PROTOCOL

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OvoVid Study

OvoVid: Effect of a multivitamin supplement with probiotic (Seidivid Ferty4®) on oocyte retrieval and quality in oocyte donors. Double-blind randomized placebo-controlled clinical trial.

OVOVID-FERTY4-2022-01

Principal Investigator and Coordinator

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IMER-Valencia/Next Fertility

Sponsor

SEID LAB

Managing CRO

CROSSDATA
SCIENTIFIC SERVICES DATA

1. SYNOPSIS

TITLE	
	OvoVid: Effect of a multivitamin supplement with probiotic (Seidivid Ferty4®) on oocyte retrieval and quality in oocyte donors. Double-blind randomized placebo-controlled clinical trial
BACKGROUND AND RATIONALE	
	BACKGROUND Micronutrient supplementation has demonstrated a positive impact on clinical outcomes of IVF therapy in terms of pregnancy rate and/or live birth rate. [1] The importance of nutrient supplementation for cell development is well established as numerous processes in DNA synthesis are dependent on minerals such as zinc, copper and selenium, as well as vitamin B, folate and other antioxidants [2]. These substances, which are involved in mitochondrial metabolism and cellular respiration, could improve oocyte mitochondrial function. Although several supplementary antioxidant molecules have shown promising results, two recent Cochrane reviews described low-quality evidence about the positive effects of oral antioxidant treatment in live birth and clinical pregnancy rates in women attending an infertility clinic. 'Combined antioxidants' available on the market, as well as melatonin, CoQ10 and L-carnitine alone, showed a positive effect on clinical pregnancy rate, probably by improving oocyte quality and their maturation process [3]. <u>ANTIOXIDANTS: Vitamins A, B, C, D, E, folates and coenzyme Q10</u> Vitamins A and E concentration has been dosed in the follicular fluid of women undergoing IVF and has been associated to a better fertilization rate and to a better quality of the embryo [4]. Moreover, the use of supplemental vitamin A has been found to have a positive effect ART outcomes [5]. The findings on the effect of Vitamin C (ascorbic acid) upon reproduction are contradictory. While some studies in animal models showed its positive effects on oocyte competence, the results of other studies have not confirmed these findings. Whether this positive effects on reproduction is due to these vitamin' antioxidant characteristics is unclear [5]. Folates effectively scavenge oxidizing free radicals by inhibiting lipid peroxidation, thus playing a key role in oocyte quality and maturation. In this regard, poor folate status seems to be detrimental also due to its involvement in cell division (e.g. of oogonia and/or granulosa cells), inflammatory cytokine production and defective methylation reaction [2]. Supplementing the diet with high levels of folate and vitamin B12 in women has been linked to improved IVF outcomes such as significantly increased pregnancy and live birth rates [6]. Vitamin D is involved in follicular fluid homeostasis, suggesting the existence of a close functional correlation among follicular protein and micronutrients [7]. Besides some studies have suggested better oocyte quality and pregnancy rate in women who received vitamin D supplementation before IVF, others have not confirmed an association between vitamin D and IVF outcomes [1] Vitamin E has been reported as a scavenger of lipid peroxyl radicals and suppresses the generation of lipid hydroperoxides from cell membrane phospholipids, by acting

synergistically with other antioxidant nutrients including vitamin C, carotene, melatonin and selenium[8]

CoQ10 is a source of superoxide anion radical, though it also acts as an antioxidant, making it both a prooxidant and an antioxidant. The reduced form of CoQ10, ubiquinol, protects biological membranes from lipid peroxidation by recycling vitamin E and is also an antioxidant [9]. The dual role of CoQ10 in controlling mitochondrial function makes it an essential molecule for cellular performance. CoQ10 plasma levels decrease with advancing age, and this decline coincides with a decline in fertility and an increase in embryo aneuploidies [10]. Oral supplementation of CoQ_{10} may increase CPR when compared with placebo or no-treatment, in women with infertility undergoing ART procedures, both with poor ovarian reserve and PCOS [11].

INOSITOLS

Myoinositol (MI) has shown an important antioxidant action (SOD, catalase and GSH increase), which improves cell morphology and growth, as well as the cell membranes synthesis. High levels of myoinositol in ovary is crucial for improved FSH signalling, oocyte maturation and embryo development. MI has been found in follicular fluid and appears to improve oocyte and embryo quality [12].
In the ovary, D-chiro inositol (DCI) is responsible for an excess production of insulin-dependent testosterone.
In patients undergoing IVF pre-treated with inositol, improvements have been found in oocyte quality and oocyte maturation, an increase in cleavage rate, embryo development (expanded blastocyst) and quality, and an increase in the pregnancy rate [13].
The adequate MI/DCI ratio for supplementation is 40:1 [13].
Countless combinations and doses of inositols that are available in market products. 1000 to 1200 mg of D-chiro-inositol has been used in the past. Also, products containing 4 g, 2 g or 1 g of myo-inositol plus 400 mcg of folic are available. A combination of 1000 mg of myo-inositol and 25 mg of D-chiro-inositol is also available. The optimal dose of inositols for reproductive purposes in PCOS and non-PCOS patients is unknown. Thorough studies on the use of inositols in non PCOS patients are lacking [14]. Nevertheless, it cannot be excluded that healthy normo-responder women could benefit from their antioxidant and oocyte maturation effect.

ZINC

Zinc has an important role for zinc, in particular with potential linking to genome stability during early embryonic development [15]. Zinc is present in available products at a dose of 11–25 mg/day.

MELATONIN

The supplementation with melatonin reduces oxidative stress through melatonin membrane receptors activation. Myo-inositol and melatonin have shown to enhance, synergistically, oocyte and embryo quality [16].

