STUDY PROTOCOL

Impact of Exercise and Mediterranean diet *vs* Aspirin on live-birth rate and cardiovascular programming in *In Vitro* Fertilization (MEDITATE-IVF): a Randomized Study.

Protocol version	Version: 2. Date: 08/06/2021
Protocol code	IIBSP-EDA-2020-18
EudraCT	2020-001069-35
Acronym	MEDITATE-IVF
Promoter	Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau – IIB Sant Pau Hospital de la Santa Creu i Sant Pau c/ Sant Quintí, 77-79 08041 Barcelona Tel: 935537869 Fax: 935537812
Principal investigators	Dra. Mónica Cruz Lemini (PI)/ Dra. Ana Polo Ramos (Co-PI) Obstetrics and Gynecology Department Hospital de la Santa Creu i Sant Pau c/ Sant Antoni Maria Claret, 167 08025 Barcelona. Tel. 932919000 Ext. 7041 E-mail: mcruzl@santpau.cat / apolo@santpau.cat
Responsible for Monitoring	UICEC Sant Pau - Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau C/ Sant Antoni Maria Claret, 167 08025 Barcelona Tel.: 935537635 Fax: 935537812
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COMPLIANCE WITH GOOD CLINICAL PRACTICE

This trial is designed to comply with Section ICH E6 (R2) Guidelines for Good Clinical Practice, as has been implemented in the European Union, on June 14th, 2017 (Directive EMA/CHMP/ICH/135/1995); directive on clinical trials Nº 536/2014 of the European Parliament, and the council of April 16th, 2014; Royal Decree 1090/2015 on clinical trials with drugs, the Ethics committees for drug research and the Spanish registry of clinical trials; and local regulatory requirements.

1. Summary

1.1. Identificación del ensayo	EudraCT: 2020-001069-35 Código del protocolo: IIBSP-EDA-2020-18 Versión: 2. Fecha: 03/06/2021
1.2 Título del ensayo clínico	Impacto del ejercicio y la dieta Mediterránea *vs* aspirina en la tasa de nacimientos vivos y programación cardiovascular en la fertilización *in vitro*: un estudio aleatorizado (MEDITATE-IVF).
1.3. Identificación del promotor	Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau – IIB Sant Pau c/ Sant Quintí, 77-79 08041 Barcelona Tel: 935537869 Fax: 935537812
1.3.1. Responsable de la monitorización	UICEC Sant Pau - Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau C/ Sant Antoni Maria Claret, 167 08025 Barcelona Tel.: 935537635 Fax: 935537812
1.4. Fuente de financiación	Instituto de Salud Carlos III, Accion Estratégica en Salud, Proyectos de Investigación en Salud, PI20/001504
1.5. Investigador coordinador o principal del estudio	Dra. Mónica Cruz Lemini (IP) / Dra. Ana Polo Ramos (Co-IP) Departamento de Ginecología y Obstetricia Hospital de la Santa Creu i Sant Pau c/ Sant Antoni Maria Claret, 167 08025 Barcelona Tel. 932919000 Ext. 7041 E-mail: mcruzl@santpau.cat / apolo@santpau.cat
1.6. CEIC de referencia	Comité Ético de Investigación Clínica del Hospital de la Santa Creu i Sant Pau.
1.7. Centros donde se prevé realizar el ensayo	Departamento de Medicina Reproductiva y Departamento de Obstericia y Ginecología, Hospital de la Santa Creu i Sant Pau, Barcelona.
1.8. Justificación y pertinencia del estudio	Este estudio se centra en la población infértil en espera de un procedimiento de fertilización *in vitro* (FIV), que aumenta continuamente en los países desarrollados. Algunos estudios han observado una mejoría en la tasa de embarazo en mujeres obesas con dieta y ejercicio, pero no existen datos en mujeres con peso normal y no hay datos sobre la administración de aspirina en la población infértil con respecto a los resultados perinatales. Ningún estudio ha analizado el impacto de la dieta Mediterránea y el ejercicio, o las intervenciones con aspirina, en mujeres que desean quedarse embarazadas. Suponemos que los factores de riesgo cardiovascular (CV) y metabólicos previos son determinantes del éxito de la FIV y los resultados del embarazo en mujeres infértiles, y que la intervención

	mostrará una mejora en la tasa acumulada de nacidos vivos, así como una disminución en los resultados perinatales adversos (hipótesis de superioridad). Nuestro estudio difiere de datos anteriores, ya que planteamos la hipótesis de que la aspirina puede mejorar los perfiles cardiovasculares e inflamatorios maternos, y que sus efectos crearán un estado de salud más favorable para la implantación y el embarazo. Planeamos administrar 6 meses antes de la FIV, 150 mg de aspirina al día, la dosis que se ha demostrado que disminuye la incidencia de preeclampsia cuando se administra entre las 11 y 36 semanas de gestación. Los resultados de este estudio evidenciarán la necesidad de cambiar los hábitos de las mujeres que se someten a estas técnicas, siendo los resultados transferibles a otras mujeres con deseo gestacional. Esperamos proporcionar evidencia que respalde el uso de estas intervenciones para mejorar las tasas de nacidos vivos en la población infértil, así como demostrar una menor incidencia de perfil angiogénico anormal, disfunción cardíaca y remodelación en la madre y el feto/niño, identificando opciones terapéuticas para el future, así como dando pie a estudios destinados a la prevención de la morbilidad CV en otras poblaciones y la morbilidad asociada con la preeclampsia en etapas posteriores de la vida. El objetivo final es mejorar las tasas de nacidos vivos con mujeres y niños sanos, reduciendo la carga que supone la enfermedad CV.
1.9. Diseño del Estudio. Fase del Estudio	Este será un ensayo clínico aleatorizado y controlado con placebo y grupos paralelos. La aleatorización será proporcionada por el Departamento de Estadística (I. Gich) que tendrá la secuencia completa para los 3 grupos. La asignación será en el momento de la inclusion; una vez que se verifiquen los criterios de inclusion, los médicos generarán la identificación de la paciente y programarán la primera visita correspondiente. Debido a la naturaleza de la intervención dietética, no es posible cegar a los participantes o médicos por completo, por lo que estarán cegados solo a la intervención de aspirina *vs* placebo.
1.10. Objetivo Principal	Evaluar el impacto del ejercicio y la dieta Mediterránea *vs* la aspirina, o la no intervención, previo a la fertilización *in vitro*, sobre la tasa acumulada de nacidos vivos.
1.11. Fármacos experimentales y control	Los pacientes serán asignados al azar a uno de los siguientes grupos de intervención, 6 meses antes del tratamiento de fertilización *in vitro*: 1. Ejercicio y dieta mediterránea: estrategias nutricionales y de ejercicio personalizadas durante los 6 meses previos al procedimiento de FIV, basadas en lo siguiente: • Programa de ejercicio físico controlado objetivamente mediante un contador de pasos proporcionado, y seguimiento por un

	experto en educación física. • Dieta Mediterránea, con suplementación de aceite de oliva virgen extra, frutos secos, medidas dietéticas recomendadas, y seguimiento nutricional por un Dietista. 2. Profilaxis con aspirina: suplementación con 150 mg de aspirina vía oral (ácido acetilsalicílico) cada 24 horas al acostarse. Se hará un seguimiento de las pacientes a los 2, 4 y 6 meses para evaluar el cumplimiento de la medicación. 3. Grupo de no intervención (placebo): tratamiento de fecundación *in vitro* 6 meses después de la inclusión, sin ninguna de las intervenciones mencionadas anteriormente. Las pacientes recibirán un placebo fabricado para que coincida con la cápsula de aspirina, con las mismas indicaciones de administración. Se hará un seguimiento de las pacientes a los 2, 4 y 6 meses para evaluar el cumplimiento de la medicación. Las intervenciones se suspenderán tras la realización de la prueba de β-hCG utilizada para confirmar el embarazo.
1.12. Variable Principal de Valoración	Tasa acumulada de recién nacidos vivos.
1.13. Población en estudio y número de pacientes	Población de estudio: mujeres infértiles en espera de un procedimiento de FIV en el Servicio de Reproducción Asistida del Hospital Santa Creu i Sant Pau de Barcelona, con infertilidad primaria que justifique el tratamiento de FIV, que deseen quedarse embarazadas, entre 18 y <38 años, que den su consentimiento por escrito y que no cumplan criterios de exclusión Las pacientes serán reclutadas por sus médicos tratantes, quienes ofrecerán el estudio a las mujeres dentro de la lista de espera para FIV. El cálculo del tamaño de la muestra se realizó en base al impacto esperado de las intervenciones, calculado como un ensayo de superioridad, hipotetizando un aumento de la tasa acumulada de nacidos vivos en los grupos de tratamiento de alrededor del 13%, con una tasa de pérdida del 10%. Como tal, para un error de tipo I (alfa) de 0.05, el error de tipo II (beta) en 0.20 y una potencia del 80%, con igual asignación a 3 grupos, el tamaño de la muestra para cada grupo se calculó de 249 sujetos; un total de 747 pacientes para el estudio. Hemos decidido redondear cada grupo a 250 pacientes**.**
1.14. Análisis Estadístico	Los análisis estadísticos se realizarán con el paquete estadístico IBM® SPSS® Statistics Versión 25 (IBM Corporation), o el software de código abierto (The R Foundation for Statistical Computing) se utilizará para todos los cálculos y la construcción de gráficos (R V2.15.1). El análisis estadístico se basará en los grupos asignados originalmente (intención de tratar). No se prevén análisis intermedios, debido al corto tiempo de la intervención y al período de tiempo hasta el nacimiento. La normalidad de la distribución de variables se evaluará

	con la prueba de Kolmogorov-Smirnov. La prueba t de Student (o prueba U de Mann-Whitney no paramétrica) y la prueba $\chi 2$ de Pearson o la prueba $\chi 2$ lineal por lineal (para tendencias entre categorías ordenadas) se realizarán para comparaciones univariadas entre grupos de variables cuantitativas o cualitativas, respectivamente. Se considerará la significancia estadística con un valor *p* por debajo de 0,05. La regresión logística también se utilizará para determinar cuáles de las variables tienen una contribución significativa para predecir los resultados perinatales adversos y de nacidos vivos.
1.15. Consideraciones Éticas	Este estudio de investigación se llevará a cabo siguiendo estrictas recomendaciones éticas internacionales para la investigación y los ensayos clínicos en humanos. Así mismo, se seguirán las normas establecidas en la Declaración de Helsinki, y se llevará a cabo según lo establecido en el protocolo y se normalizarán los procedimientos laborales que aseguren una buena práctica clínica (BPC). El (los) investigador (es) explicará al paciente o su representante legal autorizado, la naturaleza del estudio, su finalidad, procedimientos, duración estimada, posibles beneficios y riesgos relacionados con su participación, así como cualquier inconveniente que pueda implicar. Se advertirá a cada participante que su participación es voluntaria y que podrá retirarse del estudio en cualquier momento, sin que ello repercuta en su posterior tratamiento o seguimiento, ni en su relación con su médico tratante. Se ha diseñado un documento de consentimiento informado para el paciente o representante legal para este propósito, y se adjunta a este documento. El riesgo-beneficio de las intervenciones será evaluado de forma individual por los médicos tratantes y reclutadores, con el fin de establecer si el paciente cumple con los criterios de inclusión. También se informará a la paciente del seguimiento incluido en el estudio para valorar los objetivos secundarios durante el embarazo, y dispondrá de un documento de información de la paciente detallando las evaluaciones y estudios extra que ello supondrá. En este ensayo no se incluye ninguna compensación económica para los pacientes. El equipo de investigación garantizará la veracidad e integridad de todos los datos, así como todos los informes que se requieran. Los investigadores guardarán los documentos del estudio hasta al menos 25 años después de la finalización del estudio. A solicitud del monitor, auditor, CEIC o autoridad sanitaria, los investigadores dispondrán de todos los ficheros relacionados con el estudio, permitiendo el acceso directo a los datos o documentos fuente para su seguimiento, auditoría, revisión por parte del CEIC, así como la inspección del juicio por las autoridades competentes.
1.16. Duración del Tratamiento	La duración del tratamiento o la duración de la intervención es de 6 meses. La duración de la paciente en el ensayo clínico y durante los

	diferentes periodos (reclutamiento, seguimiento y cierre) variará en función del intervalo entre la intervención y el embarazo, pero será al menos 6 meses antes del embarazo y del propio embarazo (9 meses hasta el plazo), un total de 15 meses en el mejor de los casos. Este período puede extenderse o acortarse si la paciente no queda embarazada durante el primer ciclo de FIV o si hay un parto prematuro.
1.17. Evaluación de la Seguridad	Todos los eventos adversos que ocurran durante el estudio serán recopilados, registrados y evaluados. Se notificará al respectivo comité de ética sobre eventos adversos graves inesperados que puedan considerarse relacionados con el estudio. Dado que el manejo del embarazo no diferirá de la práctica actual, no se esperan riesgos adicionales para las mujeres embarazadas o los recién nacidos.
1.18. Calendario del Estudio. Duración del estudio	Inclusión del primer paciente: 01/07/2021 Inclusión del último paciente: 31/12/2022 Finalización del último paciente en el estudio: 31/03/2024 Finalización del estudio: 30/06/2024 Duración total del estudio: 3 años.

2. Index

2.1. Term glossary

AE	Adverse event
AR	Adverse reaction
ART	Assisted reproduction technologies
BMI	Body mass index
cTn	Cardiac troponins
CV	Cardiovascular
EU	European union
GCP	Good clinical practice
β-hCG	β-human chorionic gonadotropin
hs-cTn	High-sensitivity cardiac troponin I
ID	Identification
IVF	*In vitro* fertilization
IUGR	Intrauterine growth restriction
LDA	Low-dose aspirin
NTproBNP	N-terminal pro-B-type Natriuretic Peptide
OR	Odds ratio
PE	Preeclampsia
PIH	Pregnancy induced hypertension
RR	Relative risk
SGA	Small-for-gestational-age
SUAR	Serious and unexpected adverse reaction
UAR	Unexpected adverse reaction

3. General information

3.1. Study title

Title: Impact of Exercise and Mediterranean diet *vs* Aspirin on live-birth rate and cardiovascular programming in In Vitro Fertilization (MEDITATE-IVF): a Randomized Study.
Protocol code: IIBSP-EDA-2020-18
Protocol version: Version 1. Date: 12/01/2021.

