PROTOCOL: Final, v3, January 2018

EUDRA-CT: 2014- 001809-40

SCHEDULING OF IVF-ICSI CYCLES WITH OESTROGENS OR ORAL CONTRACEPTIVES IN THE LUTEAL PHASE IN PROTOCOL WITH ANTAGONIST. COMPARISON OF OUTCOMES VERSUS NO TREATMENT

FINAL REPORT

MADRID

Version 3. JANUARY, 2018

1. TITLE PAGE

STUDY TITLE: Scheduling of IVF-ICSI cycles with oestrogens or oral contraceptives in the luteal phase in protocol with antagonist. Comparison of outcomes versus no treatment.

INVESTIGATIONAL PRODUCT: Valerato de Estradiol/ Levonorgestrel-Etinilestradiol

INDICATION STUDIED: Pituitary braking in the luteal phase prior to the start of the ovarian stimulation cycle in IVF-ICSI treatments.

STUDY DESIGN: Randomised, non-blinded, phase IV study with 3 parallel treatment groups: oestradiol valerate, levonorgestrel-ethinylestradiol or no pre-treatment.

PROTOCOL IDENTIFICATION CODE: MER001

EUDRA-CT: 2014-001809-40

DEVELOPMENT PHASE OF STUDY: IV

DATE OF APPROVAL BY THE INDEPENDENT ETHICS COMMITTEE (IEC) OF THE HOSPITAL UNIVERSITARIO LA PAZ: 28th November, 2014

DATE OF APPROVAL BY THE SPANISH AGENCY OF MEDICINES AND MEDICAL DEVICES (AEMPS): 10th September, 2015

STUDY INITIATION DATE (First subject enrolled): 11st November, 2015

STUDY COMPLETION DATE (last visit last subject): 23rd July, 2018

PRINCIPAL INVESTIGATOR: Sara Fernández Prada
SPONSOR SIGNATORY: Onica Armijo Suárez/ Sara Fernández Prada

2. SINOPSIS

Sponsor: Onica Armijo Suárez / Sara Fernández Prada

Name of Active Ingredient: Valerato de Estradiol/ Levonorgestrel- Etinilestradiol

Title of Study: Scheduling of IVF-ICSI cycles with oestrogens or oral contraceptives in the luteal phase in protocol with antagonist. Comparison of outcomes versus no treatment.

Principal Investigator: Sara Fernández Prada

Recruiting Center: Hospital La Paz

Trial management: Assisted Reproduction Section. Gynaecology and Obstetrics Service. La Paz University Hospital.

Data management: Assisted Reproduction Section. Gynaecology and Obstetrics Service. La Paz University Hospital

Statistical analysis: Biostatistics Service of La Paz University Hospital

Study Period*:*

Start of the study: 11st November, 2015

End of the study: 23rd July, 2018

Study development phase: IV

Objectives**:** To evaluate the gestational outcomes (clinical gestation rate, miscarriage and live birth) obtained in patients with a normo-responder profile, undergoing IVF-ICSI treatment in an antagonist protocol with pre-treatment in previous luteal phase (oestradiol valerate or combined oral contraceptives) versus the outcomes observed in patients without previous pre-treatment.

Methodology: Randomised, non-blinded, phase IV study with 3 parallel treatment groups: oestradiol valerate, levonorgestrel-ethinylestradiol or no pre-treatment.

Number of subjects:

Patients included according to protocol:106

Patients suitable for the analysis: 86

Patients enrolled in preconceptive arm: 39

Patients enrolled in estradiol valerate arm: 32

Patients without treatment: 35

Diagnosis and main criteria for inclusion:

Inclusion Criteria:

- Patients from the Assisted Reproduction Service of the Hospital Universitario La Paz who started IVF-ICSI treatment with ovarian stimulation protocol with GnRH antagonists.
- Patients between 18-40 years of age, with previous primary infertility due to various causes such as mild-moderate male factor, tubal factor, grade I-II endometriosis, or primary infertility of unknown origin.
- Patients with a body mass index (BMI) < 30 kg/m2.
- Presence of regular ovulatory cycles (every 26-35 days).
- Previous performance of ≤ 2 IVF-ICSI cycles.
- Patients with basal hormonal values in the 1st phase of the cycle of FSH < 14 IU/ml and Estradiol < 80 pg/ml.
- Presence of both ovaries.
- Patients who give their written consent for inclusion after receiving the study information.

Exclusion criteria:

- Patients diagnosed with grade III-IV endometriosis.
- Patients with uterine malformations.
- Presence of previously unexcised hydrosalpinx.
- Severe male factor (< 100,000 EMR or testicular biopsy semen).
- Antral follicle count in 1st stage < 4 between both ovaries.