RATIONALE FOR THE STUDY

Unfortunately, there is a lack of evidence favoring the protective and enhancing qualities of antioxidants. Although some micronutrients are considered useful for some patient subgroups, such as polycystic ovary syndrome and low-responder patients, little

	has been studied regarding whether oocyte quality and outcomes of assisted reproduction techniques could be improved in normo-responder women. Therefore, there is a need for large clinical trials using micronutrient supplements alone or in combination to research their potential effects on clinical outcomes in couples undergoing IVF therapy. This study represents a new step in this line of research, being the first study designed to evaluate the effect of combining micronutrients with probiotics on the outcome of the oocyte donation program in normo-responder female donors.
OBJECTIVES	
PRIMARY OBJECTIVE	• To assess the effect of pretreatment with a multivitamin supplement with probiotics (Seidivid Ferty4®) on oocyte retrieval in normo-responder patients undergoing controlled ovarian hyperstimulation (COH) for oocyte donation.
SECONDARY OBJECTIVES	• To assess the effect of pretreatment with a multivitamin supplement with probiotics (Seidivid Ferty4®) on oocyte quality in normo-responder patients undergoing COH for oocyte donation • To assess the effect of pretreatment with a multivitamin supplement (Seidivid Ferty4®) on the biochemical profile (fasting glucose, AST, ALT, cholesterol) and hormonal profile (baseline LH) in normo-responder patients undergoing COH for oocyte donation.
STUDY DESIGN	
STUDY DESING	Prospective, randomized, double-blind, placebo-controlled clinical trial of a multivitamin food supplement with probiotics.
SETTING OF THE STUDY	Next Fertility - Valencia.
POPULATION	
INCLUSION CRITERIA	1. Women undergoing COH for egg donation cycles 2. Signing of Informed Consent 3. Age 18-34 years 4. Body mass index between 18-29.9 kg/m^2 5. Meet the criteria for inclusion in the oocyte donation program of the IMER-Next Fertility center 6. Ultrasound antral follicle count before COH of ≥15 follicles
EXCLUSION CRITERIA	1. Patients excluded from the oocyte donation program 2. Untreated sexually transmitted disease, HIV, HCV, HBV positive serology 3. Blood disorders, neurodegenerative/psychiatric diseases, fragile X syndrome, oncological diseases, endometriosis. 4. Polycystic ovary syndrome 5. Oligomenorrhea (<1 menstrual cycle in 3 months) 6. Diabetic patients

NUMBER OF SUBJECTS	• Group A (Case group): 105 subjects • Group B (Control Group): 105 subjects
MATERIALS AND METHODS	
STUDY GROUPS	• Group A: the patients included in this group will be administered Seidivid Ferty4® (no label, white blister) for at least 30 days before controlled ovarian hyperstimulation and till the day of the trigger • Group B: the patients included in this group will be administered placebo (no label, white blister) for at least 30 days before controlled ovarian hyperstimulation and till the day of the trigger
STUDY DESCRIPTION	RANDOMIZATION METHOD: White, no-label, numerated blisters randomly containing capsules of either treatment or placebo (from 1 to 210) will be sent to the clinic. Once a patient is enrolled, she receives a progressive enrolment number, is inserted in the study database, and is given the blister with the corresponding number on it. PATIENTS RECRUITMEN: On the day the patient enters the oocyte donation program, she will be assessed to verify that she meets the inclusion criteria for the study. She will be informed about the study and asked to sign a written informed consent to be enrolled in it. No extra compensation will be given to oocyte donors who accept to participate in the study. Once the informed consent has been signed, she will be given a white, no-label, numerated blister and assigned the corresponding enrolment number. She will start the assumption of the treatment immediately and will call the clinic on the first day of the following bleeding to start the oocyte donation protocol (FSHr or FSHu or hMG or a combination, Medroxyprogesterone Acetate (MPA) for pituitary suppression andGnRH agonist trigger). She will continue to assume the treatment till the day of the GnRH-a trigger. CHARACTERISTIC OF THE TREATMENT Group A treatment (multivitamin product with probiotics): • MI plus DCI combined treatment at the ratio of 40:1 (1000 mg of MI, 25 mg of DCI) • 400 μg of folic acid • Melatonin 1.9 mg • Q10 co-enzyme 100 mg • Vitamin D3, B6, B12, E • Iron 28 mg • Zinc 15 mg • L. Gasseri 1x10^9 • L. Crispatus 1x10^9

	Group B treatment (placebo): • Placebo
INSTRUMENTS / TESTS	1. Ultrasound scan 2. Operating room and tools for oocyte pick up 3. Laboratory tools for oocyte analysis and vitrification 4. IT resources 5. Blood lab test (External laboratory)
OUTCOMES VARIABLES	PRIMARY VARIABLES • Total number of oocytes retrieved in the follicular puncture • Number of mature (MII) oocytes retrieved in the follicular puncture SECONDARY VARIABLES • Number of good oocytes (following the standard grading system established in Next fertility IMER to discard oocytes). • Oocyte quality variables: oocyte morphology will be evaluated following the standard classification system established at IMER Next Fertility, according to previously published criteria [17,18]. The oocytes will be classified as normal or with dimorphisms. Dimorphisms were subdivided into intracytoplasmic and extracytoplasmic. In the first case we evaluated the presence of incorporations, refractile bodies, vacuoles, aggregation of the smooth endoplasmatic reticulum and dense granulation. The assessment of extracytoplasmic dimorphism was based on the first polar body morphology, perivitelline space size and granularity, zona pellucida defects and shape anomalies. We considered high quality oocytes those cells without any of the previously described alterations. The evaluation was carried out by two different embryologists, blinded to the study protocol. • Biochemical and hormonal profile variables, results of blood tests: fasting glucose, AST, ALT, cholesterol, baseline LH
STATISTICAL ANALYSIS	SAS System software, version 9.4, will be used for analysis.
ETHICAL CONSIDERATIONS	
ETHICS COMMITTEE	Before study start, it will be submitted for the consideration of the Medicinal Product Research Ethics Committee of Hospital General Universitario de Valencia for approval.
INFORMED CONSENT	Before inclusion in the study, the physician will have the obligation to inform the patient of the different aspects of the study for her information, and written informed consent must be signed by the patient for her participation in the study
GENERAL CONSIDERATIONS	
STUDY DURATION	• Inclusion period: the inclusion period will be 9 months (June 2022-March 2023) • Follow-up period: the follow-up period will end in May 2023

FINANCIAL DISCLOSURE	The sponsor (SEID, S.A.) will cover all expenses generated by the study, including: the treatment, the cost of the instrumental tests and the investigators' fees.
EXPECTED RESULTS, IMPLICATIONS OF THE STUDY	Better oocyte yield, in patients considered normal responders, pre-treated with Probiotics/supplements than patients who have not received it. Assess the advantages of using Probiotics/supplements in normo-responder patients to ovarian stimulation.
KEY WORDS	Probiotics, Lactobacillus, Supplements, Oocyte quality, Oocyte yield, Ovarian controlled hyperstimulation

2. INDEX

3. STUDY BACKGROUND AND RATIONALE

3.1. BACKGROUND

Micronutrient supplementation has demonstrated a positive impact on clinical outcomes of IVF therapy in terms of pregnancy rate and/or live birth rate. [1] The importance of nutrient supplementation for cell development is well established as numerous processes in DNA synthesis are dependent on minerals such as zinc, copper and selenium, as well as vitamin B, folate and other antioxidants [2]. These substances, which are involved in mitochondrial metabolism and cellular respiration, could improve oocyte mitochondrial function. Although several supplementary antioxidant molecules have shown promising results, two recent Cochrane reviews described low-quality evidence about the positive effects of oral antioxidant treatment in live birth and clinical pregnancy rates in women attending an infertility clinic. 'Combined antioxidants' available on the market, as well as melatonin, CoQ10 and L-carnitine alone, showed a positive effect on clinical pregnancy rate, probably by improving oocyte quality and their maturation process [3].