3.2. Description of the study products

3.2.1. Experimental drug or medication

International Common Denomination (ICD): Aspirin, Acetylsalicylic acid.
Chemical Denomination: Acetylsalicylic acid.
Molecular form: $C_9H_8O_4$
Composition: 150 milligrams.
Pharmaceutical form: Capsule.
Dosage and administration route: 150 milligrams daily, oral administration.

3.2.2. Medication provider

Pharmacy Department, Hospital de la Santa Creu i Sant Pau – IIB Sant Pau.

3.3. Promoter

Institut de Recerca del Hospital de la Santa Creu i Sant Pau – IIB Sant Pau
C/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel: 935537869
Fax: 935537812

3.4. Responsible for monitoring

UICEC Sant Pau - Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau
C/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel.: 935537635
Fax: 935537812

3.5. Researcher information

3.5.1. Coordinating investigator

Elisa Llurba Olivé
Obstetrics and Gynecology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel. 932919000 Ext. 7041
Mobile: +34 687743699
E-mail: ellurba@santpau.cat

3.5.2. Principal investigators

Mónica Cruz Lemini (PI)
Obstetrics and Gynecology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel. 932919000 Ext. 7041
Mobile: +34 662456145
E-mail: mcruzl@santpau.cat

Ana Polo Ramos (Co-PI)
Assisted Reproduction Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 696605770
Tel. 932919000 Ext. 7041
E-mail: apolo@santpau.cat / apolo@fundacio-puigvert.es

3.5.3. Collaborating investigators

Carmen Garrido Giménez
Obstetrics and Gynecology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel. 932919000 Ext. 7041
Mobile: +34 616816056
E-mail: cgarrido@santpau.cat

Eva Magret Descamps
Obstetrics and Gynecology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel. 932919000 Ext. 7041
Mobile: +34 644482018
E-mail: emagret@santpau.cat

Cristina Trilla Solá
Obstetrics and Gynecology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel. 932919000 Ext. 7041
Mobile: +34 644482018
E-mail: ctrilla@santpau.cat

Carlos DoSouto Capel
Assisted Reproduction Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 639922171
Tel. 932919000 Ext. 7041
E-mail: cdosouto@fundacio-puigvert.es

Beatriz Alvarado Mercadal
Assisted Reproduction Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 656843487
Tel. 932919000 Ext. 7041
E-mail: balvaro@fundacio-puigvert.es

Esther López Bernal
Neonatology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 609085013
E-mail: elopez@santpau.cat

Olga Martínez Pasarell
Embriology Department
Fundació Puigvert

c/ Cartagena 340
08025 Barcelona
Mobile: +34 679359633
E-mail: omartinez@fundacio-puigvert.es

Olga Sánchez García
Sant Pau Biomedical Research Institute (IIB-Sant Pau)
c/ Sant Quintí 77-79
08041 Barcelona
Mobile: +34 627888438
E-mail: osanchez@santpau.cat

Víctor Rodrñiguez Sureda
Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)
Sant Pau Biomedical Research Institute (IIB-Sant Pau)
c/ Sant Quintí 77-79
08041 Barcelona
Mobile: +34 679124024
E-mail: victor.sureda@vhir.org

Eulalia Urgell Rull
Biochemistry Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel: +34 935537362
E-mail: eurgell@santpau.cat

Nuria Berga Garrote
Pharmacy Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel: +34 935537453
E-mail: nberga@santpau.cat

Elena Juanes Borrego
Pharmacy Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel: +34 935537453
E-mail: ejuanes@santpau.cat

Ma. Teresa Doñate Rodríguez
Cardiology Department
Hospital de la Santa Creu i Sant Pau

c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 639388190
E-mail: mdonate@santpau.cat

Alessandro Sionis Green
Cardiology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 656380920
E-mail: asionis@santpau.cat

Susana Hernández Durán
Dietetics Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 607889960
E-mail: shernandezd@santpau.cat

3.5.4. Responsible for Data Management

Mónica Cruz Lemini (PI)
Obstetrics and Gynecology Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel. 932919000 Ext. 7041
Mobile: +34 662456145
E-mail: mcruzl@santpau.cat

Ana Polo Ramos (Co-PI)
Assisted Reproduction Department
Hospital de la Santa Creu i Sant Pau
c/ Sant Antoni Maria Claret, 167
08025 Barcelona
Mobile: +34 696605770
Tel. 932919000 Ext. 7041
E-mail: apolo@santpau.cat / apolo@fundacio-puigvert.es

3.6. Estimated duration of the trial

Estimated duration for the trial is 36 months; the last patient will end follow-up a maximum of 15 months after inclusion (6 months intervention, 9 months of pregnancy).

First patient inclusion: 1/07/2021
Last patient inclusion: 31/12/2022
Last patient visit (finalization): 31/03/2024
Study end: 30/06/2024
Total length of study: 36 months (3 years).

4. Justification and objectives

4.1. Study justification

Infertility and cardiovascular/metabolic risk factors

Infertility remains a public health problem that affects 15-20% of women attempting to conceive. Although infertility is considered a non-life-threatening disease, it brings about overwhelming suffering, social, economic, psychological and physical effects. Although most pregnancies after assisted reproduction technologies (ART) are associated with a favorable outcome for the mother and infant, reports of abnormal vascular adaptation in early pregnancy and emerging maternal and perinatal pathology warrant further investigations. Very recent studies have reported an increased cardiovascular (CV) risk in the infertile population, and an increased risk associated with in vitro fertilization (IVF).

Lifestyle factors including diet, smoking, exercise and stress affect reproductive performance, also during ART. Several recent reports have suggested that preconception dietary habits may influence IVF outcomes, such as oocyte and embryo quality, implantation and successful completion of pregnancy. There are also some epidemiological studies considering nutrition in the light of a more holistic approach that focuses on the role of dietary patterns rather than individual nutrients, foods or groups, as this approach probably better reflects long-term eating habits and behaviors. There is clear evidence that obesity has negative effects on both general and reproductive health. Natural fertility is reduced in obese couples. We recently have shown that a 12-week diet and exercise program can lead to a significant reduction in total weight and visceral adiposity and to more favorable IVF outcomes in obese women. Clark et al. (1998) suggested that reductions in body mass index values of just 5–10%, without necessarily reaching normal weight, have positive effects on reproduction. Detrimental lifestyle habits have been indicated as potential causes of reduced fertility. Recently studies have suggested an association between Mediterranean diet and increased live birth rates after ART, especially in women under 35 years of age. However, the issue remains under debate. Highly processed meat intake is negatively associated with fertilization, implantation and pregnancy rates among couples undergoing conventional IVF. Moreover, a dietary pattern high in red meat has been negatively associated with second- and third-trimester fetal growth parameters. Recent animal data have also shown that maternal olive oil increased piglet birth weight, while reducing plasma interleukin-1β and tumor necrosis factor-α levels in the offspring.

Assisted reproduction technologies: perinatal outcomes and cardiovascular disease

There is evidence that ART are associated with an increased risk of adverse perinatal outcomes, which include a higher incidence of miscarriage, low birth weight or intrauterine growth restriction (IUGR), preterm birth, increased incidence of congenital

heart disease and other congenital malformations. Recent systematic reviews and studies also suggest pregnancies achieved by ART have an increased risk for preeclampsia and patients should be counseled with regards to these outcomes before subjecting to ART procedures. There is also debate whether maternal factors associated to infertility, such as obesity, play a role in the presence of increased complications in ART pregnancies.

Preliminary evidence has also suggested that ART may be associated with long-term CV changes in both the mother and child. Prenatal studies performed in our setting have shown that children conceived by ART have CV remodeling that is already present in prenatal stages and that persists in postnatal life. Mechanisms that produce this CV remodeling are difficult to establish, and may be conditioned by associated risks of multiple pregnancy, prematurity or growth restriction in these pregnancies; however, studies have demonstrated that there exists fetal heart remodeling independent to these conditions.

Preeclampsia and cardiovascular disease

Preeclampsia (PE) remains one of the most prevalent complications of pregnancy, ~5–7% of pregnancies in the United States, and approximately 3% in Spain. Although improved obstetrical care has significantly diminished PE-associated maternal mortality, it still remains a leading cause of peripartum morbidity, and a major cause of prematurity due to life-threatening symptoms in the mother. Severe PE is directly related to cardiac injury characterized by cardiac remodeling, decreased myocardial relaxation and global left ventricular diastolic dysfunction. In addition, it is known that CV lesions are not limited to the gestational period but evolve after delivery.

CV disease is the leading cause of death in women in the Western world. Multiple epidemiological studies have shown that PE during pregnancy is associated with the development of hypertension, myocardial infarction, cerebrovascular disease, renal complications, and vascular and metabolic dysfunction years after pregnancy. Cardiac dysfunction evidenced by echocardiography has been found even in normotensive patients with a history of PE. These studies show a 2-4 times higher risk of developing CV disease in the short and long term. When PE is accompanied by preterm birth or IUGR, the adjusted risk of CV disease 5 years postpartum increased by 45%. This effect is important and comparable to that of smoking; therefore, history of PE has recently been incorporated by the American Society of Cardiology as an independent risk factor to suggest clinical follow-up; recent data supports CV follow-up starting within the fourth decade of life.

Despite the consistent evidence regarding the relationship between placental pathology and CV complications, it is not known whether PE is a cause or the consequence of a latent CV risk that manifests as PE during pregnancy. The majority of PE cases occur in patients with an a priori low risk; moreover, the association with CV disease is higher in

those patients with severe/preterm PE, rather than with mild/late/term PE, that usually is the one associated to patients with metabolic risk.

Biomarkers for cardiovascular disease

Among CV biomarkers, there are some that have been shown associated to CV disease and may provide risk information in selected populations. Cardiac troponins (cTn) are structural proteins solely present in the myocardium, whose concentrations can increase even in subclinical states of cardiac conditions. Few studies have examined cTn in pregnant women; the combination of PE effects on the heart with the ability of detecting small cTn increases in maternal circulation, offers the possibility to assay high-sensitivity cardiac troponin I (hs-cTn) as diagnostic biomarker of PE. Finally, an increase in N-terminal pro B-type Natriuretic Peptide (NTproBNP) concentrations has been associated to increased vascular resistances and lower cardiac output in PE, suggesting ventricular stress and subclinical cardiac dysfunction.

Interventions for CV risk reduction: Mediterranean diet and exercise in ART

It is well documented in literature that weight loss can improve fertility and pregnancy outcomes, particularly in IVF. The benefits for this cohort of patients include more regular menstrual cycles, better quality embryos available for transfer, less medication dosage requirements and the need for fewer treatment cycles. In 2015, the Polo group conducted a prospective, randomized and controlled study on the impact of diet and physical exercise on the results of IVF cycles in 41 women at Sant Pau Hospital in Barcelona <38 years with a body mass index (BMI) ≥30 kg/m. Patients who performed diet and exercise for 12 weeks obtained a rate of evolutionary gestation and cumulative gestation higher than that of the women in the control group. Although weight loss has been the most used parameter in recent years, it is necessary to focus attention and aim at changes in body composition towards a more favorable phenotype that includes a decrease in pro-inflammatory abdominal fat, with consequent improvement of the endocrine metabolic state, and this requires a nutritional approach that in many cases aims to improve insulin sensitivity and a concept of metabolic flexibility. In women, the pre-pregnancy period offers a good opportunity to try to change the lifestyle, since these are especially receptive to lifestyle advice in this period of time. Most studies have been carried out in obese women, with none focusing on the general infertile population.

Aspirin prophylaxis in ART and pregnancy

Aspirin is an antiplatelet agent that exerts its effects via enhancement of prostacyclin synthesis, which in turn causes vasodilation and improves blood perfusion in many organs. Aspirin can cause this effect at low doses (75–325 mg/day). Preconception use of low-dose aspirin (LDA) has been reported to improve endometrial growth and vascularization in women undergoing IVF. Aspirin has been used to treat some reproductive outcomes in IVF; however, two meta-analyses showed that LDA had no significant effect on clinical

pregnancy rate (relative risk, RR 1.03, 95%CI 0.91–1.17) or live-birth rate (RR 0.91, 0.72–1.15) in women undergoing IVF. Results of a systematic review with less stringent quality criteria, showed that although pregnancy rates were increased with LDA (odds ratio, OR 1.19, 95%CI 1.01–1.39), live-birth rates were not affected (OR 1.08, 95%CI 0.82–1.68). However, a recent randomized control trial showed that LDA was associated with an increase of 10% in live-birth rate in women with a single, well documented pregnancy loss before 20 weeks' gestation, and was probably due to increased conception or implantation rates in women treated with LDA. A considerable amount of publications has tried to determine whether LDA can improve the outcomes of IVF, but none have assessed pregnancy outcomes. LDA before conception might positively affect downstream pregnancy outcomes during a crucial treatment window. LDA also appears to be safe and well-tolerated by women who have already become pregnant. Heterogeneity between doses of LDA is also a key factor when evaluating outcomes in previous studies, since initially doses of 81mg daily were used, and more recently 100mg. LDA at a 150mg dose is currently recommended for pregnant women at high-risk for preeclampsia, starting before 16 weeks of gestation (ideally 12 weeks) to reduce the incidence of the disease, and is deemed safe during pregnancy. Several studies, both for prevention of cardiovascular events and prevention of preeclampsia, have shown that the effect of LDA on blood pressure and platelets is closely related to the moment of administration. Administration at night, before going to bed, is associated with a greater decrease in blood pressure in normotensive and hypertensive patients, and in pregnant women at high risk of preeclampsia, in addition to a decrease in preterm delivery and fetal growth restriction. This is why most societies that recommend its use, and new studies that are carried out currently, recommend administration at bedtime.