Test Product*:* Valerato de estradiol 2 mg and levonorgestrel 150 mcg-etinilestradiol 30 mcg

Duration of treatment:

- Valerato de estradiol: 4-10 days
- Levonorgestrel-Etinilestradiol: 12-28 days

Reference therapy: Not defined

Criteria for evaluation:

To assess the gestational outcomes observed in the 3 study groups, patients using oral contraceptives prior to the start of ovarian stimulation, patients using oestradiol valerate and, finally, patients without pre-treatment.

To evaluate the different response parameters during the ovarian stimulation cycle in each of the study groups and to assess whether there are differences between them.

Results:

Efficacy (Primary efficacy variables): After analysing the clinical gestation rate, miscarriage rate and live birth rate after fresh, delayed and cycle transfer in the different study groups, no statistically significant differences were detected, although there was a tendency towards worse outcomes in the group pretreated with oestrogens.

These outcomes could be related to the treatment regimen selected in our study and the time of exposure to oestradiol valerate in this study group.

After analysing this last aspect, we observed that patients with more days of oestrogen exposure had higher gestation rates.

Efficacy (Secondary efficacy variables): We did not find statistically significant differences in the cycle response parameters between the different study groups (days of stimulation, gonadotropin dose, E2 or progesterone levels on the day of the ovulatory trigger, total number of oocytes obtained, number of mature oocytes, number of embryos obtained or cancellation rate).

Safety: No clinically relevant alterations were found in phisical examination or vital signs. No side effects were reported.

Conclusions:

- No statistically significant differences in gestational rates were detected between the different study groups. We have analysed these rates both after transfer in the same stimulation cycle and with delayed embryo transfer to assess the possible effect that these medications could have on implantation. However, the outcomes observed are comparable in both scenarios.
- Finally, we did not observe statistically significant differences in the cumulative gestational rates, coinciding with the tendency to worse outcomes in the group pretreated with oestrogens in all cases. In this subgroup, it appears that more days of exposure to this pre-treatment is associated with better gestational outcomes.
- No differences were observed in any of the response parameters included in the secondary endpoints between the different groups studied.

3. TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITION TERMS

IVF-ICSI (In vitro fertilization- Intracytoplasmic sperm injection)
IECCR (Independet Ethics Committee on Clinical Research)
AEMPS (Spanish Agency of Medicines and Medical Devices)
GnRH (Gonadotropin-releasing hormone)
BMI (Body mass index)
FSH (Follicle stimulating hormone)
MSR (Motile Sperm Recovery)
E2 (Oestrogens)
OHHS (Ovarian Hyperstimuation syndrome)
LH (Luteinizing Hormone)
HMG (Human menopausal gonadotropine)
HCG (Human Chorionic gonadotropine)
AMH (Antimullerian hormone)
IMP (Investigational medicinal products)
NIMP (Non-investigational medicinal products)
CRF (Case Reprot Form)
SAE (Serious adverse event)
GCP (Good Clinical Practice)
AI (Artificial Insemination)
OCP (Oral contraceptives)
ET (Embryo transfer)
MII (Metaphase II)

4. ETHICS

4.1. INDEPENDENT ETHICS COMMITTEE (IECCR)

The final approved protocol and the Informed Consent Form were reviewed by the Independent Ethics Committee on Clinical Research (IECCR) of the Hospital Universitario La Paz. Protocol (Versión 001, 3rd June, 2014) and Informed Consent Form (Version MER001 3rd June, 2014) were approved on November 2014.

4.2. ETHICAL CONDUCT OF THE STUDY

The study was carried out according to the procedures of the Clinical Trials Unit of Hospital Universitario La Paz, according to the Spanish legislation referring to clinical trials in human beings, and according to the ICH guidelines of Good Clinical Practices c (CPMP/ICH/135/95) and current Helsinki Declaration (Fortaleza, Brazil, October 2013).

4.3. PATIENT INFORMATION AND CONSENT

Patients were informed about the clinical trial by a member of the research team who explained, in comprehensive terms, the procedures of the study, characteristics of the medicinal product and its possible derivative adverse effects. Patients gave their written consent prior to the pre-study screening examination. They were also informed about their right to discontinue the study at any moment.

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study has been carried out in the Reproductive Assisted Unit from Hospital Universitario La Paz, Madrid. The starting date was 11st November 2015 and the end date was 23rd July 2018.

The study management was carried out in collaboration with the clinical trials unit of La Paz Hospital and the statistical analysis was carried out in collaboration with the biostatistics service of La Paz Hospital.

SPONSOR: Onica Armijo Suárez/ Sara Fernández Prada

PRINCIPAL INVESTIGATOR: Sara Fernández Prada

STATISTICAL ANALYSIS OF DATA: Biostatistics Service of La Paz University Hospital

6. INTRODUCTION

Assisted reproduction is currently a booming field with an important increase in the use of the different techniques to obtain pregnancy,
despite the fact that there does not seem to be an increase in sterility rates.