ANTIOXIDANTS: Vitamins A, B, C, D, E, folates and coenzyme Q10

Vitamins A and E concentration has been dosed in the follicular fluid of women undergoing IVF and has been associated to a better fertilization rate and to a better quality of the embryo [4]. Moreover, the use of supplemental vitamin A has been found to have a positive effect ART outcomes [5].
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Folates effectively scavenge oxidizing free radicals by inhibiting lipid peroxidation, thus playing a key role in oocyte quality and maturation. In this regard, poor folate status seems to be detrimental also due to its involvement in cell division (e.g. of oogonia and/or granulosa cells), inflammatory cytokine production and defective methylation reaction [2]. Supplementing the diet with high levels of folate and vitamin B12 in women has been linked to improved IVF outcomes such as significantly increased pregnancy and live birth rates [6].

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3.2. RATIONALE FOR THE STUDY

Unfortunately, there is a lack of evidence favoring the protective and enhancing qualities of antioxidants. Although some micronutrients are considered useful for some patient subgroups, such as polycystic ovary syndrome and low-responder patients, little has been studied regarding whether oocyte quality and outcomes of assisted reproduction techniques could be improved in normo-responder women.

Therefore, there is a need for large clinical trials using micronutrient supplements alone or in combination to research their potential effects on clinical outcomes in couples undergoing IVF therapy.

This study represents a new step in this line of research, being the first study designed to evaluate the effect of combining micronutrients with probiotics on the outcome of the oocyte donation program in normo-responder female donors.

4. OBJECTIVES

4.1. PRIMARY OBJECTIVE

To assess the effect of pretreatment with a multivitamin supplement with probiotics (Seidivid Ferty4®) on oocyte retrieval in normo-responder patients undergoing controlled ovarian hyperstimulation (COH) for oocyte donation, considering both the total number of oocytes retrieved and the number of mature oocytes retrieved, that is, in metaphase II (MII).

4.1. SECONDARY OBJECTIVES

- To assess the effect of pretreatment with a multivitamin supplement with probiotics (Seidivid Ferty4®) on oocyte quality in normo-responder patients undergoing COH for oocyte donation.

- To assess the effect of pretreatment with a multivitamin supplement with probiotics (Seidivid Ferty4®) on the biochemical profile (fasting glucose, AST, ALT, cholesterol) and hormonal profile (baseline LH) in normo-responder patients undergoing COH for oocyte donation.

5. STUDY METHODOLOGY

5.1. TYPE OF STUDY

A prospective, single-center, randomized, double-blind, placebo-controlled clinical trial with a dietary supplement with probiotics, without medicinal products or medical devices.

5.2. STUDY DESIGN

A single-center, randomized, double-blind, placebo-controlled clinical trial to assess the effect of pretreatment with a multivitamin supplement with probiotics (Seidivid Ferty4®) on oocyte quality in normo-responder patients undergoing COH for oocyte donation.

Patients will be recruited on the day they are going to start the egg donation program. The investigator will verify that the patient meets all inclusion and exclusion criteria and will inform the patient about the characteristics and objectives of the study. Before inclusion, the patient must sign the written informed consent form to participate.

Once the informed consent is signed, the patient will be assigned a patient code and will be given the treatment box (white and with no label) corresponding to her code.

The patient will start taking the study treatment (multivitamin supplement with probiotics or placebo) immediately after inclusion.

On the first day of her next menstruation/bleeding, the subject will call the site to initiate the oocyte donation protocol (approximately 30 days after enrollment). Ovarian stimulation cycles for oocyte donors may begin on any day of the menstrual cycle, though the early follicular phase is preferred.

The egg donation protocol will be performed according to standard practice in line with the IMER (Next Fertility) center's donation program:

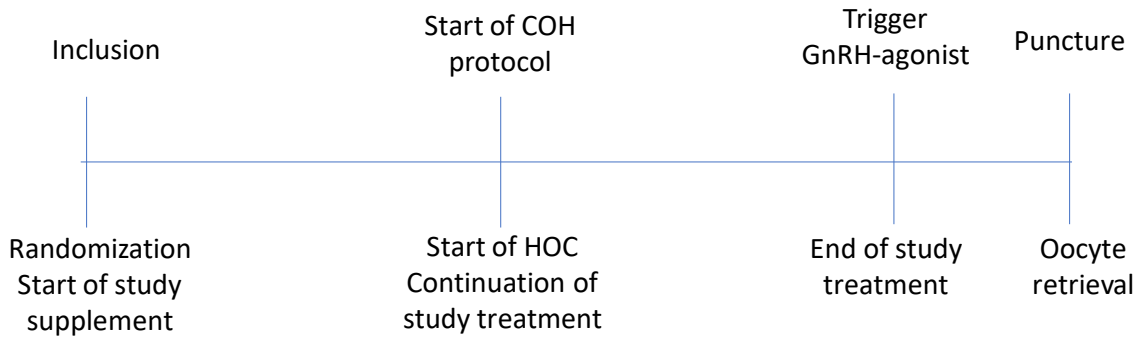
- Stimulation with rFSH or uFSH or hMG or a combination. The standard dose of FSH will be 225 IU/day (standard protocol), but it may be modified or increased in case of suspected inadequate response, suboptimal response in previous cycles or suspected poor compliance.

- Medroxyprogesterone acetate (MPA) for pituitary suppression. Dose of 10 mg/day p.o. starting on the first day of the COH and continuing up to and including the day of trigger

- GnRH-agonist trigger: Triptorelin acetate (Decapeptyl®) 0.2 mg, single administration, 36 hours before puncture.