Justification of the current study

This study focuses on infertile population awaiting an IVF procedure, which is continuously growing in high-income countries. Some studies have observed an improvement in pregnancy rates in obese women with diet and exercise, but no data exist in women with normal weight, and there is no data on the administration of LDA in the infertile population with regards to perinatal outcomes. Mediterranean diet, physical activity and LDA prophylaxis have consistently showed an improvement in CV and metabolic parameters in the general population. No studies have analyzed the impact Mediterranean diet and exercise or LDA interventions in women wishing to become pregnant.

We hypothesize that previous CV and metabolic risk factors are determinants of IVF success and pregnancy outcomes in infertile women and that intervening will show improvement in the cumulative live-birth rate, as well as a decrease in adverse perinatal outcomes. Our study differs from previous data, since we hypothesize that LDA may improve maternal CV and inflammatory profiles, and that its effects will create a more

favorable health status for implantation and pregnancy. Many previous studies used lower dosages of aspirin (i.e. 81 mg) that have not been associated with favorable perinatal outcomes; we plan to administer 6 months before IVF 150mg of aspirin daily, the dose shown to decrease the incidence of PE when administered from 11-36 weeks' gestation. Given our secondary objectives evaluating prevention of placental complications (preeclampsia, growth restriction) and improvement of parameters related to cardiovascular health of these patients, we will administer LDA at bedtime. We also plan to stop LDA intake when β-human chorionic gonadotropin (β-hCG) testing is performed after IVF, and only treat during pregnancy those women with increased risk for PE, according to our established clinical protocol (standard-of-care) based on an algorithm including maternal risk factors, uterine artery Doppler, maternal blood pressure and angiogenic markers in the first trimester of pregnancy.

The results of this study will provide evidence of the need to change habits of women undergoing these techniques, being the results transferable to other women with a gestational desire. We expect to provide evidence supporting the use of these interventions to improve live-birth rates in the infertile population, as well as demonstrate lower incidence of abnormal angiogenic profile, cardiac dysfunction and remodeling in the mother and fetus/child, identifying therapeutic options for future studies aiming at the prevention of CV morbidity in other populations and morbidity associated with PE later in life. The ultimate goal is to improve live-birth rates with healthy women and children, reducing the burden of CV disease.

Main hypothesis

The implementation of an intervention (exercise and Mediterranean diet, or LDA) 6 months on women awaiting IVF procedures will increase the cumulative live-birth rate, when compared to a non-intervention group.

Secondary hypotheses

- Maternal CV risk parameters in the intervention groups will show improvement when compared to the non-intervention group before IVF and during pregnancy.
- Placental function biomarkers will show improvement when compared to the non-intervention group during pregnancy.
- Maternal cardiac function parameters will show improvement when compared to the non-intervention group before IVF and during pregnancy.
- Fetal cardiac function parameters will show improvement when compared to the non-intervention group at the 3rd trimester of pregnancy.
- The implementation of an intervention (exercise and Mediterranean diet, or LDA) 6 months on women awaiting IVF procedures will decrease the incidence of adverse perinatal outcomes, when compared to a non-intervention group.

4.2.Study objectives

Main objective

To evaluate the impact of exercise and Mediterranean diet *vs* LDA *vs* no intervention 6 months previous to IVF, on cumulative live-birth rate.

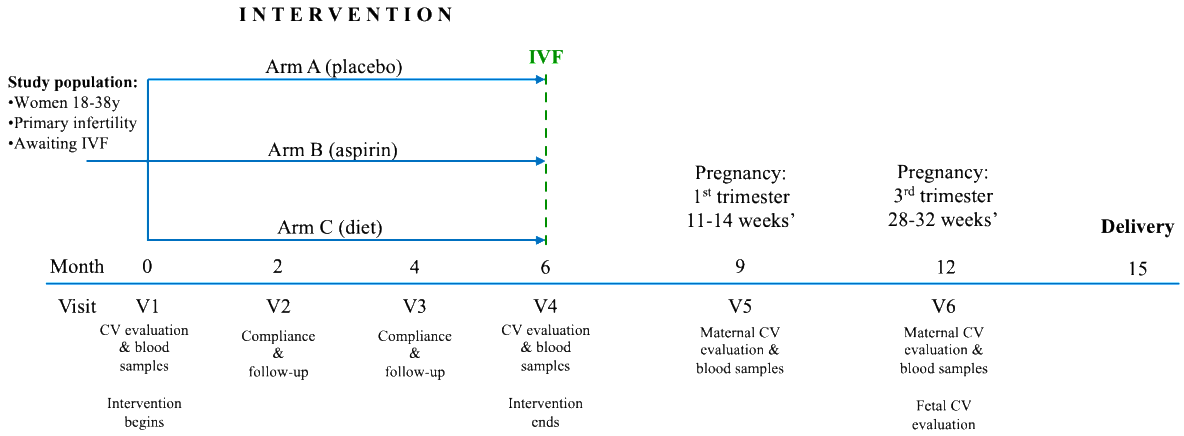
Secondary objectives

- To evaluate basal CV risk parameters in women awaiting IVF procedures.
- To evaluate CV risk parameters in women awaiting IVF procedures 6 months after pre-conception interventions.
- To evaluate placental function biomarkers in the 1st and 3rd trimester of all study groups.
- To evaluate maternal cardiac function parameters upon inclusion, previous to IVF, at 1st and 3rd trimester of pregnancy and compare between study groups.
- To evaluate fetal cardiac function parameters at 3rd trimester of pregnancy and compare between study groups.
- To describe the incidence of adverse perinatal outcomes, maternal and fetal, in the study groups.

5. Study design and type

5.1. Study design

This will be a Phase III national, unicentric, open-label, placebo controlled, randomized clinical trial with parallel groups. Study design is summarized in the following figure:



5.2. Randomization

This will be an open-label placebo controlled randomized clinical trial with parallel groups. Randomization will be provided by the Statistics Department (I. Gich) who will have the complete sequence for all 3 groups. Allocation will be at enrolment, once inclusion criteria are verified; recruiting physicians will generate patient ID and schedule the patient for the corresponding first visit. In the first visit, the patient will be directed to either the Pharmacy Department for medication capsules, or to the Dietist/Excersise specialists; due to the nature of the dietary intervention, it is not possible to blind participants or physicians completely, so they will be blinded only to the LDA *vs* placebo intervention. For patients allocated to the medication groups, Pharmacy will provide the capsules, so both the physician and patient will be blinded to treatment. Once the intervention is complete, IVF management and obstetric management will be equal in all 3 groups, and physicians performing follow-up will be blinded to the pre-conception intervention.

5.3. Development of the study

The following is a chronological description of the study:

5.3.1. Visit 0 (study explanation and patient inclusion)

- The patient will be invited to participate by the Assisted Reproduction Department collaborators, who will explain study details, verify inclusion and exclusion criteria and, if she decides to accept, sign the informed consent form.

- Researchers will introduce patient data in the database and a patient ID will be assigned.
- Researcher will record demographic data and medical history for the patient.
- Researchers will schedule Visit 1 within the following 2 weeks, and provide the requisition form for pre-intervention blood samples for basal biomarkers, as well as the initial evaluation questionnaires.

5.3.2. Visit 1 (Pre-intervention evaluation)

- Patient physical examination (complete anthropometry, vital signs, body composition).
- Patient cardiovascular evaluation (echocardiography).
- Evaluation of results of the pre-intervention blood analyses.
- Intervention:
    - If the patient was randomized to the Diet/Exercise group: visit the same day by the Dietist and Exercise experts, for evaluation of questionnaire results and instructions on this intervention. Dietary supplements will be provided in this visit for the first 2 months, as well as monitoring devices to register exercise variables (see Annex 14.3).
    - If the patient was randomized to any of the Medication groups: the patient will be accompanied to the Pharmacy Department, who will provide the appropriate vial based on the ID assigned, for 2 months. Physicians and patients will be blinded to the medication administered.
- Follow-up visits at 6 months will be scheduled.

5.3.3. *Visit 2 (2-month follow-up)*

- Follow-up visit will be based on the intervention applied:
    - If the patient was randomized to the Diet/Exercise group: follow-up visit by the Dietist and Exercise experts, for evaluation questionnaires and compliance for this intervention. Dietary supplements will be provided for the next 2 months.
    - If the patient was randomized to any of the Medication groups: the patient will be scheduled in the Pharmacy Department to provide the appropriate vial based on the ID assigned, for 2 months, and evaluate compliance to treatment.
- Follow-up visit at 4 months will be scheduled.

5.3.4. *Visit 3 (4-month follow-up)*

- Follow-up visit will be based on the intervention applied:
    - If the patient was randomized to the Diet/Exercise group: follow-up visit by the Dietist and Exercise experts, for evaluation questionnaires and

compliance for this intervention. Dietary supplements will be provided for the next 2 months.

- If the patient was randomized to any of the Medication groups: the patient will be scheduled in the Pharmacy Department to provide the appropriate vial based on the ID assigned, for 2 months, and evaluate compliance to treatment.

- Patient will be reminded of follow-up visit at 6 months.

5.3.5. *Visit 4 (6-month follow-up, intervention ends)*

- Patient physical examination (complete anthropometry, vital signs, body composition).
- Patient cardiovascular evaluation (echocardiography).
- If the patient was randomized to the Diet/Exercise group: visit by the Dietist and Exercise experts, for evaluation questionnaires and final compliance for this intervention.
- If the patient was randomized to any of the Medication groups: the patient will be scheduled in the Pharmacy Department to return the vial, and evaluate compliance to treatment.
- Researchers will provide the requisition form for post-intervention blood samples for biomarkers.

5.3.6. *Visit 5 (11-14 weeks' pregnancy)*

- Patient physical examination (complete anthropometry, vital signs, body composition).
- Patient cardiovascular evaluation (maternal echocardiography).
- Researchers will provide the requisition form for blood samples for biomarkers.

5.3.7. *Visit 6 (28-32 weeks' pregnancy)*

- Patient physical examination (complete anthropometry, vital signs, body composition).
- Patient cardiovascular evaluation (maternal echocardiography).
- Fetal cardiovascular evaluation (fetal echocardiography).
- Researchers will provide the requisition form for blood samples for biomarkers.
- This will be the last visit the patient will perform for the study.

Perinatal results will be obtained from the patient's case file after the estimated date of delivery has passed, in order to evaluate the live-birth rate and perinatal outcomes. If any patient does not deliver at our Hospital, they will be contacted by phone in order to obtain as much information as possible on the delivery.

5.4.End of the study

Conclusion and closure of this randomized trial will be considered when patients included in the study have reached a final outcome: not achieving pregnancy after IVF, or if pregnancy is achieved, when it has come to a conclusion. Once pregnancy outcome data is obtained for patients included in the study, we will close data on that patient. Patient participation also ends at this point in time.

6. Subject selection and withdrawal

6.1. Subject selection

Infertile women awaiting an IVF procedure at the Assisted Reproduction Department of Santa Creu and Sant Pau Hospital in Barcelona will be invited to participate in the study if they fulfill the following criteria:

6.1.1. Inclusion criteria

Patients with primary infertility justifying IVF treatment, who wish to become pregnant, aged between 18 and <38 years, candidates for single embryo transfer in day 5, who give written consent when invited to participate in the study protocol.

6.1.2. Exclusion criteria

Low ovarian reserve (defined as anti-Mullerian hormone <1 ng/mL), pregnancy at the moment of inclusion, presence of comorbidities that contraindicate IVF, pregnancy or childbirth, known allergies to any of the interventions to be applied, previous unsuccessful IVF cycles, probability of loss to follow-up, probability of delivery in another hospital or center.

Patients with conditions where LDA administration is contraindicated will also be excluded; some of these conditions or comorbidities would also contraindicate IVF.

- Allergy to acetylsalicylic acid or any of the other components.
- Gastric ulcer, duodenal or recurrent gastric symptoms.
- Allergic asthmatic reactions when taking anti-inflammatories, acetylsalicylic acid, other analgesics, as well as the dye tartrazine.
- Hemophilia or other blood clotting problems that predispose to bleeding.
- Severe kidney and / or liver failure.
- Asthmatic crises, with or without nasal polyps, after ingestion of acetylsalicylic acid.
- Diagnosis of glucose-6-phosphate dehydrogenase deficiency.
- Treatment with oral anticoagulants.
- Treatment with anti-inflammatories or other types of drugs that can interact with LDA.
- Hypertension treated with diuretics and angiotensin converting enzyme (ACE) inhibitors.

Likewise, patients will be asked about possible drug interactions that could increase the possibility of adverse effects, or contraindicate IVF and pregnancy, such as: immunosuppressants (cyclosporine and tacrolimus), methotrexate, other pain relievers or non-steroidal anti-inflammatory drugs, anticoagulants (warfarin), probenecid, chronic use of corticosteroids, diuretics, antidepressants (selective serotonin reuptake inhibitors), ACE inhibitors, hypoglycemic agents, vancomycin, alpha-interferon, lithium, chronic use of anti-acids, digoxin, barbiturates, zidovudine, phenytoin and valproic acid, metamizole, and habitual consumption of alcohol in significant quantities (three or more alcoholic beverages - beer, wine, liquor, etc. per day).

6.2. Sample size estimation

Sample size calculation was performed based on the impact expected of the interventions, calculated as a superiority trial, hypothesizing an increase of cumulative live-birth rate in the treatment groups of around 13%, with a 10% drop-rate. As such, for achieving a type I (alpha) error of 0.05, type II (beta) error at 0.20 aiming for a power of 80%, with equal allocation to 3 groups, the sample size for each group was calculated of 249; a total of 747 patients for the study. We have decided to round up each group to 250 patients.

