IVIRMA Global

Clinical Trial Research Protocol

Follicular Esteroidogenesis in Controlled Ovarian Stimulation

EudraCT: 2015-005762-28

NCT: NCT02738580

PROTOCOL REVISION HISTORY

Version	Date	State
1	17-DEC-2015	Final

IVI Foundation

Project Title

Follicular Esteroidogenesis in Controlled Ovarian Stimulation

Acronym:	**¡Error! No se encuentra el origen de la referencia.**ESTEFOL
EudraCT:	2015-005762-28**¡Error! No se encuentra el origen de la referencia.**
Internal Code:	**¡Error! No se encuentra el origen de la referencia.**1512-VLC-066-EB
Development Phase:	Fase IV**¡Error! No se encuentra el origen de la referencia.**
Investigational Medicinal Product (IMP):	Recombinant FSH, Highly Purified HMG, Ganirelix, Triptorelin
Research Area:	Ovarian function and stimulation
Report entry:	12/17/2015

key words:

Estradiol, Pregnenolone, Progesterone, Testosterone, Androstenodione, ovarian stimulation

Sponsor Information

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Responsibilities and Signatures

By signing this protocol from the project entitled: *Follicular Esteroidogenesis in Controlled Ovarian Stimulation*

Those undersigning state that:

- This clinical trial respects the ethical and legal rules and follows good clinical practice in its implementation
- It has the material and human resources needed to carry out the study, without interfering in other studies or clinical tasks usually entrusted to them
- They are committed that each subject is treated and controlled according the approval granted by the Ethics Committee for Clinical Research, Institutional Review Board, remaining committees and the involved authorities
- Collaborators included in this study are adequately trained for its implementation, they will have an active participation, and they consent thereto.

Sponsor

Clínica IVI Valencia Dr/a. Amparo Ruiz Director/a IVI Valencia	12/17/2015

Principal Investigator

Ernesto Bosch Aparicio Servicio Ginecología y Reproducción Humana Clínica IVI Valencia	12/17/2015

Protocol Synopsis

Protocol Title: Follicular Esteroidogenesis in Controlled Ovarian Stimulation

EudraCT Code: 2015-005762-28

Sponsor´s protocol version and date: 1.0 of 12/1/2015

Sponsor:

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Phase: Fase IV

Centros participantes:

IVI Valencia

Investigational Medicinal Product (IMP): Recombinant FSH, Highly Purified HMG, Ganirelix, Triptorelin

Primary Objective: To compare the serum concentrations of the different hormones involved in follicular steroidogenesis during an EOC cycle between FSHr and hpHMG.

Secundary Objectives:

- Define the area under the curve for each of the follicular steroids along the EOC in cycles stimulated with FSHr and hpHMG.

- Calculate the contribution to the circulation of each of the follicular steroids per unit of follicular volume.

- To identify the metabolic pathways of follicular steroidogenesis as a function of the type of gonadotrophin used for COC.

- To compare the concentration of steroids in follicular liquid in stimulated cycles with FSHr and hpHMG.

- Correlate the hormone levels observed in serum with the concentration in follicular fluid]

Methodology: 110 women will be included (55 in each study group, i) FSHr; ii) hpHMG) with normal ovarian function, who will follow ovarian stimulation in cycle with GnRH Antagonists. For greater uniformity in the study population, oocyte donors will be used.

Serum extractions and transvaginal ultrasound for folliculometry will be performed on the days of stimulation 1, 4, 6, 8 and the day of administration of the triptorelin. In the follicular puncture for the extraction of the oocytes, the first follicle will be individually aspirated to obtain the follicular fluid. The samples obtained will be frozen and stored at -80ºC for later analysis.

In each serum sample they will be determined: Pregnenolone, Progesterone, 17-OH-Progesterone, Dehydroepiandrostenodione, Androstenodione, Testosterone, Estrone and Estradiol.

Each day's hormone concentration will be compared between the two groups, so that the profile of each of the hormones can be defined for both stimulation protocols, and thus the follicular steroidogenesis profile. The area under the curve during ovarian stimulation will be calculated for each of the hormones included in the study, and both groups will be compared. The proportion of patients with elevated Progesterone on the last day of stimulation (>1.5 ng/mL) will be compared between both groups.

Follicular fluid concentrations will be defined for each of the steroids analyzed, and the values between both groups will be compared, as well as their correlation with the levels observed in serum.

Clinical Trial Prospective randomized, open, parallel groups.

Number of subjects: 110

A previous study conducted by our group (Bosch et al, 2008) in which these two same protocols were compared in terms of clinical outcomes, showed that the serum P on the last day of stimulation was 0.73 ± 0.42 in the HMG group vs 0.99 ± 0.48 in the FSH group ($p<0.001$). According to these data, to determine a difference of this magnitude with a 95% confidence level (5% alpha error) and 80% power (20% beta error), 48 patients per group are required. Assuming a loss rate of 15%, 55 patients should be randomly assigned to each group, i.e. 110 patients in total

Inclusion criteria:

- Women 18-35 years old
- Good state of psycho-physical health
- Normal menstrual cycle (25-35 days)
- Normal ovarian reserve, determined by an AMH=10-30 pMol/L)
- BMI < 25.0
- Other criteria to be met by oocyte donors

Exclusion criteria:

- Kidney failure
- Polycystic ovary
- BMI >=25.0
- Any systemic or metabolic disorder that contraindicates the use of gonadotrophins.
- Any reason for exclusion from the oocyte donation program.