Throughout the COH period of the protocol (usual mean duration: 11 days) the patient will continue taking the study supplement (multivitamin with probiotics or placebo), without interruption until the day of the GnRH-agonist trigger.

The patients will undergo the regular ultrasound controls of the egg donation program for evolutive control of the number and growth of follicles. The trigger (GnRH-agonist) will be administered when the donor completes at least 9 days of COH and the presence of at least 10 follicles larger than 15 mm with at least 4 with a diameter ≥20 mm is confirmed. Administration of the agonist will be as a single dose, 36 hours before follicular puncture for the retrieval of mature eggs.



The retrieved oocytes will be denuded and examined by light microscopy to assess their quality. Mature oocytes (metaphase II, or MII) will be counted and subsequently classified according to previously published criteria [17,18] into: normal or good quality oocytes (useful) and dysmorphic oocytes. Dimorphisms were subdivided into intracytoplasmic and extracytoplasmic. In the first case we evaluated the presence of incorporations, refractile bodies, vacuoles, aggregation of the smooth endoplasmatic reticulum and dense granulation. The assessment of extracytoplasmic dimorphism was based on the first polar body morphology, perivitelline space size and granularity, zona pellucida defects and shape anomalies. We considered high quality oocytes those cells without any of the previously described alterations. The evaluation was carried out by two different embryologists, blinded to the study protocol.

The results of the routine laboratory tests set out in the site's donation protocol and taking place in the assessment phase for potential donors and at the start of the COH program will be recorded. These analyses include the following parameters: Blood count, GOT/GPT, total cholesterol and LDL cholesterol, triglycerides, creatinine, prothrombin time, cephalin time, blood group and Rh, LH (COH start only), glucose (COH start only).

5.3. STUDY SITE

The study will be conducted at the Next Fertility IMER-Valencia Institute of Reproductive Medicine. Av. de Burjassot, 1, 46009 Valencia.

5.4. TREATMENT GROUPS

Patients will be randomized into two treatment groups of the same size:

- Group A: patients included in this group will be administered Seidivid Ferty4® (white box with no label) for at least 30 days prior to controlled ovarian hyperstimulation and until the day of the agonist trigger (GnRH-a).
- Group B: patients included in this group will be administered placebo (white box with no label) for at least 30 days prior to controlled ovarian hyperstimulation and until the day of the agonist trigger (GnRH-a).

5.5. RANDOMIZATION PROCESS

Patient allocation to Group A (study) or Group B (control) will be based on an automatically generated randomization list, at a 1:1 ratio. According to this list, the treatment boxes will be prepared and identified only with the study code and patient code.

As they are included in the study, patients will be assigned a numeric, correlative code and will be given the box of medication with the corresponding patient code number.

5.6. TYPE OF CONTROL

The control will consist of a placebo with no active ingredients, whose presentation and external appearance will be identical to that of the dietary supplement under study.

5.7. BLINDING PROCEDURE

To maintain the double-blinding, patients will be supplied the treatment at the clinic. The active treatment (multivitamin with probiotics) and the placebo will be provided in identical white boxes for both groups, marked only with the patient code and the study code. Neither the investigator nor the patient will know the contents.

The investigator will have an encrypted randomization list, so that, if necessary, he/she can determine the treatment group assigned to a patient.

6. STUDY POPULATION

6.1. SUBJECT SCREENING

Normo-responder patients (*) who are going to start the egg donation program, who meet all inclusion criteria and none of the exclusion criteria and who agree to participate in the study by signing the informed consent will be included.

(*) In the donation program at the Next Fertility centers, healthy female donors under 35 years of age with an ultrasound antral follicle count ≥15 follicles are considered normo-responder patients.

6.2. INCLUSION CRITERIA

1. Women undergoing COH for egg donation cycles
2. Signing of Informed Consent

3. Age 18-34 years
4. Body mass index between 18-29.9 kg/m^2
5. Meet the criteria for inclusion in the oocyte donation program of the IMER-Next Fertility center
6. Ultrasound antral follicle count before COH of ≥15 follicles

6.3. EXCLUSION CRITERIA

1. Patients excluded from the oocyte donation program
2. Untreated sexually transmitted disease, HIV, HCV, HBV positive serology
3. Blood disorders, neurodegenerative/psychiatric diseases, fragile X syndrome, oncological diseases, endometriosis.
4. Polycystic ovary syndrome
5. Oligomenorrhea (<1 menstrual cycle in 3 months)
6. Diabetic patients

6.4. PLANNED NUMBER OF SUBJECTS

A total of 210 patients are planned, n=105 patients in Group A (cases) and n=105 patients in Group B (controls).

6.5. RATIONALE FOR SAMPLE SIZE

Based on previous studies, we know that the mean number of mature oocytes retrieved in the study population is approximately 16.9 ± 7.7. Considering this study as a non-inferiority versus placebo study, and defining the non-inferiority margin as 3 mature oocytes, assuming an SD of 7.7 points, a statistical power of 80% and an alpha of 0.025 for a one-sided test, 105 patients per treatment group would be required, and thus a total of 210 patients would be required to conduct the study.

6.6. STUDY COMPLETION OR STOPPING CRITERIA

Subjects will be withdrawn from the study at their own request or at the discretion of the investigator if one of the following situations occurs:

1. **Voluntary withdrawal from the study by the patient**: The patient may voluntarily withdraw from the study at any time for any reason, without this affecting the treatment she should receive.
2. **Voluntary withdrawal from the donation program by the patient**: If the patient decides to withdraw from the donation program by her own decision and for any reason, she will be withdrawn from the study.
3. **Cancellation of the oocyte donation program** due to medical criteria (program cancellation criteria)
4. At **the investigator's medical discretion**: The investigator may withdraw any subject at his/her discretion for safety reasons, protocol deviations, non-adherence or for administrative reasons.
5. Any intercurrent **active or unstable disease** that may interfere with the conduct of the study or that may affect the development or outcome of the oocyte donation program.

6. Occurrence of any **Serious Adverse Effect** (the "*SAE Form*" should be completed and reported to the study monitor and sponsor within 3 days of its identification.
7. For **non-adherence**: patients who do not comply with the administration of the study treatment (less than 80% of the prescribed doses or less than 80% of the treatment days).
8. **Protocol breach**. Violation of the restrictions described or non-compliance with any of the inclusion criteria or occurrence of exclusion criteria throughout the study.

Subjects excluded or withdrawn from the study for any of the above reasons will not be replaced.