6.3. Withdrawal criteria an analyses

Withdrawal criteria will be voluntary termination of pregnancy or a patient wishing to withdraw from the study. Subjects that withdraw from the study will not be replaced. If patient withdrawal occurs due to pregnancy before treatment completion, patients will be offered follow-up and will be analyzed as intention-to-treat. If withdrawal occurs due to adverse events with treatment, these will be reported and recorded, accordingly.

Assignment of participants into analyses populations will be performed prior to database lock. The following populations will be obtained:

- **Intention-to-treat -** this population will include all randomized women and will be the target population for the outcome analysis. Following the intention-to-treat principle, patients will be analyzed according to the treatment they were assigned to at randomization.
- **According-to-protocol -** this population will include all women who fulfill all inclusion and exclusion criteria, completed the treatment, and for whom data is available for the analysis.
- **Safety -** this population will include all patients who received at least one dose of their intervention and had at least one follow-up visit. Participant's safety will be analyzed by trial arm.

7. Treatment/intervention description

7.1. Dosage, posology, administration route and pharmaceutical form

7.1.1. Experimental treatment

Name: Aspirin / acetylsalicylic acid
Posology: 150 milligrams daily
Administration route: Oral
Pharmaceutical form: Capsules with micropellets of prolonged liberation
Treatment duration: 6 months

7.1.2. Control treatment

Name: Placebo / Microcrystalline cellulose
Posology: 140 milligrams daily
Administration route: Oral
Pharmaceutical form: Capsules with microcrystalline cellulose
Treatment duration: 6 months

7.1.3. Dietary and exercise intervention

Dietary interventions will be based on the study published by Estruch R, et al. in the New England Journal of Medicine (the PREDIMED trial), which are described in full in the Annexes section (Annex 14.3). The dietary supplements specified will be provided by the researchers every 2 months during the study intervention period. The following summarizes the primary dietary interventions proposed:

Mediterranean Diet

- -Extra-virgin olive oil, >=50 grams (4 tbsp) daily, including cooking oil for frying (30Lt per 6 months).
- -Nuts/Walnuts, 15 grams, at least 3 times per week (4kg per/patient in 6 months).
- Almonds 7.5 grams, (at least 3 times per week (2kg per/patient in 6 months).

Exercise

- The average number of steps per week will be recorded with an exercise monitoring band provided by the researchers, as well as the basal heart rate for the patient, and total sleep hours. Personalized exercise advice and goals will be determined by a specialist and based on the patient's basal status. The intervention is detailed in the Annexes section (Annex 14.3.2).

7.1.4. Blinding techniques of the study treatments

The Pharmacy Department of Hospital e la Santa Creu i Sant Pau will fabricate placebo capsules identical to the experimental treatment. The dietary intervention applied cannot be blinded.

7.1.5. Rescue medication

None.

7.2. Concomitant therapy

7.2.1. Allowed medication

- Antihistamines
- Antibiotics
- Vitamins and oligoelements
- 3Required medications for maternal conditions (i.e. levothyroxine)

7.2.2. Forbidden medication

- Concomitant use of non-steroidal anti-inflammatory drugs (i.e. ibuprofen) and aspirin is not recommended, due to increased risk for gastritis and blockage of aspirin's availability, although it is not forbidden.

7.3. Special management and storage required for medication

None.

7.4. Evaluation of treatment compliance

If the patient was randomized to the Diet/Exercise group participants will be asked regarding excess amounts of olive oil and supplements at each follow-up visit. Compliance will also be assessed by means of specially designed questionnaires for both interventions (Annexes 14.4, 14.5), applied in person or by telephone call. Exercise monitoring and surveillance will be performed by a specialist, and recorded by means of questionnaires regarding lifestyle and an exercise monitoring band provided by the researchers. The exercise monitoring band will be equipped with a step counter, heart rate monitor and sleep monitoring. The average number of steps per week will be recorded, as well as the basal heart rate for the patient, and total sleep hours. Participants will be encouraged to report any concerns or side effects in a diary for review at each trial visit.

If the patient was randomized to any of the Medication groups: Participants will be asked to bring their trial medication to each follow-up/refill visit at the Pharmacy Department. Compliance will be assessed by the Pharmacy trial team by counting remaining tablets. Participants will be encouraged to report any concerns or side effects in a diary for review at each trial visit.

8. Response evaluation

8.1. Efficacy evaluation

8.1.1. Main outcome variables

- Cumulative live-birth rate: defined as the number of deliveries that result in a live born neonate, expressed per 100 embryo transfers.

8.1.2. Secondary outcome variables

- Adverse perinatal outcome: defined as the presence of either of the following: miscarriage, stillbirth, low birth weight, preterm birth, preeclampsia, congenital malformations and/or admission to neonatal ICU.
- High risk of PE according to first trimester screening: a logistic regression-based predictive model for early- and late-onset PE will be used, according to a validated algorithm including maternal characteristics, levels of pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) at 8–12 weeks, and blood pressure and uterine artery Doppler at 11.0–13.6 weeks. Details of this screening method have previously been described and are available for clinical use.
- Preeclampsia: categorized according to the guidelines of the Royal College of Obstericians and Gynaecologists. Briefly, hypertension will be defined as the repeated measurement of systolic blood pressure of ≥140 mm Hg (Korotkoff I) and diastolic blood pressure of ≥90 mm Hg (Korotkoff V). Proteinuria will be defined as the excretion of 300 mg protein or greater per day in the 24-hour urine collection or a repeated dipstick of ≥1+. Pregnancy-induced hypertension (PIH) will be defined as the new onset of hypertension after 20 weeks of gestation, and preeclampsia (PE) will be defined when additional proteinuria was diagnosed according to International Society for the Study of Hypertension in Pregnancy.

8.2. Safety evaluation

The incidence rates of adverse events and serious adverse events, and their relationship to trial drugs, will be summarized by treatment group. The proportion of women discontinuing treatment will be summarized by reason and by treatment group.

8.2.1. Aspirin

- Safety of low-dose aspirin has been demonstrated in large cohort and case– control studies, both in pregnant and non-pregnant patients, which have reported that the drug is not associated with increase in risk of congenital heart defects or other structural or developmental anomalies. Randomized studies report that ~10% of

women receiving low-dose aspirin present with gastrointestinal symptoms; however, there is no evidence of increase in any type of maternal bleeding.

8.2.2. Dietary intervention.

- Patients with known allergies to any of the supplements provided will be excluded from entering the study. Safety and tolerance of dietary interventions will be evaluated at follow-up visits; the most commonly reported side effects are bloating, fullness, indigestion, altered bowel habit. In the PREDIMED study, some patients reported difficulties in chewing the nuts, resolved by crushing and mixing them.

8.2.3. Exercise intervention.

- Patients will receive a personalized template with daily and weekly objectives in terms of the total number of steps, including the exercises indicated for the warm-up and cool-down phases that will help to fine-tune the body to avoid possible injuries derived from sports practice. Exercise consisting of a more or less vigorous walk, in the case of poorly trained women, and a gentle trot for trained women, should not involve any excessive overload in joints, that can cause injuries.

9. Statistical analysis

Sample size calculation was performed based on the impact expected of the interventions, calculated as a superiority trial, hypothesizing an increase of cumulative live-birth rate in the treatment groups of around 13%, with a 10% drop-rate. As such, for achieving a type I (alpha) error of 0.05, type II (beta) error at 0.20 aiming for a power of 80%, with equal allocation to 3 groups, the sample size for each group was calculated of 249; a total of 747 patients for the study. We have decided to round up each group to 250 patients.

Once all evaluations have been performed and laboratory determinations have been completed, evaluation and analysis of the results will be carried out, with assistance of the Bioinformatics Department of the Institut de Recerca, and the Research Support Unit. Statistical analyses will be performed with the statistical package IBM® SPSS® Statistics Version 25 (IBM Corporation) or Open-source software (The R Foundation for Statistical Computing) will be used for all computations and graph construction (R V2.15.1). Statistical analysis will be based on the originally assigned groups (intention-to-treat).

No intermediate analyses are planned, due to the short time of intervention and the time period till birth. Multiple imputations will not be used in case of missing values or subjects, and analysis will be performed as intention-to-treat, including all randomized women that will be the target population for the outcome analysis. Following the intention-to-treat principle, patients will be analyzed according to the treatment they were assigned to at randomization. Normality of the distribution of variables will be evaluated with the Kolmogorov-Smirnov test. Student's t-test (or non-parametric Mann-Whitney U test) and Pearson's $\chi 2$ test or linear-by-linear $\chi 2$ test (for trends across ordered categories) will be performed for univariate between-group comparisons of quantitative or qualitative variables, respectively. Statistical significance will be considered with a threshold p value below 0.05.

Logistic regression also will be used to determine which of the variables have a significant contribution for predicting live-birth and adverse perinatal outcomes. In these regression models, stepwise algorithms will be performed to select variables at p-value cut-off of 0.05 for inclusion and 0.1 for exclusion, with tolerance level>0.01.

9.1 Database creation

The database system that will be used is the Clinapsis system; the person responsible for creation of the database will be Judit Solá.

Each area or medical specialty will have a unique login to introduce their relevant data in the database, without being able to modify data not related to their specialty. Principal

investigators will have access to all data and editor privileges. We contemplate database access as follows:

- Assisted Reproduction Department: individual researcher logins (2).
- Pharmacy Department: 1 login.
- Nutrition Department: 1 login.
- Exercise Coordinator: 1 login.
- Obstetrics Department: individual logins (3).
- Cardiology Department: 1 login.

10. Adverse events

10.1. Definitions

10.1.1. Adverse event (AE)

Any incidence detrimental to a patient's health or a clinical trial subject treated with a drug, even if it does not necessarily have a causal relationship with said treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of an investigational drug, whether or not it is related to the investigational drug.

10.1.2. Adverse reaction (AR)

An AR is any unintended and harmful reaction to an investigational drug, regardless of the dose administered. Unlike an AE, in the case of an adverse reaction there is a suspected causal relationship between the investigational drug and the adverse event.

10.2. Description

10.2.1. Intensity

According to intensity of the process, AEs and ARs can be classified into:

- **Mild:** they do not cause any limitation of usual activities.
- **Moderate**: cause certain limitations of usual activities.
- **Severe:** make usual activities impossible.

10.2.2. Severity

According to the severity of the process, AEs and ARs may be classified as:

- **Serious:** Any adverse event or adverse reaction that, at any dose: causes death, threatens the life of the subject, requires hospitalization of the patient or prolongs an existing hospitalization, causes permanent or significant disability, or results in an abnormality or congenital malformation. For the purposes of notification, those suspected adverse events or adverse reactions that are considered important from a medical point of view, even if they do not meet the above criteria, will also be treated as serious, including important medical events that require intervention to prevent one of the consequences described above from occurring. Likewise, all suspicions of transmission of an infectious agent through a drug will be reported as serious.
- **Non-serious:** an adverse event that does not meet the previous severity criteria.

10.2.3. Causality

The causal relationship of an adverse event with the study medication will be established according to the following definitions:

- **True:** A clinical event or laboratory test anomaly, which appears in a reasonable time sequence after drug administration and which cannot be explained by concurrent disease or other drugs or chemicals. The response to drug discontinuation must be clinically plausible. The event must be definitively pharmacological or phenomenological using, if necessary, an appropriate re-exposure procedure.
- **Likely:** A clinical event or laboratory test anomaly, which appears in a reasonable time sequence after drug administration and which is unlikely to be explained by concurrent disease or other drugs or chemicals. The response to drug discontinuation is clinically plausible. Information on re-exposure is not required to meet this definition.
- **Possible:** A clinical event or laboratory test anomaly, which appears in a reasonable time sequence after drug administration but which could also be explained by concurrent disease or other drugs or chemicals. Information about exposure interruption may be missing or unclear.
- **Unlikely:** A clinical event or laboratory test anomaly, with a temporal relationship with the administration of the drug that makes a causal relationship unlikely (but not impossible). The underlying disease, other drugs, or chemicals provide plausible explanations.
- **Conditional / Not classified:** A clinical event or laboratory test anomaly, reported as an adverse event, for which more data is essential for a proper evaluation or additional data is being evaluated.
- **Not assessable / Not classifiable:** Report that suggests an adverse event, which cannot be assessed due to insufficient or contradictory data, and which cannot be supplemented or verified.

In a simplified way, for the purpose of notification to regulatory authorities, the following definitions regarding causation will be adopted:

- **Related / Suspected:** The temporal relationship of an AE with the study medication indicates a possible causal relationship and cannot be explained by other factors such as the patient's clinical status, therapeutic interventions or concomitant medication.

  Unrelated / Not Suspected: The temporal relationship of an AE with study medication indicates an unlikely causal relationship, or else other factors (medication or concomitant conditions), other therapeutic interventions provide a satisfactory explanation for the AE.

10.2.4. Other definitions

Unexpected Adverse Reaction (UAR):

An Unexpected Adverse Reaction (UAR) is defined as any adverse reaction whose nature, intensity or consequences do not correspond to the reference information for the drug (i.e., the investigator's manual in the case of an investigational drug not authorized for marketing, or the technical data sheet of the product in the case of an authorized medicine).

Serious and Unexpected Adverse Reaction (SUAR):

Serious Unexpected Adverse Reaction (SUAR), in which the nature, severity or consequences of an UAR do not correspond to the reference safety information.

10.3. Recording of adverse events

Any adverse event that occurs during a study, and is voluntarily reported by the subject or observed by the investigator, must be recorded on the adverse events page included in the data collection notebook, regardless of the opinion of the researcher regarding its relationship to treatment. The investigator will determine the relationship between the adverse event and the drugs under study, and will record their conclusions in the corresponding section of the data collection notebook.