There are several techniques available to achieve this, such as IVF or ICSI, in both cases it is necessary to obtain mature oocytes in order to cultivate them later with the male gametes.

The probabilities of success of this technique depend on several intrinsic factors such as the age of the woman, considered the most important single factor, the ovarian reserve and a third factor, which is unknown until the patient undergoes a stimulation cycle, the ovarian response.

There are different patterns of ovarian stimulation with GnRH analogues to achieve the highest number of mature oocytes in each case.
The pattern with the best gestational results has classically been the use of agonists in a long protocol, in spite of higher risk of ovarian hyperstimulation.

For this reason, new protocols have been explored to improve the results,
one of the most important of these is the use of antagonists, which has become very popular in the last decade. With this regimen, gonadotropin stimulation with exogenous gonadotrophins is started at the beginning of the cycle and not in the previous cycle as in the first protocol, which greatly reduces the doses and duration of stimulation with greater patient comfort.; in addition, there is a drastic reduction in severe OHSS, the cycle is started knowing that there is no inadvertent gestation, and greater follicular development is obtained as there is no inhibition of the ovarian receptors at the beginning of the stimulation.

The latest Cochrane review on ovarian stimulation protocols with antagonists and subsequent articles concludes that they have similar efficacy in terms of gestation rates to those obtained with agonist guidelines in a long protocol but with a significant reduction in ovarian hyperstimulation rates.

with a significant reduction in ovarian hyperstimulation rates.

In spite of this, the use of antagonists has disadvantages such as the difficulty in programming the punctures and embryo transfers at the optimum time coinciding with a working day, as the stimulation begins on the first days of the menstrual cycle; in addition to the asynchronism in follicular development due to the existence of FSH levels not suppressed in the previous luteal phase that allow the follicular growth to begin earlier, therefore we obtain fewer mature oocytes despite the fact that the total number of follicles stimulated is greater.

In an attempt to solve the problems of antagonists, the idea arises of using steroid hormones in the luteal phase of the previous cycle to inhibit the reproductive axis and thus prevent asynchronous follicular growth and to initiate ovarian stimulation, taking into account the optimal time for punctures and embryo transfers. This may be particularly relevant in patients with low ovarian reserve, in whom it has also been observed that a greater number of oocytes of better quality are obtained.

Both oral contraceptives and synthetic progesterone have long been used to programme cycles, primarily inhibiting LH levels and requiring several days of post-treatment flushing; more recently, estrogens have been introduced for this purpose, which have an inhibitory action on FSH.

Several small sample studies have been conducted on the effect of contraceptives as pre-treatment with conflicting outcomes in terms of pregnancy rates. In response to this, several meta-analyses have been published, including a recent Cochrane review of data from six randomised clinical trials, which concludes that pregnancy rates are affected by contraceptive use. However, this meta-analysis presents confounding factors such as the use of different contraceptives with different doses and different patterns of administration in terms of days of treatment and washout period.

The use of luteal phase oestrogens has recently been proposed as a programming technique based on the inhibitory effect of oestradiol on follicular growth by inhibiting endogenous FSH levels, which is stopped by withdrawal of treatment. his oestrogen regimen offers the advantage of less pre-treatment time and avoids the contraceptive

effect in the previous cycle, which gives the couple an extra chance to achieve spontaneous conception. Previous studies have shown no difference in gestational rates compared to those obtained in non-pretreated groups, although the statistical power of these studies was limited. The optimal number of days of treatment to achieve the best gestational outcomes also remains to be defined as there are few studies.

A recent study has been published comparing the outcomes between the oestrogen and contraceptive groups with no statistically significant differences, although the statistical power was low due to the small sample size.

Our aim is to compare the gestational outcomes obtained in the three groups under study, i.e. the pre-treated with oestradiol, the pre-treated with contraceptives and the non-pre-treated group to find out if it is possible to schedule cycles in a way that is not only efficient but also effective for the couples, without affecting the gestational outcomes or even improving them, and therefore to find out which strategy is the most appropriate to carry out.

In conclusion, more clinical studies are needed to better understand the effect of steroid treatment in the luteal phase on endometrial receptivity, the variations in hormone levels it causes and the oocyte quality it determines in order to establish a critical choice of treatment in the endometrial phase.

to be able to establish a critical choice in the programming of FIV-ICSI cycles.

7. STUDY OBJECTIVES

7.1. PRIMARY OBJECTIVES

To evaluate the gestational outcomes (clinical gestation rate, miscarriage and live birth) obtained in patients with a normo-responder profile, undergoing IVF-ICSI treatment in an antagonist protocol with pre-treatment in previous luteal phase (oestradiol valerate or combined oral contraceptives) versus the outcomes observed in patients without previous pre-treatment.