7. SOURCE OF INFORMATION

Data will be collected from the patient's clinical history, using physician-patient interviews and instrumental tests (ultrasound, oocyte study, blood tests). All information will be recorded in the patient's clinical history, in addition to the paper Case Report Form.

8. STUDY CONDUCT

8.1. SUBJECT RECRUITMENT

On the day the patient enters the oocyte donation program, she will be assessed to verify that she meets the inclusion criteria for the study. She will be informed about the study and asked to sign a written informed consent to be enrolled in it.

No extra compensation will be given to oocyte donors who accept to participate in the study.

Once the informed consent has been signed, she will be given a white, no-label, numerated blister and assigned the corresponding enrolment number.

8.2. PARTICIPANT FOLLOW-UP

Patients will be followed up for up to 45 days (+3 days), with data recorded in 5 control visits:

- Control 1 – Screening and Inclusion Visit – Day 1.
- Control 2 – COH Start Visit – Day 30 ± 2.
- Control 3 – Ultrasound Monitoring of Follicular Development Visit – Day 7 of stimulation
- Control 4 – End of COH and Trigger Visit – Day 40 (±3 days).
- Control 5 – End of Study Visit – Day of Follicular Puncture (42 ± 3 days).

Control 1 - Screening and Inclusion Visit (Day 1):

- Submission of the Patient Information Sheet (PIS), patient information on the study and signing of the Informed Consent Form (ICF).
- Review of inclusion and exclusion criteria.
- Assignment of the patient code
- Recording of demographic data (age, weight, height, BMI, toxic habits)

- Recording of clinical data: number of previous COH cycles for donation, number of living children
- Blood tests
- Ultrasound for antral follicle count
- Dispensing of treatment assigned per patient code
- Scheduling, with the patient, the next visit (approximately 30 days later) for the start of the COH. The patient will call the site on the first day of menstruation to confirm a visit.

Control 2 – COH Start Visit (Day 30 ± 2).

- Treatment compliance with the study supplement
- Recording of potential supplement-related effects or adverse events
- Confirmation of criteria to start COH
- Prescribing treatment for COH as per site protocol
- Dispensing of last box of study treatment (supplement/placebo) with patient code

Control 3 – Ultrasound Monitoring of Follicular Development Visit – Day 7 of Stimulation (Day 37±2).

- Treatment compliance
- Recording of potential supplement-related effects or adverse events
- Ultrasound findings: number of follicles of different sizes (10 mm, 14 mm, 16 mm, 20 mm, etc.)

Control 4 – End of COH and Trigger Administration Visit– (Day 40 ± 3 days).

- Treatment compliance
- Ultrasound findings: number of follicles of different sizes (10 mm, 14 mm, 16 mm, 20 mm, etc.)
- Confirmation of criteria to finalize COH: no less than 9 days of ovarian stimulation and presence of at least 10 follicles larger than 15 mm with at least 4 follicles with a diameter ≥20 mm
- Recording of day of GnRH-agonist trigger administration
- End of treatment with the study supplement and collection of packaging
- Recording of potential supplement-related effects or adverse events
- Patient evaluation of the study supplement (organoleptic properties, tolerability)
- Scheduling of the day of puncture

Control 5 – End of Study Visit – Day of Follicular Puncture (42 ± 3 days).

- Oocyte retrieval count
- Oocyte quality assessment and classification

- Final destination of oocytes
- Recording of potential adverse effects or events
- Study completion data

9. TREATMENT DESCRIPTION

9.1. TREATMENT DESCRIPTION

- **Study dietary supplement: Seidivid Ferty4®.** The study supplement consists of a multivitamin compound containing myo-inositol, D-chiro-inositol, coenzyme Q10, melatonin and vitamin D, which help in the production of oocytes and contribute to their maturation. It also incorporates methylated folate (Quatrefolic®) and micronutrients (iron, iodine, zinc), as well as probiotics of the endometrial microbiota (Lactobacillus gasseri and Lactobacillus crispatus). It is available as capsules with the following composition:

  Myo-inositol.....1 g
  1D-chiro-inositol .....25 mg
  Melatonin .....1,9 mg
  Folic acid (Quatrefolic®)..... 400 µg
  Vitamin D3 .....25 µg
  Co-enzyme Q10.....100 mg
  Vitamin B12 .....2,5 µg
  Vitamin B6 .....1,4 mg
  Vitamin E .....12 mg
  Iron (Fe) .....28 mg
  Iodine (I) ..... 200 µg
  Zinc (Zn) .....15 mg
  L. crispatus ..... 1x109 UFC
  L. gasseri ..... 2x109 UFC

- **Placebo.** The placebo is supplied as capsules whose external appearance is identical to that of the study supplement. The placebo consists of:

  Microcrystalline cellulose PH 102 (E-460) ..... 450 mg

9.2. TREATMENT DOSAGE

Patients will start treatment on the day of study inclusion and will continue treatment until the GnRH-agonist (GnRH-a) trigger is administered at the end of the COH.

The dosing regimen will be 2 capsules per day, as a single dose, together with a glass of water (150 ml). They can be taken at any time of the day, but it is recommended that the daily dose be taken before or during one of the main meals so that the subject does not forget to take it.

9.3. PACKAGING AND LABELING OF THE TREATMENT

All study products will be supplied by the sponsor. The study multivitamin product with probiotics (Seidivid Ferty4®) and placebo will be supplied as capsules with an identical external appearance packaged in blisters in a white box with no label, marked only with the study code and patient code.

Labeling will be performed according to the predetermined randomization list generated automatically by CROSSDATA prior to study start, using IBM SPSS Statistics software.

9.4. TREATMENT ASSIGNMENT

Treatment assignment will be performed once the patient has been enrolled in the study. After signing the informed consent, the investigator will assign a sequential patient code number in order of inclusion. The codes will start with number 01 for the first patient, number 02 for the second patient, number 03 for the third patient and so on. The investigator will then give the patient the box of medication assigned to her code and explain the dosing regimen and treatment duration to the patient.

It is the responsibility of the investigator to dispense study treatments according to the randomization code.

9.5. STORAGE AND COUNTING

Records and inventory of clinical research treatments must be kept by the investigator according to the following standards:

1. The investigator must store the clinical research products in the pharmacy or store them in a secure place at room temperature, accessible only to persons authorized to dispense the investigational products.
2. Inventory will be performed by the investigator or another person designated by the investigator. It will include details of the material received, as well as clearly indicating when and to which subject it was dispensed.
3. At the end of the clinical research, the investigator must return all materials and the rest of the containers to the sponsor, regardless of if they are empty or if they still contain clinical research product.
4. The investigator agrees not to supply the clinical research products to any person other than his/her research team and the study participants.