AE Collection and Assessment Methods

Every AE should, in principle, be documented in the section of the data collection notebook reserved for this purpose, and not as a comment collected anywhere in said data collection notebook. The following aspects will be collected:

- Beginning;
- Duration and, where applicable,
- Completion of the AE;
- Description of the AE;
- Any factor considered as a possible causal agent of AE;
- Concomitant medication; and
- Assessment of the relationship of intensity, severity, causality and expected condition made by the researcher.

10.4. Notifications

Any serious adverse event must be notified to the monitor and promoter, by telephone or fax within 24 hours from the moment in which they become aware of said events, unless for certain serious adverse events, the protocol provides that immediate communication is not required.

The investigator will complete the Adverse Events form of the CRD and Annex D (modified version), and will send it to the monitor and the promoter via fax and email within a period of no more than 24 hours. This communication will be made within that period even if not all the information provided for in the form is available, which must be completed within 10 days. The form should include an assessment of the intensity, severity, causality and the expected condition between the investigational drug and / or a concomitant treatment and the AE.

Follow-up reports to the promoter on the evolution of the adverse event will continue until the event in question has disappeared or the clinical situation has stabilized. If necessary, any additional information will be provided.

In the initial and follow-up communications, the subjects of the trial will be identified only by the identification code of the subject of the trial, specific for each one of them.

In the event that the death of a subject participating in the clinical trial has been reported, the investigator will provide the sponsor and the CEIm with all the additional information requested.

The investigator is obliged to report each serious adverse event immediately, by phone or fax to:

Head of pharmacovigilance:
Claudia E. Delgado Espinoza
Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau
C/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel.: 93 553 76 34
Fax: 93 553 78 12
e-mail: cdelgadoe@santpau.cat

Responsible for monitoring:
UICEC Sant Pau
Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau
C/ Sant Antoni Maria Claret, 167
08025 Barcelona
Tel.: 93 553 76 35
Fax: 93 553 78 12

Notification will be done using the CRD Adverse Event Reporting Form and Annex D modified version.

The fact that a serious AE is considered related or not related to the investigational drug(s), and is considered expected or not expected, will be determined by the person in charge of pharmacovigilance of the UICEC Sant Pau according to the reference documents, which for this study will be the technical file of the study drug.

If it is a suspicion of SUAR, the promoter will notify the Agencia Española de Medicamentos y Productos Sanitarios and the competent body for Pharmacovigilance of the Autonomous Community where it occurred.

The reporting period of suspected serious and unexpected adverse reactions by the sponsor to the organisms mentioned above will depend on the severity of the reaction and will be determined as follows:

a) In the event of suspected serious and unexpected fatal or life-threatening adverse reactions, as soon as possible and, in any case, within seven days after the promoter became aware of the reaction.
b) In the event of suspected serious and unexpected non-fatal or non-life-threatening adverse reactions, no later than fifteen days after the promoter became aware of the reaction.
c) In the event of suspected serious and unexpected, fatal or life-threatening adverse reactions, which were not initially considered such, as soon as possible and, in any case, no later than seven days after the promoter has learned that the reaction is fatal or life-threatening.

When necessary to guarantee rapid notification, the promoter may make an incomplete initial notification that will be completed as far as possible within the following eight days.

Adverse events that are not serious or that are considered unrelated to the treatments under trial or that are considered expected should be reported in tabulated form in the final report of the clinical trial.

The principal investigator and promoter will prepare an annual safety report evaluating the safety of the investigational drug taking into account all available information. Said report will be communicated to the Agencia Española de Medicamentos y Productos Sanitarios and to the CEIm.

Regardless of the annual safety report, the principal investigator together with the promoter will prepare an «ad hoc» evaluation report whenever there is a relevant safety

problem. This report will be presented immediately to the Agencia Española de Medicamentos y Productos Sanitarios and to the CEIm.

10.5. Specific aspects for safety assessment

The incidence rates of adverse events and serious adverse events, and their relationship to trial drugs or interventions, will be summarized by treatment group. The proportion of women discontinuing treatment will be summarized by reason and by treatment group.

10.5.1. Aspirin

- Patients with a history of known allergies, suspicion of allergy or pathologies that may contraindicate the use of aspirin, will be excluded from the study.
- Randomized studies report that ~10% of women receiving low-dose aspirin present with gastrointestinal symptoms; however, there is no evidence of increase in any type of maternal bleeding.
- Safety of low-dose aspirin has been demonstrated in large cohort and case– control studies, both in pregnant and non-pregnant patients, which have reported that the drug is not associated with increase in risk of congenital heart defects or other structural or developmental anomalies.

10.5.2. Dietary intervention.

- Patients with known allergies to any of the supplements provided will be excluded from entering the study.
- Safety and tolerance of dietary interventions will be evaluated at follow-up visits; the most commonly reported side effects are bloating, fullness, indigestion, altered bowel habit. In the PREDIMED study, some patients reported difficulties in chewing the nuts, resolved by crushing and mixing them.

10.5.3. Exercise intervention.

- Patients will receive a personalized template with daily and weekly objectives in terms of the total number of steps, including the exercises indicated for the warm-up and cool-down phases that will help to fine-tune the body to avoid possible injuries derived from sports practice. Exercise consisting of a more or less vigorous walk, in the case of poorly trained women, and a gentle trot for trained women, should not involve any excessive overload in joints, that can cause injuries.

11. Ethical aspects

11.1. General considerations

The study will be carried out in strict accordance with international ethical recommendations for research and clinical trials in humans. The researcher will be responsible for ensuring that the clinical trial is carried out in accordance with the standards set out in the Declaration of Helsinki and following the recommendations of the Spanish Ministry of Health regarding clinical trials.

Before including a subject in the study, the Comité de Ética de la Investigación con medicamentos (CEIm) and the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) must approve the study protocol, the information that will be given to the subject and the informed consent model to be used.

The study should be carried out in accordance with the protocol and with the Standard Work Procedures (PNTs) that ensure compliance with the Good Clinical Practice (GCP) standards, as described in the ICH Tripartite Harmonized Standards for Good Clinical Practice 1996.

The CEIm and the AEMPS must be informed of any subsequent amendment to the protocol and their opinion must be requested in the event that a new evaluation of the ethical aspects of the trial is necessary.

11.2. Patient information and informed consent

It is the responsibility of the investigator to obtain the informed consent of the patient. The patient cannot participate in any specific study procedure before obtaining their consent or that of their legal guardian / family member when the patient is unable to give consent due to their clinical situation.

If, in the follow-up phase of the study, the patient is still unable to give written consent, oral consent may be requested before a witness.

Before the start of the trial, and before obtaining informed consent, the investigator or the person designated by the investigator, will explain to the potential trial subject or their legal guardian / family, the objectives, methods and potential risks of the study and any discomfort that it may cause.

The explanation about the nature, scope and possible consequences of the study will be made in understandable language.

The prospective trial subject or her legal guardian / family member should have time to reflect on her participation in the study, and have the opportunity to ask questions. After this explanation, and before entering the trial, consent must be properly recorded by the signature of the subject or her legal guardian / family member.

The information provided must include:

- Explanation that the test involves an experimental procedure.
- Explanation of the purpose of the trial.
- Description of the drug to be studied and random assignment. Statement that the treatment may not be the study drug (i.e., in cases where placebo or another comparator drug is given in a randomized trial).
- Description of the procedures to follow, including invasive ones. Duration of the subject's participation. Approximate number of subjects who will participate in the trial.
- Responsibilities of the subject.
- Reasonably foreseeable risks and discomforts for the subject (if applicable for the embryo or fetus) and planned remedial measures.
- Description of the benefits for the subject / society.
- Availability of alternative treatments with their potential risks and benefits.
- Compensation to subjects: coverage of risks, medical treatment of possible damages, financial compensation.
- Knowledge of any additional costs for the subject that may arise from their participation in the research.
- Conditions of participation: Consent expressed according to their free will, right to abandon the trial at any time, right to refuse to participate without prejudice to the subject.
- Explanation that the identity of the subject is confidential but that the clinical chart can be reviewed by the trial monitor, the auditors and can be made known to the health authorities.
- Statement that new relevant findings will be made available to the subject.
- Identification of whom and what service they can go to obtain answers regarding any aspect of the trial or the rights of the subject (name and telephone number).
- Description of the circumstances in which the investigator may discontinue a subject's participation in a trial.
- In the case that the subject is female, if deemed necessary, the promoter will provide additional information to prevent the possibility of pregnancy during the process of selecting, developing and monitoring a trial.

Patient Information Sheet and Informed Consent Form are presented in the Annexes section.

11.3. Evaluation of the foreseeable benefits and risks for trial subjects and other possible patients

At this time there is no clear recommendation that any of these interventions will provide benefit in IVF patients to achieve pregnancy, however previous studies suggest this possibility. In this study we want to act preventively, that is, before the technique itself: for 6 months we will intervene on the patient's lifestyle. We know today that 80% of health problems are due to lifestyle; the way we live affects our hormones, our metabolism and our ability to be receptive to a new life.

We have chosen the Mediterranean diet and moderate exercise because there is unquestionable evidence about the health benefits: decreased stress, longevity, decreased risk of death from cardiovascular disease and cancer. In addition, we have published that in obese women, eating a similar diet for only 12 weeks, even without losing weight, improves the likelihood of becoming pregnant by ART or even spontaneously. We also want to evaluate low-dose aspirin because it is a drug that avoids the risk of death in patients with cardiovascular risk factors, and during pregnancy the risk of preeclampsia is reduced by half. In addition, in a recent study, treatment with aspirin 6 months before ART seemed to increase the success of having a child by 10%.

The ultimate goal is to improve the quality of life and health of future mothers, to demonstrate that it is decisive in achieving pregnancy and improve the perinatal results. The results of this trial could change recommendations in the infertile population regarding lifestyle and dietary modifications previous to an ART procedure, or could provide evidence for the routine use of aspirin in this population awaiting IVF.

11.4. Considerations on treatment of biological samples

Regarding the obtention, handling, identification and storage of biological samples, the provisions of the Biomedical Research Law 14/2007 of July 3, specifically in chapters III and IV of Title V, as well as the provisions of provided in Royal Decree 1716/2011, of November 18, specifically in Chapter I of Title II, which establishes the treatment of biological samples of human origin for biomedical research purposes.

In accordance with these regulations, the obtainment of biological samples for biomedical research purposes may only be carried out when the written consent of the source subject has been previously obtained and prior information on the consequences and risks that such obtainment may entail for their health. Such consent will be revocable. Once the study is finished, the remaining biological samples will be stored in the C.0004338 collection of the IIB Sant Pau Biobank, after the specific consent of the participant.

11.5. Data confidentiality

The treatment, communication and transfer of personal data of all participating subjects will comply with the provisions of EU Regulation No. 2016/679, Organic Law 3/2018, of December 5th, Personal Rights on Data Protection and Guarantee of Digital Rights, with the Royal Decree that approves the Regulations for the development of the Organic Law and other current regulations.

Both the center and the promoter are respectively responsible for the processing of the personal data of the participating subjects and undertake to comply with the data protection regulations in force. To preserve the confidentiality of the subjects' personal data, only the principal investigator, his collaborators, and the technical personnel participating in the study will have access to their identity. For the same reason, the complete affiliation data and the written consent will be kept in the file of the center researcher.

The names of the participants will not appear in any of the study reports. The identity of the participants will not be revealed to any person except to fulfill the purposes of the study, and in the case of medical emergency or legal requirement. Any personal information that can be identifiable will be kept and processed by computerized means under secure conditions by the study researchers. Access to such information will be restricted and will always be done under conditions of confidentiality. The results of the study may be communicated to the health authorities and, eventually, the scientific community, without the identity of the participating subjects being recorded in any case.

In accordance with current legislation, the participating subject may exercise the rights of access, modification, opposition and cancellation of data. You can also limit the processing of data that are incorrect, request a copy or transfer to a third party (portability) the data he has provided for the study. To exercise such rights, the participating subject may contact the main investigator of the study. Likewise, you will have the right to contact the Data Protection Agency if you are not satisfied.

Information regarding the identity of patients will be considered confidential for all purposes. The identity of the patients cannot be revealed or disclosed. The data of the patients collected in the Data Collection Notebook during the study, must be documented anonymously and dissociated, linking to a code (patient number), so that only the researcher can associate such data with an identified person or identifiable.

The database generated by the study will not contain any identification of the patient, other than a numerical code by which it will not be possible to reveal their identity. The information collected in the study will always be treated as grouped data and never as individual or personal data, thus maintaining anonymity and confidentiality.

The researcher and the sponsor are obliged to keep the data collected for the study for at least 25 years after its completion. Subsequently, the personal information will only be kept by the health care center and by the sponsor for other scientific research purposes if the participating subject has given their consent to do so, or if this is permitted by law and the applicable ethical requirements.

If the encrypted data is transferred outside the EU to the entities of our group, to service providers or to scientific researchers who collaborate with us, the data of the participating subjects will be protected with safeguards such as contracts or other mechanisms by the authorities on data protection. Participants will have the right to request more information, for which they may contact the Principal Investigator of the study.

11.6. Insurance

The promoter of the study will ensure that the subject of the trial is compensated for any damages suffered as a result of the trial. In this sense, the promoter of the study will contract an insurance policy, which will cover any type of risk derived from the study, both for subjects and for participating physicians, complying with the points specified in this regard in Royal Decree 1090/2015..

12. Practical considerations

12.1. Responsibilities of trial participants

Researchers/Investigators

The principal investigators will be responsible for carrying out the trial in accordance with current regulations for clinical trials in Spain, being solely responsible for the execution of the trial. The principal investigators and their collaborators agree to practice each and every one of the explorations and complementary tests specified in the protocol on all the subjects included in the trial.

The auxiliary personnel who collaborate in the study must be informed by the principal investigator of their responsibilities towards the subject.

Auxiliary staff responsibilities

The auxiliary personnel who collaborate in the study will comply with the general rules established for conducting the trial and will follow the investigator's instructions at all times.

