251.

5 Esmaeilzadeh S, Maryam Gholinezhad-Chari M, Reza Ghadimi R (2017) The Effect of Metformin Treatment on the Serum Levels of Homocysteine, Folic Acid, and Vitamin B12 in Patients with Polycystic Ovary Syndrome. J Hum Reprod Sci 10(2): 95–101.

6 Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN (2017) The metabolism and significance of homocysteine in nutrition and health. Nutrition & Metabolism, 14:78.

7 Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL (2007) Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update 13(3): 225-38.

8 Berker B, Kaya C, Aytac R, Satıroglu H (2009) Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction. Hum Reprod 24(9): 2293–2302.

9 Schiuma N et al (2020) Micronutrients in support to the one carbon cycle for the modulation of blood fasting homocysteine in PCOS women. J Endocrinol Invest 43(6): 779-786

10 The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Human Reproduction 2004; 19(1): 41-47.