10. OUTCOME VARIABLES

10.1. PRIMARY VARIABLES

- Total number of oocytes retrieved in the follicular puncture
- Number of mature (MII) oocytes retrieved in the follicular puncture

10.2. SECONDARY VARIABLES

- Oocyte quality variables: oocyte morphology will be evaluated following the standard classification system established at IMER Next Fertility, according to previously published criteria [17,18].

  - Rate of normal or good quality oocytes
  - Rate of oocytes with dimorphisms
  - Rate of oocytes with intra-cytoplasmic and extra-cytoplasmic dimorphisms
  - Percentage of oocytes with intra-cytoplasmic dimorphisms with each of the following changes:
    - Cytoplasmic inclusions
    - Refractile bodies
    - Vacuoles
    - Aggregation of smooth endoplasmic reticulum
    - Dense granulation

  - Percentage of oocytes with extra-cytoplasmic dimorphisms with each of the following changes:
    - Alterations of the first polar corpuscle
    - Changes in size and granularity of the perivitelline space
    - Defects and abnormalities in the shape of the zona pellucida
- Biochemical and hormonal profile variables: results of blood tests at the time of study inclusion and at the time of starting COH:
  - Fasting glucose
  - AST
  - ALT
  - Cholesterol
  - Baseline LH (only at time of COH start)
- Sociodemographic and clinical variables:
  - Age (years)
  - Weight (kg)
  - BMI (kg/m^2)
  - Toxic habits
  - Number of previous COH cycles for egg donation
  - No. of living children
- Antral follicles:
  - Total number of antral follicles at study inclusion
  - Total number of antral follicles at the time of COH start
  - Total number of follicles at the time of trigger administration
  - Number of follicles of each size at the time of trigger administration

11. ENDPOINTS

11.1. PRIMARY ENDPOINTS

- Raw and percent difference in total number of oocytes retrieved between the study group and the control group
- Raw and percent difference in the number of mature MII oocytes between the study group and the control group
- Raw and percent difference in the percentage of mature MII oocytes between the study group and the control group

11.2. SECONDARY ENDPOINTS

- Difference between both groups in the percentage of normal or good quality oocytes and oocytes with dimorphisms obtained at puncture
- Difference between both groups in the percentage of oocytes with intra-cytoplasmic and extra-cytoplasmic dimorphisms
- Difference between both groups in the percentage of oocytes with intra-cytoplasmic dimorphisms with each of the described alterations
- Difference between both groups in the percentage of oocytes with extra-cytoplasmic dimorphisms with each of the described alterations
- Changes in levels of biochemical parameters between the time of inclusion and when starting COH, in both groups
- Differences in LH hormone levels when starting COH between both groups.

12. STATISTICAL ANALYSIS

All patients meeting the screening criteria will be included in the analysis, and the patients considered not evaluable for the analysis and the reason for their exclusion will be listed. The study variables will be described. Absolute and relative frequency distributions of qualitative variables, as well as measures of central tendency and dispersion (mean, standard deviation, median, minimum and maximum) of quantitative variables will be presented. The 95% confidence intervals (CIs) will be presented for the primary outcome variables associated with the primary objective and the primary and secondary variables.

Missing data will not be imputed and will be left as lost. Subgroup analyses will not be defined a priori; if any particular subgroup of patients is of interest, sub-analyses may be performed post-hoc for these groups in line with the analyses proposed for the general population.

SAS System software, version 9.4, will be used for analysis. When an inferential analysis is required, parametric tests will be used for continuous variables, and non-parametric tests will be used for ordinal, categorical or non-parametric variables. For variables with a normal (or parametric) distribution, t-tests will be used to compare paired data. Mann-Whitney hypothesis tests will be performed for those not fitting a normal (non-parametric) distribution. Contingency tables and comparison of proportions and/or frequency distributions will be analyzed using the chi-squared test (or Fisher's exact test where appropriate).

12.1. PRIMARY ENDPOINTS

- Raw and percent difference in the total number of oocytes retrieved between the study group and the control group: This objective will be assessed using the Mann-Whitney U test. This evaluation will be performed under the non-inferiority hypothesis
- Raw and percent difference in the number of mature MII oocytes between the study group and the control group: : This objective will be assessed using the Mann-Whitney U test in the event of a raw difference, and the Student’s t-test for the percentage assessment. This evaluation will be performed under the non-inferiority hypothesis
- Raw and percent difference in the percentage of mature MII oocytes between the study group and the control group: This objective will be assessed using the Mann-Whitney U test in the event of a raw

difference, and the Student's t-test for the percentage assessment. This evaluation will be performed under the non-inferiority hypothesis

12.2. SECONDARY ENDPOINTS

- Difference between both groups in the percentage of normal or good quality oocytes and oocytes with dimorphisms obtained at puncture: This objective will be assessed using a Student's t-test. This evaluation will be performed under the non-inferiority hypothesis
- Difference between both groups in the percentage of oocytes with intra-cytoplasmic and extra-cytoplasmic dimorphisms: This objective will be assessed using a Student's t-test. This evaluation will be performed under the non-inferiority hypothesis
- Difference between both groups in the percentage of oocytes with intra-cytoplasmic dimorphisms with each of the described changes: This objective will be assessed using a Student's t-test. This evaluation will be performed under the non-inferiority hypothesis
- Difference between both groups in the percentage of oocytes with extra-cytoplasmic dimorphisms with each of the changes described: This objective will be assessed using a Student's t-test. This evaluation will be performed under the non-inferiority hypothesis
- Changes in the levels of biochemical parameters between the time of inclusion and the time of starting COH, in both groups: This objective will be assessed using a Student's t-test, assessing the differences between the time of starting COH and the time of inclusion by treatment group.
- Differences in LH hormone levels at the time of starting COH between both groups: This objective will be assessed using a Student's t-test.

13. ADVERSE EVENT REPORTING

All adverse effects and events experienced by patients during their time in the study will be recorded in the Case Report Form, specifying:

- Type of reaction
- Severity: mild (no limitation of usual activities; patient may experience slight discomfort), moderate (some limitation of usual activities; patient may experience greater discomfort), severe (inability to carry out usual activities; patient may experience intolerable pain or discomfort).
- Duration
- Causal relationship with the treatment with the study supplement: definite, probable, possible, unlikely, unrelated, unknown.
- Treatment administered
- Resolution

For an adverse reaction considered serious (see attached box), the physician must complete a specific form (Annex 6) with all available data and report it immediately (if possible, within 24-48 h) by phone or email to the sponsor and CRO.