12.2. Data archive conditions and corrections

The data obtained must be transcribed in the Data Collection Notebook (CRD) and these data will be considered valid information for the subsequent evaluation of the efficacy and safety data of the treatments under study. The notebooks must be filled in correctly and in case data that has already been transcribed must be corrected, they will be crossed out and the correct value will be noted to the side. Corrections must always be dated and validated by the signature of the principal investigator or his collaborators.

The clinical trial master file will comply with the provisions of articles 57 and 58 of Regulation (EU) No. 536/2014 of the European Parliament and Council, of April 16th, 2014. Its content will take into account the supplementary guidelines published by the European Commission.

The promoter and the researcher will keep the content of the master file in paper or digital format of this clinical trial for at least twenty-five years after the end of the trial.
The content of the master file will be preserved in such a way that it can be easily made available to the competent authorities, should they request it.

The clinical chart of the trial subject must be kept in accordance with the provisions of Law 41/2002, of November 14th, and in accordance with the maximum period allowed by the hospital.

Any transfer of ownership of the content of the master file will be documented and the new owner will assume the responsibilities set forth in this article.

12.3. Monitoring, audits and inspections

12.3.1. Monitoring

- The study is planned to be monitored by the monitor designated by the promoter.
- Monitoring will include visits to the center and telephone communication with the research team in order to ensure proper compliance with the protocol, the GCP guidelines and the regulations of the health authorities.
- During the monitoring visits, the most relevant aspects of the study will be reviewed, such as the procedure followed for obtaining informed consent, a verification of the documentation in the investigator's file and a percentage of the data recorded in the CRD (these data must be correctly completed and be truthful with source documents), inclusion/exclusion criteria and adverse events to date.
- Monitoring findings will be reported to the promoter or the delegated structure so that appropriate measures can be taken, through the monitoring visit reports. A follow-up letter will be sent to the investigator, where the most significant findings and issues that may have been pending will be reported.
- At the end of the study, a report will be prepared with the overall results about quality and reliability of the data, as well as the adherence to the protocol and the good clinical practice procedures observed.
- At the end of the study, the data collection notebooks will be sent to the Study Promoter or to the corresponding delegated structure for archiving.

12.3.2. Audits

The clinical trial will be included in the IR-HSCSP Quality Assurance Program, in relation to Clinical Research, analyzing in each case the criticality of the trial and the audits to be performed.

12.3.3. Inspections

Both the Researcher and the Promoter will allow direct access to the source data or documents for monitoring, auditing, review by the CEIC, as well as the inspection of the test by the health authorities if they are required.

12.4. Corrections to the clinical trial protocol

Any change made to the study protocol will always take the form of an amendment or addendum in writing. For its formalization, the approval of all the people responsible for the study that also signed the protocol is required; if there were relevant modifications, the express approval of the Ethics Committee and health authorities may be necessary.

12.5. Breaches of the study protocol

Non-compliances or deviations from the study protocol are not allowed, especially with regard to prescription of drugs or doses not programmed in the study, as well as other modes of administration, other indications, or longer treatment periods.

Serious breaches of protocol that have occurred will be reported by the promoter, without undue delay and at the latest within seven calendar days from the date on which they became aware of the breach, to the Spanish Agency for Medicines and Health Products and to the CEIm.

For these purposes, serious non-compliance will be understood as one that may significantly compromise the safety and rights of the test subjects or the reliability and solidity of the data obtained in the clinical trial.

12.6. Medication control

The entry record of the received medication, as well as the inventory of its use, must be kept by the hospital pharmacy, observing the following rules at all times:

a) The investigator must keep the study medication in the hospital pharmacy, accessible only to the persons authorized to administer said drugs.
b) The hospital pharmacist will carry out an inventory that will include details about the medication, and clear indications about the time of its administration and in which individual it was used. This record will also indicate the amount and type of medication that is available at any time during the trial, throughout the duration of the trial.
c) The hospital pharmacist will carry out an inventory of the excess medication at the end of the clinical trial, and will record the results in the Drug Accounting Form.
d) The researcher accepts that he will not provide the study drugs to anyone, with the exception of his collaborators in the research and the patients under study.

12.7. Identification of samples and sample labeling

The different types of labels designed for this phase of the study will be printed using the ETIK program, before starting the final packaging.

The person responsible for preparing the medication will have the randomization list. The medication will be labeled and identified according to the randomization sequence.

Label data:

- ***Code:*** IIBSP-EDA-2020-18
- ***EudraCT:*** 2020-001069-35

- ***Promoter:*** IIB-Sant Pau
- ***Address:*** Sant Quintí, 77-79 08041 Barcelona
- ***PI:*** Mónica Cruz Lemini / Ana Polo Ramos
- Tromalyt 150 mg vs Placebo
- ***Pharmaceutical form:*** capsules
- ***Route of administration:*** oral
- ***Batch:***
- ***Expiration:*** month / year
- ***Patient code (identification number):***
- Exclusive sample for clinical trial.
- Store at room temperature.
- Keep away from children.

12.8. Assignment of study treatments

The randomization list will be generated by the Methodological and Statistical Support Unit of the Institut de Recerca.

The randomization list will be generated in such a way that all treatments have an equal probability of being assigned.

The patients will be included in the study consecutively from the inclusion of the first eligible patient according to the selection criteria. When a patient is included in the study, the researcher will assign a patient ID number; this ID must be consecutive with respect to the previous assigned number, and taking as a reference the date and time when the intervention is planned.

The randomization codes will be assigned in a correlative way as the patients are included in the study.

The center is recommended to keep a register of both included and non-included patients in order to ensure that no bias is introduced into the selection process. This registry will also provide information on the reasons for not including patients who have undergone an intervention.

If the randomized treatment is discontinued, the investigator may prescribe other treatment based on his clinical judgment. The patient will no longer be able to contribute to the drug exposure phase study for the main analysis, but the patient will remain in the follow-up phase.

12.9. Trial interruption

The clinical trial can be interrupted by the principal investigator and / or by the promoter in any of the following cases:

- Ineffectiveness of the studied treatment.
- Appearance of adverse events unknown to date, as well as known adverse events whose nature, severity, duration or incidence are not as expected.
- Insufficient number of patients included in the study.

12.10. Publishing conditions

The results obtained as consequence of the clinical research with the product under study will be reviewed and discussed by the research team and the promoter for subsequent publication. The data obtained will not be disclosed to third parties until an agreement is reached with the promoter for its disclosure, either in the form of a conference, communication to congress, or publication. As an exception to the previous paragraph, researchers may include the title of the essay in their respective Curriculum Vitae, as long as it does not include any information about the promoter.

12.11. Preparation of the final report

After obtaining the conclusions of the study, a final report will be prepared in collaboration with the promoter. Said report will include the statistical analysis and a medical evaluation of the results. This report will be based on the objectives stated in the study protocol.

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14. Annexes

14.1. Chronogram for study evaluations

Procedure \ Visit	V0 Inclusion	V1 Intervention begins	V2 Follow-up	V3 Follow-up	V4 Intervention ends	V5 Pregnancy 11-14 wks	V6 Pregnancy 28-32 wks
Inclusion/exclusion criteria verification	X						
Informed consent form signature	X						
Patient ID assignation and Randomization	X						
Demographic data recording	X						
Questionnaires provided	X		X	X	X		
Maternal biometric parameters		X			X	X	X
Maternal blood analyses: CV and lipid profiles		X			X	X	X
Maternal CV evaluation		X			X	X	X
Intervention begins: capsules *vs* Diet/Exercise		X					
Intervention follow-up: capsules *vs* Diet/Exercise			X	X	X		
Fetal CV evaluation							X

14.2. Informed consent form

HOJA DE INFORMACIÓN A LA PACIENTE

TÍTULO DEL ESTUDIO: Impacto del ejercicio y la dieta Mediterránea *vs* aspirina en la tasa de nacimientos vivos y programación cardiovascular en la fertilización *in vitro*: un estudio aleatorizado.
ACRÓNIMO: MEDITATE-IVF.
CÓDIGO DEL ESTUDIO: IIBSP-EDA-2020-18.
PROMOTOR: Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau – IIB Sant Pau.
INVESTIGADOR PRINCIPAL: Dra. Mónica Cruz Lemini / Dra. Ana Polo Ramos.
CENTRO: Hospital de la Santa Creu i Sant Pau.

INTRODUCCIÓN:

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que se le invita a participar. El estudio ha sido aprobado por un Comité de Ética de la Investigación con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo a la legislación vigente, el Real Decreto 1090/2015 de 4 de diciembre y el Reglamento Europeo 536/2014 de 16 de abril, por los que se regulan los ensayos clínicos con medicamentos.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda decidir si acepta o no participar en este estudio. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir.

Además, puede consultar con las personas que considere oportuno.

PARTICIPACIÓN VOLUNTARIA

Le invitamos a participar en el estudio porque usted se encuentra en lista de espera de un procedimiento de reproducción asistida para poder embarazarse, llamado fertilización *in vitro* o FIV, y su tiempo de espera aproximado para el mismo es de alrededor de 6 meses.

Debe saber que su participación en este estudio es voluntaria y que puede decidir NO participar. Si decide participar, puede cambiar su decisión y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su atención sanitaria

OBJETIVO DEL ESTUDIO

El objetivo principal de nuestro estudio es evaluar 2 opciones de manejo en las pacientes que están en espera de realizar una FIV, para conocer si tienen un beneficio con una mayor tasa de embarazo y recién nacidos vivos. Como objetivo secundario, se buscará también valorar si existen menos complicaciones en esos embarazos, y mayor salud cardiovascular en la madre y los niños. El objetivo final es mejorar la calidad de vida y la

salud de las futuras mamás para demostrar que es determinante en poder embarazarse y mejorar los resultados de la gestación y del bebé.

DESCRIPCIÓN DEL ESTUDIO

Este estudio está dirigido a mujeres entre los 18 y 38 años de edad, que estén en espera de una FIV en nuestro centro, y que no sean alérgicas a ninguno de los componentes que utilizaremos. Buscamos reclutar un total de 750 pacientes, 250 por grupo de estudio, y la duración de la intervención será de 6 meses.

En este estudio se pretenden comparar tres manejos clínicos distintos. La asignación a cada uno de estos manejos estará determinada por el azar, con lo cual su médico no intervendrá en este proceso. Usted tendrá una probabilidad del 33% de ser asignada a uno de los tres grupos contemplados en este estudio:

- **Grupo 1:** se le proveerá de una cápsula que deberá tomarse durante 6 meses, diariamente por las noches antes de dormir, la cual contendrá aspirina a una dosis que se ha visto puede reducir complicaciones durante el embarazo y es segura de tomar.
- **Grupo 2:** se le proveerá de una cápsula que deberá tomarse durante 6 meses, diariamente por las noches antes de dormir, que tendrá el mismo aspecto que la aspirina, pero que no contiene sustancia farmacológicamente activa y por tanto no se espera que tenga efecto. Esta cápsula, llamada placebo, contendrá únicamente celulosa microcristalina, que es lo que contienen comúnmente las cápsulas de vitaminas comerciales, u otros medicamentos como excipiente.
- **Grupo 3:** se le proveerá de una serie de suplementos nutricionales (aceite de oliva extra-virgen, nueces y almendras) así como de asesoramiento por una Dietista, para llevar a cabo una dieta Mediterránea durante todo el periodo de estudio (6 meses). Así mismo, se le proveerá de una pulsera que monitoriza el ejercicio y sueño, y un seguimiento por una especialista en Salud Física, para aconsejar un aumento en su actividad física y monitorearlo.

Dado que es imposible no saber si usted está en el grupo de la Dieta/Ejercicio, este estudio no se considera "cegado"; sin embargo, si Usted estuviera en alguno de los 2 grupos que debe tomar una cápsula por la noche, ni Usted ni su médico sabrán cuál de los dos tratamientos va a recibir. Los médicos que realicen sus estudios ecográficos, así como quien procese sus muestras de sangre, también desconocerán el grupo de estudio al cual pertenece.

En todos los grupos se realizará el procedimiento de fertilización *in vitro* de la misma manera que en las pacientes que no participen en el estudio. Así mismo, de quedar embarazada, el seguimiento se realizará en el servicio de Obstetricia del Hospital de la Santa Creu i Sant Pau, de como el de cualquier otro embarazo obtenido por FIV.

ACTIVIDADES DEL ESTUDIO

La duración del tratamiento en este estudio es de 6 meses; los 6 meses previos a su fertilización *in vitro*.

Durante los 6 meses del estudio, realizaríamos las siguientes visitas, que no se realizan de manera rutinaria:

1. *Visita 1:* analítica de sangre y ecografía de su corazón, así como mediciones corporales (i.e. peso, tensión arterial, composición corporal). Le daremos sus cápsulas o suplementos para los siguientes 2 meses.
2. *Visita 2:* 2 meses; le proporcionaremos cápsulas o suplementos para los siguientes 2 meses, y le pediremos que conteste algunos cuestionarios.
3. *Visita 3:* 4 meses; le proporcionaremos cápsulas o suplementos para los siguientes 2 meses, y le pediremos que conteste algunos cuestionarios.
4. *Visita 4:* analítica de sangre y ecografía de su corazón, así como mediciones corporales. Le realizaremos algunos cuestionarios sobre sus hábitos y se terminaría el periodo de tratamiento del estudio.

Posterior a este periodo, usted realizará su tratamiento de estimulación ovárica, fertilización *in vitro* y transferencia de embriones, igual que cualquier otra paciente que no ha participado en el estudio. Si fuera exitoso el procedimiento, se ofrecerá realizar el seguimiento de su embarazo en el servicio de Obstetricia del Hospital de la Santa Creu i Sant Pau, y se harían 2 visitas extras a las rutinarias del embarazo:

5. *Visita 5:* en semana 11-14 del embarazo, nueva analítica de sangre y ecografía de su corazón.
6. *Visita 6:* en semana 28-32 del embarazo, nueva analítica de sangre, ecografía de su corazón y una ecografía especializada del corazón de su bebé.