Test Product, Dose and Mode of Administration:

Arm 1:

MEDICATION: Recombinant FSH

Therapeutic group: Gonadotrophins for ovarian stimulation

Route of administration: subcutaneous

Dose: 225 IU/day

Arm 2:

MEDICATION: Highly purified HMG

Therapeutic group: Gonadotrophins for ovarian stimulation

Route of administration: subcutaneous

Dose: 225 IU/day

Reference Therapy, dose and Mode of administration:

Duration of study:

- Recruitment period: April 2016-July 2016
- Estimated end-of-treatment date of the last patient: October 2016
- End of study date: December 2016

Schedule of Assessments:

The patients will be recruited from the population of oocyte donors of the IVI Valencia programme. Patients will come to the clinic on their 2nd day of menstruation of the cycle in which ovarian stimulation is to be performed. At that time patients will be randomly assigned 1:1 to start COCs with 225 IU/day of FSHr (Gonal F) or hpHMG (Menopur). On the 6th day of stimulation, all patients will start a daily dose of 0.25 mg of the antagonist (Orgalutran), until the day of the final induction of oocyatric maturation. When at least 8 follicles reach a diameter of at least 17 mm, a bolus of 0.2 mg of triptorelin (Decapeptyl) should be administered. Follicular puncture will be performed 36 hours later, according to usual clinical practice.

Serum extractions and transvaginal ultrasound for folliculometry will be performed on the days of stimulation 1, 4, 6, 8 and the day of administration of the triptorelin, and the follicular liquid of the first aspirated follicle will be individually aspirated. The samples obtained will be frozen and stored at -80ºC for later analysis.

Each serum sample shall be determined:

- Pregnenolone
- Progesterone
- 17-OH-Progesterone
- Dehydroepiandrostenodiona
- Androstenodione
- Testosterone
- Strona
- Estradiol
- FSH
- LH

Statistical Methods and Data Analysis

1. Population

It will include 110 women (55 in each study group) with normal ovarian function, aged between 18 and 35 years, who will follow ovarian stimulation in cycle with GnRH antagonists. For greater uniformity in the study population, oocyte donors will be used.

2. Sample size

A previous study conducted by our group (Bosch et al, 2008) in which these two same protocols were compared in terms of clinical outcomes, showed that the serum P on the last day of stimulation was 0.73 ± 0.42 in the HMG group vs 0.99 ± 0.48 in the FSH group ($p<0.001$). According to these data, to determine a difference of this magnitude with a 95% confidence level (5% alpha error) and 80% power (20% beta error), 48 patients per group are required. Assuming a loss rate of 15%, 55 patients should be randomly assigned to each group, i.e. 110 patients in total.

3. Study Variables and Database

3.1. Main variable:

Area under the serum progesterone curve during ovarian stimulation

3.2. Secondary variables:

- Area under the serum estradiol curve during ovarian stimulation
- Area under the serum Pregnenolone curve during ovarian stimulation
- Area under the 17-OH serum progesterone curve during ovarian stimulation
- Area under the serum Androstenodione curve during ovarian stimulation
- Area under the serum Dehydroandrostenodione curve during ovarian stimulation
- Area under the serum Testosterone curve during ovarian stimulation
- Area under the curve of Serum Strone during ovarian stimulation
- Area under the serum FSH curve during ovarian stimulation
- Area under the serum LH curve during ovarian stimulation
- Concentration of P in follicular liquid
- Concentration of Estradiol in follicular liquid
- Concentration of Pregnenolone in follicular liquid
- Concentration of 17-OH Progesterone in follicular liquid
- Concentration of Androstenodione in follicular liquid
- Concentration of Dehydroepiandrostenodione in follicular liquid
- Concentration of Testosterone in follicular liquid
- Concentration of Estrone in follicular liquid

- Concentration of FSH in follicular liquid
- Concentration of LH in follicular liquid

3.3. Control variables

- Total number of oocytes
- Number of mature oocytes (metaphase II)
- Number of follicles > 10 mm on the last day of stimulation
- Total follicular mass defined by the sum of all follicular volumes (defined by the formula 4/3 x πr3 , where r=radius=diameter/2) in each of the ultrasound controls.

3.4. Descriptive variables:

- Age
- Body Mass Index
- Stimulation days
- Total dose of gonadotrophins
- Days with antagonist GnRH

4. Statistical analysis

4.1. Descriptive data analysis:

Description of continuous quantitative variables (all study variables are) in their central parameters (mean and median) and dispersion values (standard deviation, p10, p25, p75, p90, maximum and minimum).

4.2. Intergroup homogeneity analysis:

Comparison of study groups from the point of view of basal and demographic characteristics using Student Test "t" after checking the assumption of normality using Levéne Variance Equality Test. In the case of non-parametric distribution, the Mann-Whitney test will be used.

4.3. Analysis of the working hypothesis:

Each hormonal determination of each day of stimulation in which it is determined will be compared by means of Student "t" for independent samples, once the normal distribution of the values by means of the tests of Kolmogorov-Smirnov and Shapiro-Wilks has been verified. Otherwise, comparisons will be made with the Mann-Whitney test. The area under the curve during ovarian stimulation will be calculated for each of the hormones included in the study, and both groups will be compared equally. The proportion of patients with elevated Progesterone on the last day of stimulation (>1.5 ng/mL) will

be compared between both groups by Chi-square test. For this particular event the relative risk and its 95% confidence interval will also be calculated.