Sponsor Contact:

Responsible Person: Andrea Martinez

Phone +34938445730

Email: farmacovigilancia@lab-seid.com

CRO Contact:

Montse Vidal (CROSSDATA – PUNTA ALTA)

Phone +34 663 825 890

Email: montsevidal@crossdata.es

SERIOUS ADVERSE REACTION: any adverse reaction that can be classified into one or more of the following categories:

- Fatal
- Life-threatening
- Results in persistent or significant disability/incapacity
- Results in hospitalization or prolongation of existing hospitalization

It also includes congenital anomalies/birth defects and serious adverse clinical consequences associated with use under conditions other than those laid down in the Summary of Product Characteristics (SmPC), overdose or abuse.

Medical discretion must be used when deciding if an event or reaction is serious in other situations. Significant reactions or adverse events that are not immediately life-threatening or do not result in death or hospitalization, but that could endanger the patient, should be considered serious.

In the event of the appearance of a serious or unexpected adverse reaction related to the use of the drugs administered for controlled ovarian hyperstimulation or trigger (rFSH or uFSH or hMG or a combination, medroxyprogesterone acetate, triptorelin acetate), the physician must report it to the authorities, following the current pharmacovigilance regulations with the spontaneous reporting of suspected adverse drug reactions. Reporting shall be effected using the online or paper Yellow Card, which shall be sent to the regional pharmacovigilance center of the Valencian Community (http://www.san.gva.es/web/dgfps/farmacovigilancia).

14. ETHICAL CONSIDERATIONS

14.1. GENERAL CONSIDERATIONS

This study will be conducted in accordance with current regulations, accepted international ethical standards of Good Clinical Practice (CPMP/ICH/135/95), the principles laid down in the latest version of the Declaration of Helsinki and *Ley 14/2007, de 3 de julio, de Investigación biomédica* [the Biomedical Research Act].

14.2. EVALUATION BY A RESEARCH ETHICS COMMITTEE

Before study start, it will be submitted for the consideration of the Medicinal Product Research Ethics Committee of Hospital General Universitario de Valencia for approval.

14.3. RISK-BENEFIT ASSESSMENT FOR RESEARCH SUBJECTS

The study product consists of a multivitamin supplement with probiotics whose components have been tested and studied on multiple occasions. All are considered safe substances when administered at controlled doses, so no serious side effects are expected. In addition, the multivitamin compound with probiotics under study is a marketed product that is currently used in standard clinical practice and for which no serious or unexpected side effects have been reported.

It is important to note that there will be no change in the usual medication or drug treatment regimen prescribed according to the oocyte donation program. Furthermore, by participating, no laboratory tests or analyses other than those usually forming part of the donation program will be performed.

Therefore, participation in this study is not expected to increase the risk of developing undesirable effects or inconveniences beyond those considered to be commonly associated with controlled ovarian stimulation within the donation program.

In contrast, the components of the supplement have been associated with better outcomes in assisted reproduction programs, so patients can be expected to show some improvement in the outcome of the egg donation program.

14.4. PATIENT INFORMATION SHEET AND INFORMED CONSENT

Before inclusion in the study, the physician will have the obligation to inform the patient of the different aspects of the study for her information, and written informed consent must be signed by the patient for her participation in the study.

14.5. DATA CONFIDENTIALITY

Confidentiality will be maintained throughout the study, and data referring to each participant will be transmitted, if necessary, in encrypted form.

The identity of the participant will be coded in the study forms. The investigator physician will identify the subjects at the Inclusion Visit (Day 1) using the assigned code. The forms will not include any personal data that could be used to identify the participants once the study is completed, thus guaranteeing the confidentiality of the clinical data recorded. The investigator physician will keep a separate confidential record linking the identification codes with the name of the participants and an identification number (National ID number or clinical history number). The data collected will be processed confidentially in accordance with the General Data Protection Regulation [GDPR (EU) 2016/679] and the *Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales* [the Spanish Personal Data Protection and Digital Rights Guarantee Act]. According to this law, the personal data required from the patients are those necessary to cover the objectives of the study for which the patient gives his/her consent.

The investigator and the sponsor will be responsible for data processing and undertake to comply with the applicable data protection regulations. The data collected for the study will be identified with a code and will not include information that could identify the patient. Only the study investigator and his/her collaborators will be able to link this data to the participant and her clinical history. Patient identity will therefore not be revealed to any other person except if required by the health authorities or in case of medical emergency. Access to personal information will be restricted to the clinical research ethics committees, inspectors from

the health authority and authorized staff from the sponsor to verify personal data, the study procedures and compliance with the legislation and good clinical practice standards (maintaining the confidentiality of the information at all times).

14.6. COMPENSATION FOR DAMAGES

This study meets the criteria for a low-intervention clinical trial described in Royal Decree 1090/2015 of 4 December 2015, regulating clinical trials with medicinal products,

- The investigational dietary supplement, excluding the placebo, is approved
- The investigational dietary supplement is used in accordance with the terms of the marketing authorization,
- The use of multivitamin supplements and probiotics is supported by published scientific evidence on the safety and efficacy of these products
- Additional diagnostic or follow-up procedures do not pose an additional risk or burden to subject safety compared to the standard clinical practice of the egg donation protocol of the Institute of Reproductive Medicine Next Fertility IMER-Valencia.

For this reason, the sponsor will assume responsibility and will ensure that the trial subject is compensated for any damages suffered as a result of the trial.

15. RESOURCES AND FUNDING

15.1. RESOURCES

The investigators will conduct this study at the facilities of the Institute of Reproductive Medicine Next Fertility IMER-Valencia. Patients admitted to the oocyte donation program who meet the inclusion and exclusion criteria of this study, who have been informed of the characteristics and objectives of the study and who agree to participate by signing the informed consent will be selected.

The sponsor will be responsible for providing the study treatment (multivitamin supplement with probiotics and placebo) free of charge in an amount sufficient to treat 210 patients during the maximum 45 days of the study.

15.2. FUNDING

This study will be funded by the sponsor SEID, S.A. The sponsor (SEID, S.A.) will cover all expenses generated by the study, including: the treatment, the cost of the instrumental tests and the investigators' fees.