Finalmente, le pediremos autorización para que después del parto podamos realizar una toma de muestras de sangre del cordón umbilical y la placenta. Las visitas propuestas de este estudio están resumidas en una tabla a continuación:

	V1 Inicio	**V2 Seguimiento 2 meses**	**V3 Seguimiento 4 meses**	**V4 Final 6 meses**	**V5 Embarazo 11-14 sem**	**V6 Embarazo 28-32 sem**
Cuestionarios	X	X	X	X		
Mediciones corporales	X			X	X	X
Análisis de sangre	X			X	X	X
Ecocardiografía	X			X	X	X
Recoger suplementos/cápsulas		X	X	X		
Ecocardiografía fetal						X

RIESGOS Y MOLESTIAS DERIVADOS DE SU PARTICIPACIÓN EN EL ESTUDIO

La aspirina es un medicamento bastante común y que está comercializado ampliamente como analgésico. En nuestro medio, la dosis baja la utilizan pacientes con riesgo cardiovascular como los que padecen hipertensión, para prevenir complicaciones. De manera

más reciente, se utiliza durante el embarazo en pacientes con riesgo alto de presentar enfermedades placentarias como la preeclampsia. Existe una experiencia amplia en humanos, por lo tanto, de su seguridad. Las molestias más comúnmente asociadas a la aspirina son de tipo gástrico; no la deben tomar pacientes que tengan gastritis crónica o úlcera gástrica. También en algunas enfermedades como el asma, puede existir una mayor probabilidad de tener un episodio, aunque esto no se considera una contraindicación para su uso.

La dieta Mediterránea consiste en realizar un cambio de alimentación, incorporando una mayor cantidad de aceite de oliva extra-virgen, nueces y almendras, y concientizando el número de porciones de pescado, legumbres, etc. que se ingieren por semana. No existe una contraindicación como tal para la misma, aunque pacientes con intolerancias alimentarias pueden presentar algunas molestias. La contraindicación principal para esta intervención es tener una alergia a alguno de los elementos que la componen, principalmente a las nueces o almendras. Las molestias más comunes son distensión, sensación de plenitud, indigestión, o cambios en sus hábitos intestinales (estreñimiento o diarrea), y usualmente duran por poco tiempo.

En cuanto a otras molestias que pueden ser dadas por su participación en el estudio, las principales serán derivadas de la toma de sangre para análisis de laboratorio, y el acudir a sus visitas de seguimiento cada 2 meses. Las visitas 1 y 4 tendrán una duración aproximada de 30 minutos, dado que se deberán realizar mediciones corporales y la ecografía de su corazón; las visitas 2 y 3 serán más cortas o inclusive puede ser que se le propongan vía telefónica, contestando los cuestionarios por esta vía o por internet. En este caso, la paciente será responsable de acudir por las cápsulas o suplementos para los siguientes 2 meses de tratamiento.

Se consideran responsabilidades directas de la paciente participante:

- Cumplir con las visitas y actividades del estudio.
- Notificar cualquier evento adverso que le suceda o cambios en medicación.

Excepto en caso de una urgencia, se solicitará que no modifique la medicación que se le otorgue, ni tome otros medicamentos o "plantas medicinales" sin consultar antes con nosotros.

POSIBLES BENEFICIOS

Es posible que de su participación en este estudio no obtenga un beneficio directo del tratamiento que le proporcionemos. Sin embargo, la estrategia nutricional se ha probado previamente en pacientes obesas con mejoría en la tasa de embarazos por FIV, aunque no se ha comprobado en pacientes con peso normal. La aspirina también se ha probado en pacientes con enfermedades concomitantes y mejora las probabilidades de embarazo en la FIV, pero no se utiliza de rutina. La evaluación de estas dos estrategias nos permitirá saber si deberían de implementarse o sugerirse en pacientes que buscan el embarazo por FIV, además de que secundariamente sabremos si le beneficia a la madre en su función cardiovascular y al bebé, con lo cual pueden existir beneficios a largo plazo para los dos.

TRATAMIENTOS ALTERNATIVOS

Actualmente no existen tratamientos alternativos a los propuestos en este estudio, ya que a las pacientes en lista de espera para una FIV no se les propone ninguna intervención en el periodo antes de iniciar el ciclo de tratamiento.

SEGURO

De acuerdo con el Real Decreto 1090/2015 por el que se regulan los ensayos clínicos con medicamentos, "los daños y perjuicios sobre el sujeto de estudio que pudieran resultar como consecuencia de un ensayo clínico de bajo nivel de intervención no precisarán estar cubiertos por un contrato de seguro o garantía financiera si los mismos estuvieran cubiertos por el seguro de responsabilidad civil profesional individual o colectivo o garantía financiera equivalente del centro sanitario donde se lleve a cabo el ensayo clínico".

Por tanto, dado que en este ensayo se utilizarán medicamentos autorizados, en indicaciones respaldadas por datos científicos, y que los procedimientos adicionales a los de la práctica habitual suponen un riesgo mínimo comparado con el de la práctica habitual, es decir se cumplen los criterios de ensayo clínico de bajo nivel de intervención, la póliza del estudio será la póliza asistencial del centro dónde se realiza la investigación.

PROTECCIÓN DE DATOS PERSONALES

En cuanto a la confidencialidad de sus datos personales, debe saber que el tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se ajustará a lo dispuesto en el Reglamento (UE) nº 2016/679 y a la Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los Derechos Digitales, con el Real Decreto por el que se aprueba el Reglamento de desarrollo de la Ley Orgánica y la demás normativa vigente.

Tanto el Centro como el Promotor son responsables respectivamente del tratamiento de sus datos y se comprometen a cumplir con la normativa de protección de datos en vigor. Los datos recogidos para el estudio estarán identificados mediante un código y sólo su médico del estudio/colaboradores podrán relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna salvo excepciones, en caso de urgencia médica o requerimiento legal.

El promotor adoptará las medidas pertinentes para garantizar la protección de su privacidad y no permitirá que sus datos se crucen con otras bases de datos que pudieran permitir su identificación.

El acceso a su información personal quedará restringido al médico del estudio/colaboradores, autoridades sanitarias (Agencia Española de Medicamentos y Productos Sanitarios, autoridades sanitarias extranjeras), al Comité de Ética de la Investigación y personal autorizado por el promotor (monitores del estudio, auditores), cuando lo precisen para comprobar los datos y procedimientos del estudio, pero siempre manteniendo la confidencialidad de los mismos de acuerdo a la legislación vigente.

De acuerdo a lo que establece la legislación de protección de datos, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos. Además puede

limitar el tratamiento de datos que sean incorrectos, solicitar una copia o que se trasladen a un tercero (portabilidad) los datos que usted ha facilitado para el estudio. Para ejercitar sus derechos puede dirigirse al investigador principal del estudio. También puede ejercitar sus derechos remitiendo una comunicación por escrito a la siguiente dirección: c/Sant Quintí 77-79 08041 Barcelona. Así mismo tiene derecho a dirigirse a la Agencia de Protección de Datos si no quedara satisfecho/a.

Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos. Sin embargo, debe tener en cuenta que los datos no se pueden eliminar aunque deje de participar en el estudio, para garantizar la validez de la investigación y cumplir con los deberes legales.

El investigador y el promotor están obligados a conservar los datos recogidos para el estudio al menos hasta 25 años tras su finalización. Posteriormente, su información personal sólo se conservará por el centro para el cuidado de su salud y por el promotor para otros fines de investigación científica si usted hubiera otorgado su consentimiento para ello, o si así lo permite la ley y los requisitos éticos aplicables.

Los datos codificados pueden ser transmitidos a terceros y a otros países de la UE pero en ningún caso contendrán información que le pueda identificar directamente, como nombre y apellidos, iniciales, dirección, nº de la seguridad social, etc. En el caso de que se produzca esta cesión, será para los mismos fines del estudio descrito o para su uso en publicaciones científicas pero siempre manteniendo la confidencialidad de los mismos de acuerdo a la legislación vigente. Si realizáramos transferencia de sus datos codificados fuera de la UE a las entidades de nuestro grupo, a prestadores de servicios o a investigadores científicos que colaboren con nosotros, los datos del participante quedarán protegidos con salvaguardas tales como contratos u otros mecanismos por las autoridades de protección de datos. Si el participante quiere saber más al respecto, puede contactar con el Investigador Principal del estudio o con el Delegado de Protección de Datos del promotor mediante correo electrónico a dpo_ir@santpau.cat.

GASTOS Y COMPENSACIÓN ECONÓMICA

El promotor del estudio es el responsable de gestionar la financiación del mismo. Para la realización del estudio el promotor del mismo ha firmado un contrato con el centro donde se va a realizar. Usted no tendrá que pagar por los medicamentos ni por pruebas específicas del estudio. Su participación en el estudio no le supondrá ningún gasto adicional a la práctica clínica habitual.

TRATAMIENTO A RECIBIR TRAS FINALIZAR EL ENSAYO CLÍNICO

Cuando termine su participación en este estudio, Usted recibirá el mejor tratamiento disponible y que su médico considere el más adecuado para su infertilidad. El seguimiento y manejo de su embarazo también se realizará como a cualquier paciente que haya realizado una FIV y no haya participado en el estudio.

OBTENCIÓN Y UTILIZACIÓN DE MUESTRAS BIOLÓGICAS

La obtención, manejo, identificación y almacenamiento de muestras biológicas se realizará en cumplimiento de la Ley de Investigación Biomédica 14/2007 y el Real Decreto 1716/2011. De acuerdo a la legislación mencionada, la obtención de muestras biológicas con fines de investigación biomédica podrá realizarse únicamente cuando se haya obtenido previamente el consentimiento escrito del paciente y puede ser revocado.

Una vez terminado el ensayo las muestras que se haya/n recogido de forma específica para este se guardarán en la colección C.0004338 del biobanco del IIB Sant Pau, si usted lo autoriza mediante la firma del consentimiento específico (documento aparte).

OTRA INFORMACIÓN RELEVANTE

Una descripción de este ensayo clínico estará disponible en http://reec.aemps.es, según exige la legislación española.

Cualquier nueva información referente a los fármacos utilizados en el estudio y que pueda afectar a su disposición para participar en el estudio, que se descubra durante su participación, le será comunicada por su médico lo antes posible.

Debe saber que puede ser excluido del estudio si el promotor o los investigadores del estudio lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca por la medicación en estudio o porque consideren que no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo que ha ocasionado su retirada del estudio.

En caso de que Usted abandone el estudio sin retirar su consentimiento (por ejemplo, no acuda a las visitas de seguimiento, realice una FIV de manera externa o realice el parto en otro hospital), quisiéramos su autorización para poder llamarle o acceder a su historia clínica compartida para ver el desenlace de su embarazo.

Debe usted saber que es posible que su médico de Atención Primaria tenga conocimiento de su participación en este estudio.

CONTACTO EN CASO DE DUDAS

Si durante su participación tiene alguna duda o necesita obtener más información, póngase en contacto con cualquiera de las dos investigadoras principales:

- Dra. Ana Polo Ramos, Reproducción Asistida, Hospital de la Santa Creu i Sant Pau – Fundació Puigvert, apolo@santpau.cat, , teléfono 932919000, Ext. 7041.
- Dra. Mónica Cruz Lemini, Obstetricia y Ginecología, Hospital de la Santa Creu i Sant Pau, mcruzl@santpau.cat, teléfono 932919000, Ext. 7041.

Al firmar la hoja de consentimiento adjunta, se compromete a cumplir con los procedimientos del estudio que se le han expuesto.

CONSENTIMIENTO INFORMADO

TÍTULO DEL ESTUDIO: Impacto del ejercicio y la dieta Mediterránea *vs* aspirina en la tasa de nacimientos vivos y programación cardiovascular en la fertilización *in vitro*: un estudio aleatorizado.

Yo, ……*(nombre y apellidos del participante)*

He leído la hoja de información que se me ha entregado sobre el estudio.

He podido hacer preguntas sobre el estudio.

He recibido suficiente información sobre el estudio.

He hablado con …….. (*nombre y apellidos del investigador)*

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

- Cuando quiera.
- Sin tener que dar explicaciones.
- Sin que esto repercuta en mis cuidados médicos.

Presto libremente mi conformidad para participar en el estudio y doy mi consentimiento para el acceso y utilización de mis datos en las condiciones detalladas en la hoja de información.

Deseo que me comuniquen la información derivada de la investigación que pueda ser relevante para mí salud:

☐ SÍ ☐ NO

Recibiré una copia firmada y fechada de este documento de consentimiento informado

Firma del participante	**Firma del investigador**
Fecha:	**Fecha**:

(copia de la paciente)

CONSENTIMIENTO INFORMADO

TÍTULO DEL ESTUDIO: Impacto del ejercicio y la dieta Mediterránea *vs* aspirina en la tasa de nacimientos vivos y programación cardiovascular en la fertilización *in vitro*: un estudio aleatorizado.

Yo, ……*(nombre y apellidos del participante)*

He leído la hoja de información que se me ha entregado sobre el estudio.

He podido hacer preguntas sobre el estudio.

He recibido suficiente información sobre el estudio.

He hablado con …….. (*nombre y apellidos del investigador)*

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

- Cuando quiera.
- Sin tener que dar explicaciones.
- Sin que esto repercuta en mis cuidados médicos.

Presto libremente mi conformidad para participar en el estudio y doy mi consentimiento para el acceso y utilización de mis datos en las condiciones detalladas en la hoja de información.

Deseo que me comuniquen la información derivada de la investigación que pueda ser relevante para mí salud:

☐ SÍ ☐ NO

Recibiré una copia firmada y fechada de este documento de consentimiento informado

Firma del participante	**Firma del investigador**
Fecha:	**Fecha**:
	(copia del investigador)

14.3. Diet and exercise interventions

14.3.1. Dietary interventions

Dietary interventions will be based on the study published by Estruch R, et al. in the New England Journal of Medicine (the PREDIMED trial).