SEID, S.A. will provide the study treatment (multivitamin supplement and placebo) in a sufficient amount so that all participants can complete the study period. No extra compensation will be given to oocyte donors who agree to participate in the study.

16. PUBLICATION AND DISSEMINATION

The results obtained as a result of this clinical research will be reviewed and discussed by the research team and the sponsor for subsequent publication.

After obtaining the conclusions of the study, the research team will prepare a final report. This report will include the statistical analysis and a medical assessment of the results. This report will be based on the objectives stated in the study protocol.

Once approved by the Medicinal Product Research Ethics Committee, the study will be registered in the international ClinicalTrials.gov registry.

Once the study is completed, at least one manuscript will be prepared with the results obtained for publication in a Biomedical journal.

17. REFERENCES

[1] S. K. Arhin, Y. Zhao, X. Lu, M. Chetry, and J. Lu, "Effect of micronutrient supplementation on IVF outcomes: a systematic review of the literature," Reprod. Biomed. Online, vol. 35, no. 6, pp. 715–722, Dec. 2017, doi: 10.1016/j.rbmo.2017.08.018.

[2] I. M. W. Ebisch, C. M. G. Thomas, W. H. M. Peters, D. D. M. Braat, and R. P. M. Steegers-Theunissen, "The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility," Human Reproduction Update, vol. 13, no. 2. Oxford Academic, pp. 163–174, Mar. 01, 2007, doi: 10.1093/humupd/dml054.

[3] M. G. Showell, R. Mackenzie-Proctor, V. Jordan, and R. J. Hart, "Antioxidants for female subfertility," Cochrane Database Syst. Rev., vol. 2020, no. 8, 2020, doi: 10.1002/14651858.CD007807.pub4.

[4] P. Skowrońska, M. Kunicki, E. Pastuszek, L. Konieczna, T. Bączek, and K. Łukaszuk, "Follicular fat-soluble vitamins as markers of oocyte competency," Syst. Biol. Reprod. Med., vol. 66, no. 2, pp. 112–121, Mar. 2020, doi: 10.1080/19396368.2020.1718244.

[5] A. Kazemi, F. Ramezanzadeh, and M. H. Nasr-Esfahani, "The relations between dietary antioxidant vitamins intake and oxidative stress in follicular fluid and ART outcomes.," Iran. J. Reprod. Med., vol. 13, no. 9, pp. 533–40, Sep. 2015, Accessed: Mar. 18, 2021. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/26568757.

[6] A. J. D. Gaskins et al., "Dietary Folate and Reproductive Success Among Women Undergoing Assisted Reproduction," Obs. Gynecol, vol. 124, no. 4, pp. 801–809, 2014, doi: 10.1097/AOG.0000000000000477.

[7] L. Bianchi et al., "Protein pathways working in human follicular fluid: The future for tailored IVF?," Expert Reviews in Molecular Medicine, vol. 18. Cambridge University Press, Jan. 18, 2016, doi: 10.1017/erm.2016.4.

[8] L. A. Pham-Huy, H. He, and C. Pham-Huy, "Free radicals, antioxidants in disease and health," International Journal of Biomedical Science, vol. 4, no. 2. Master Publishing Group, pp. 89–96, Jun. 2008, Accessed: Mar. 18, 2021. [Online]. Available: www.ijbs.org.

[9] A. M. James, R. A. J. Smith, and M. P. Murphy, "Antioxidant and prooxidant properties of mitochondrial Coenzyme Q," Archives of Biochemistry and Biophysics, vol. 423, no. 1. Academic Press Inc., pp. 47–56, Mar. 01, 2004, doi: 10.1016/j.abb.2003.12.025.

[10] M. V. Miles et al., "Age-related changes in plasma coenzyme Q10 concentrations and redox state in apparently healthy children and adults," Clin. Chim. Acta, vol. 347, no. 1–2, pp. 139–144, Sep. 2004, doi: 10.1016/j.cccn.2004.04.003.

[11] P. Florou, P. Anagnostis, P. Theocharis, M. Chourdakis, and D. G. Goulis, "Does coenzyme Q10 supplementation improve fertility outcomes in women undergoing assisted reproductive technology procedures? A systematic review and meta-analysis of randomized-controlled trials," Journal of Assisted Reproduction and Genetics, vol. 37, no. 10. Springer, pp. 2377–2387, Oct. 01, 2020, doi: 10.1007/s10815-020-01906-3.

[12] T. T. Y. Chiu, M. S. Rogers, E. L. K. Law, C. M. Briton-Jones, L. P. Cheung, and C. J. Haines, "Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: Relationship with oocyte quality," Hum. Reprod., vol. 17, no. 6, pp. 1591–1596, 2002, doi: 10.1093/humrep/17.6.1591.

[13] P. Merviel et al., "Impact of myo-inositol treatment in women with polycystic ovary syndrome in assisted reproductive technologies," Reproductive Health, vol. 18, no. 1. BioMed Central Ltd, Dec. 01, 2021, doi: 10.1186/s12978-021-01073-3.

[14] F. Lisi, "Pretreatment with myo-inositol in patients undergoing gonadotropins multiple follicular stimulation for IVF," Acta Medica Int., vol. 3, no. 1, p. 8, 2016, doi: 10.5530/ami.2016.1.4.

[15] Y. Ménézo et al., "Zinc concentrations in serum and follicular fluid during ovarian stimulation and expression of Zn 2+ transporters in human oocytes and cumulus cells," 2011, doi: 10.1016/j.rbmo.2011.03.015.

[16] A. Pacchiarotti, G. Carlomagno, G. Antonini, and A. Pacchiarotti, "Effect of myo-inositol and melatonin versus myo-inositol, in a randomized controlled trial, for improving in vitro fertilization of patients with polycystic ovarian syndrome," Gynecol. Endocrinol., vol. 32, no. 1, pp. 69–73, Jan. 2016, doi: 10.3109/09513590.2015.1101444.

[17] Mikkelsen AL, Lindenberg S. Morphology of in-vitro matured oocytes: impact on fertility potential and embryo quality. Hum Reprod. 2001 Aug;16(8):1714-8. doi: 10.1093/humrep/16.8.1714.

[18] Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, Sapienza F, Baroni E, Litwicka K, Greco E. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril. 2008 Nov;90(5):1692-700. doi: 10.1016/j.fertnstert.2007.09.024.