Table 1. Summary of Dietary Recommendations to Participants in the Mediterranean-Diet Groups and the Control-Diet Group.

Food	Goal
Mediterranean diet	
Recommended	
Olive oil*	≥4 tbsp/day
Tree nuts and peanuts†	≥3 servings/wk
Fresh fruits	≥3 servings/day
Vegetables	≥2 servings/day
Fish (especially fatty fish), seafood	≥3 servings/wk
Legumes	≥3 servings/wk
Sofrito‡	≥2 servings/wk
White meat	Instead of red meat
Wine with meals (optionally, only for habitual drinkers)	≥7 glasses/wk
Discouraged	
Soda drinks	<1 drink/day
Commercial bakery goods, sweets, and pastries§	<3 servings/wk
Spread fats	<1 serving/day
Red and processed meats	<1 serving/day
Low-fat diet (control)	
Recommended	
Low-fat dairy products	≥3 servings/day
Bread, potatoes, pasta, rice	≥3 servings/day
Fresh fruits	≥3 servings/day
Vegetables	≥2 servings/day
Lean fish and seafood	≥3 servings/wk
Discouraged	
Vegetable oils (including olive oil)	≤2 tbsp/day
Commercial bakery goods, sweets, and pastries§	≤1 serving/wk
Nuts and fried snacks	≤1 serving /wk
Red and processed fatty meats	≤1 serving/wk
Visible fat in meats and soups¶	Always remove
Fatty fish, seafood canned in oil	≤1 serving/wk
Spread fats	≤1 serving/wk
Sofrito‡	≤2 servings/wk

* The amount of olive oil includes oil used for cooking and salads and oil consumed in meals eaten outside the home. In the group assigned to the Mediterranean diet with extra-virgin olive oil, the goal was to consume 50 g (approximately 4 tbsp) or more per day of the polyphenol-rich olive oil supplied, instead of the ordinary refined variety, which is low in polyphenols.
† For participants assigned to the Mediterranean diet with nuts, the recommended consumption was one daily serving (30 g, composed of 15 g of walnuts, 7.5 g of almonds, and 7.5 g of hazelnuts).
‡ Sofrito is a sauce made with tomato and onion, often including garlic and aromatic herbs, and slowly simmered with olive oil.
§ Commercial bakery goods, sweets, and pastries (not homemade) included cakes, cookies, biscuits, and custard.
¶ Participants were advised to remove the visible fat (or the skin) of chicken, duck, pork, lamb, or veal before cooking and the fat of soups, broths, and cooked meat dishes before consumption.

Mediterranean Diet

- Extra-virgin olive oil, >=50 grams (4 tbsp) daily, including cooking oil for frying
- Nuts/Walnuts, 15 grams, at least 3 times per week
- Almonds 7.5 grams, at least 3 times per week

Questionnaire

Follow-up and compliance to the diet will be evaluated by Dietists by means of the validated 14-item questionnaire of adherence to the Mediterranean diet.

14.3.2. Exercise interventions

Exercise monitoring band

- Through the evaluation of the REGICOR questionnaire and the initial personal interview, in which we will ask questions regarding the state of health and possible previous injuries, the patient will receive a personalized template with the daily and weekly objectives, in terms of the total number of steps, including the exercises indicated for the warm-up and cool-down phases. These exercises will help to fine-tune the body to avoid possible injuries derived from sports practice.
- We must take into account that sports practice consisting of a more or less vigorous walk, in the case of poorly trained women, and a gentle trot for trained women, should not involve any excessive overload at the joint level that can cause injuries.
- The nature of the exercise does not imply the learning of a complex technique, since it involves movements present in the daily life of patients.
- The average number of steps per week will be recorded with the exercise monitoring band provided by the researchers, as well as the basal heart rate for the patient, and total sleep hours. This information will be recorded in all follow-up visits.

Questionnaires

- Physical activity questionnaire (REGICOR): validated questionnaire for quantifying physical activity in our population.
- Perceived Stress Scale (PSS) Questionnaire: validated questionnaire for quantifying perceived stress in our population.
- FAGERSTROM Questionnaire: validated questionnaire for quantifying dependence on smoking in our population.

14.4. Mediterranean Diet Adherence Questionnaire

	Dieta y frecuencia de consumo	Criterios para 1 punto (0 si no se cumple)	Ptos
1.	¿Usa usted el aceite de oliva como principal grasa para cocinar?	Sí	
2.	¿Cuánto aceite de oliva consume en total al día (incluyendo el usado para freír, comidas fuera de casa, ensaladas, etc)?	4 o más cucharadas	
3.	¿Cuántas porciones de verdura u hortalizas consume al día? (*1 porción=200g; guarniciones=1/2 porción*)	2 o más (al menos una de ellas en ensalada o crudas)	
4.	¿Cuántas piezas de fruta (incluyendo zumo natural) consume al día?	3 o más	
5.	¿Cuántas porciones de carnes rojas, hamburguesas, o embutidos (salchichas, jamón) consume al día? (*1 porción=100 - 150g*)	Menos de 1	
6.	¿Cuántas porciones de mantequilla, margarina o nata consume al día? (*1 ración=12g*)	Menos de 1	
7.	¿Cuántas bebidas carbonatadas y/o azucaradas consume al día?	Menos de 1	
8.	¿Cuántas copas de vino consume a la semana?	7 o más copas	
9.	¿Cuántas porciones de legumbres consume a la semana? (*1 porción=150g*)	3 o más	
10.	¿Cuántas porciones de pescado o mariscos consume a la semana? (*1 porción=100-150g de pescado, o 4-5 unidades o 200g de marisco*)	3 o más	
11.	¿Cuántas veces consume repostería comercial (no casera) como galletas, flanes, dulces o pasteles a la semana?	Menos de 3	
12.	¿Cuántas porciones de frutos secos (incluyendo cacahuetes) consume a la semana? (*1 porción=30g*)	3 o más	
13.	¿Consume usted preferentemente carne de pollo, pavo o conejo en vez de ternera, cerdo, hamburguesas o salchichas?	Sí	
14.	¿Cuántas veces a la semana consume vegetales, pasta, arroz u otros platos aderezados con sofrito (salsa de tomate con cebolla, puerro o ajo, elaborada con aceite de oliva?	2 o más	
		PUNTUACIÓN	

14.5. REGICOR Questionnaire

Cuestionario abreviado de REGICOR de la actividad física en el tiempo libre

A continuación le preguntaremos sobre actividades físicas realizadas en un mes normal.

En un mes normal:

1.a. ¿Cuántos días pasea o camina tranquilamente?† |__|__| días

1.b. De promedio, ¿cuántos minutos al día? |__|__|__| minutos/día

2.a. ¿Cuántos días camina deprisa? |__|__|__| minutos/día

3.a. ¿Cuántos días camina campo a través, va de excursión? |__|__| días

3.b. De promedio, ¿cuántos minutos al día? |__|__|__| minutos/día

4.a. ¿Cuántos días sube escaleras? |__|__| días

4.b. De promedio, ¿cuántos pisos al día? |__|__|__| pisos/día

5.a. ¿Cuántos días trabaja en el huerto o en el jardín? |__|__| días

5.b. De promedio, ¿cuántos minutos al día? |__|__|__| minutos/día

6.a. ¿Cuántos días hace ejercicios, deportes al aire libre o en casa o en el gimnasio? |__|__| días

6.b. De promedio, ¿cuántos minutos al día? |__|__|__| minutos/día

† NOTA: Incluye caminar al trabajo. También se puede incluir ir en bicicleta al trabajo o pasear en bicicleta.

Comportamiento sedentario

- De promedio, durante un día laborable y fuera del trabajo ¿cuántas horas al día está viendo la televisión/ordenador/videojuegos? |__|__| horas/día
- De promedio, durante un día no laborable ¿cuántas horas al día está viendo la televisión/ordenador/videojuegos? |__|__| horas/día
- ¿Cuántos días trabaja a la semana? |__| días

Source: Molina et al., 2017.

14.6. Perceived Stress Scale (PSS) Questionnaire

	Nunca	Casi nunca	De vez en cuando	A menudo	Muy a menudo
1. En el último mes, ¿con qué frecuencia ha estado afectado por algo que ha ocurrido inesperadamente?	0	1	2	3	4
2. En el último mes, ¿con qué frecuencia se ha sentido incapaz de controlar las cosas importantes en su vida?	0	1	2	3	4
3. En el último mes, ¿con qué frecuencia se ha sentido nervioso o estresado?	0	1	2	3	4
4. En el último mes, ¿con qué frecuencia ha manejado con éxito los pequeños problemas irritantes de la vida?	0	1	2	3	4
5. En el último mes, ¿con qué frecuencia ha sentido que ha afrontado efectivamente los cambios importantes que han estado ocurriendo en su vida?	0	1	2	3	4
6. En el último mes, ¿con qué frecuencia ha estado seguro sobre su capacidad para manejar sus problemas personales?	0	1	2	3	4
7. En el último mes, ¿con qué frecuencia ha sentido que las cosas le van bien?	0	1	2	3	4
8. En el último mes, ¿con qué frecuencia ha sentido que no podía afrontar todas las cosas que tenía que hacer?	0	1	2	3	4
9. En el último mes, ¿con qué frecuencia ha podido controlar las dificultades de su vida?	0	1	2	3	4
10. En el último mes, ¿con que frecuencia se ha sentido que tenía todo bajo control?	0	1	2	3	4
11. En el último mes, ¿con qué frecuencia ha estado enfadado porque las cosas que le han ocurrido estaban fuera de su control?	0	1	2	3	4
12. En el último mes, ¿con qué frecuencia ha pensado sobre las cosas que le quedan por hacer?	0	1	2	3	4
13. En el último mes, ¿con qué frecuencia ha podido controlar la forma de pasar el tiempo?	0	1	2	3	4
14. En el último mes, ¿con qué frecuencia ha sentido que las dificultades se acumulan tanto que no puede superarlas?	0	1	2	3	4

Source: Cohen et al., 1983.

14.7. FAGERSTROM Questionnaire

Pregunta	Respuesta	Puntuación
¿Cuánto tiempo después de despertarse fuma su primer cigarrillo?	Menos de 5 minutos 6-30 minutos 31-60 minutos Más de 60 minutos	3 2 1 0
¿Encuentra dificultad para no fumar en los sitios donde está prohibido (cine...)?	Sí No	1 0
¿Qué cigarrillo le desagrada más dejar de fumar?	El primero Otros	1 0
¿Cuántos cigarrillos fuma cada día?	Más de 30 21-30 11-20 Menos de 11	3 2 1 0
¿Fuma más durante las primeras horas tras levantarse que durante el resto del día?	Sí No	>1 0
¿Fuma aunque esté tan enfermo que tenga que guardar cama la mayor parte del día?	Sí No	1 0

Source: Fagerström, 1978.

14.8. Maternal cardiovascular evaluation

14.8.1. Echocardiographic assessment

Performed by a Cardiologist specialized in cardiac imaging according to the usual standard protocol. High-resolution images will be acquired and post-processed with dedicated software for speckle tracking analysis.

The following 2D echocardiographic parameters will be measured: heart rate, end-systolic and diastolic diameters and volumes, interventricular septum and posterior wall thickness, left ventricular ejection fraction obtained by Teichholz and Simpson methods, left atrial diameters and volume by Simpson method, left ventricle outflow tract diameter, TAPSE, mitral valve diameter, aortic valve diameter, trans-mitral and trans-aortic Doppler flow velocities, with continuous and pulsed Doppler. Derived parameters: left ventricular shortening fraction, stroke volume and cardiac output.

Offline analysis: 2D clips will be obtained for offline analysis with speckle tracking software (Automated Cardiac Motion Quantification (aCMQ-STRAIN) by Philips). 4-chamber, 2-chamber and 3-chamber views will be analyzed. The software will automatically provide a Region of Interest (ROI) so that the strain of the selected tissue can be evaluated. The user may also manually specify a ROI template by identifying three points – one point on each side of the mitral annulus and one point at the apex. The system will then automatically identify the endocardial boundary and create an ROI that extends from the endocardial boundary a fixed distance outward toward the epicardium. Tissue motion will be tracked over a cardiac cycle using a speckle tracking algorithm. We will use the global longitudinal strain to predict maternal heart function. Longitudinal strain is calculated as the change in length of each of the regions as compared to its relaxed length.

14.8.2. Carotid intima-media thickness (cIMT)

Transverse longitudinal evaluation of the common carotid artery, its bifurcation and internal carotid artery. Thickness of the intima layer is measured at the middle third of the common carotid artery and is considered the measurement between the tunica intima and tunica media, the innermost two layers of the wall of an artery, in millimeters.

14.9. Fetal cardiovascular evaluation

Performed by a Fetal Medicine Specialist according to the usual standard protocol. The following echocardiographic parameters will be evaluated:

- Conventional fetoplacental Doppler evaluation: uterine arteries, umbilical artery, middle cerebral artery, ductus venosus.
- Cardiovascular morphometric parameters: cardiothoracic ratio, atrial areas, cardiac and ventricular sphericity indexes and wall thicknesses in systole and diastole.
- Systolic function parameters: stroke volume, cardiac output, combined cardiac output, cardiac index, shortening and ejection fractions, mitral/tricuspid annular plane systolic excursion (MAPSE/TAPSE) and systolic annular peak velocities (S').
- Diastolic function parameters: peak early (E) and late (A) transvalvular filling velocities, E/A ratio, early-diastolic (E') and atrial contraction (A') annular peak velocities, E/E' ratio, E'/A' ratio, ventricular filling times and fractions, E wave deceleration time, A wave duration time.
- Global function parameters: myocardial performance index by pulsed and tissue Doppler.
- Myocardial deformation: strain and strain-rate by offline 2D speckle tracking analysis.