7.2. SECONDARY OBJECTIVES

- To assess the cancellation rate due to insufficient response or absence of viable embryos observed in the different study groups.

- To assess the number of ovarian follicles observed ultrasonographically at the end of the stimulation, the hormonal values on the day of the ovulatory trigger, the number of oocytes obtained, the oocyte maturity rate and the number of embryos evolved in the different study groups.

- To evaluate the days necessary to complete the controlled ovarian stimulation and the doses of gonadotrophins used in the different study groups.

- To establish a fixed treatment pattern in terms of doses and pre-treatment days, which will allow a homogeneous group to be obtained and, therefore, an adequate statistical study to be carried out.

- To evaluate the possible association of the exposure time to the different pre-treatments with the reproductive outcomes.

8. INVESTIGATIONAL PLAN

8.1. OVERALL STUDY DESIGN AND PLAN- DESCRIPTION

Non-blinded randomised controlled clinical trial, with 3 intervention arms in which patients with indication for IVF-ICSI treatment in an antagonist protocol for controlled ovarian stimulation will be included. Depending on randomisation, patients will receive oral oestradiol valerate, combined oral contraceptive or no pre-treatment in the luteal phase prior to the start of the cycle. The study was conducted at the Assisted Reproduction Unit of the Hospital La Paz.

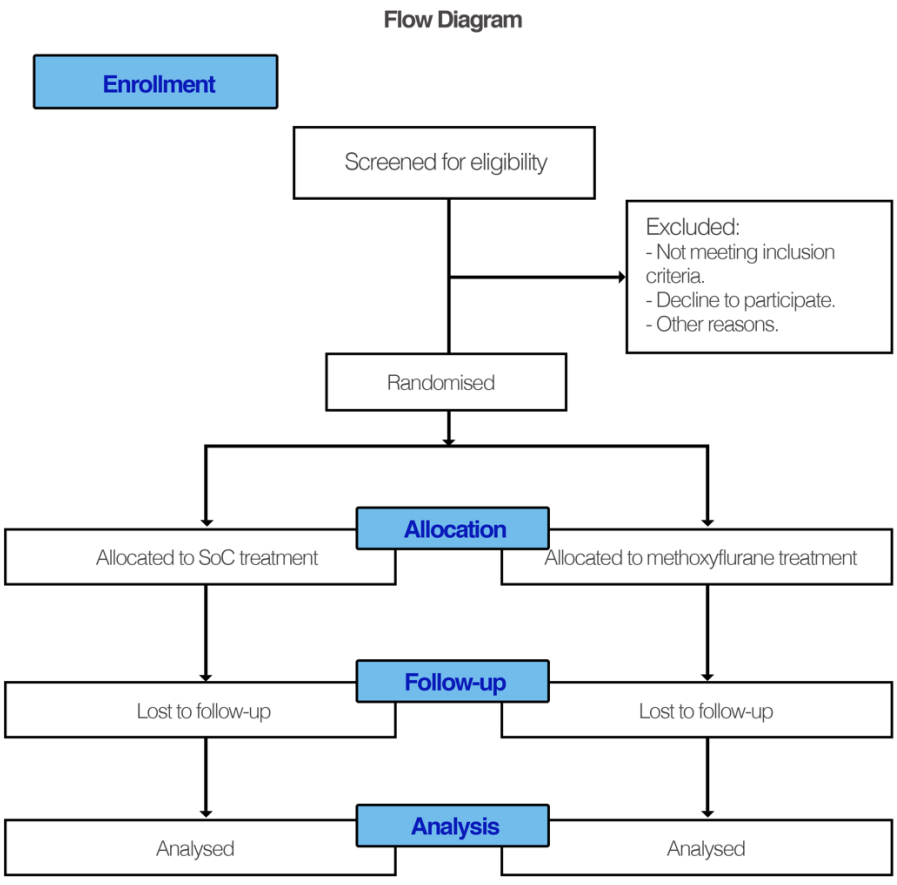


Figure 1. Flow diagram of the study

In accordance with current legislation, all patients were informed both verbally and in writing about the study in which they were proposed to participate and signed informed consent. The following steps were carried out:

- Review of inclusion and exclusion criteria
- Randomisation
- Review of medical history and concomitant medication.
- Recording of drug exposure time
- Recording of ultrasound and hormonal response of the patients.
- Recording of stimulation response parameters and gestational outcomes
- Recording of adverse events

In total 150 patients were offered to participate in the study, 106 patients accepted inclusion. Of the 44 patients not included, 6 did not meet any of the criteria and 38 refused to participate.

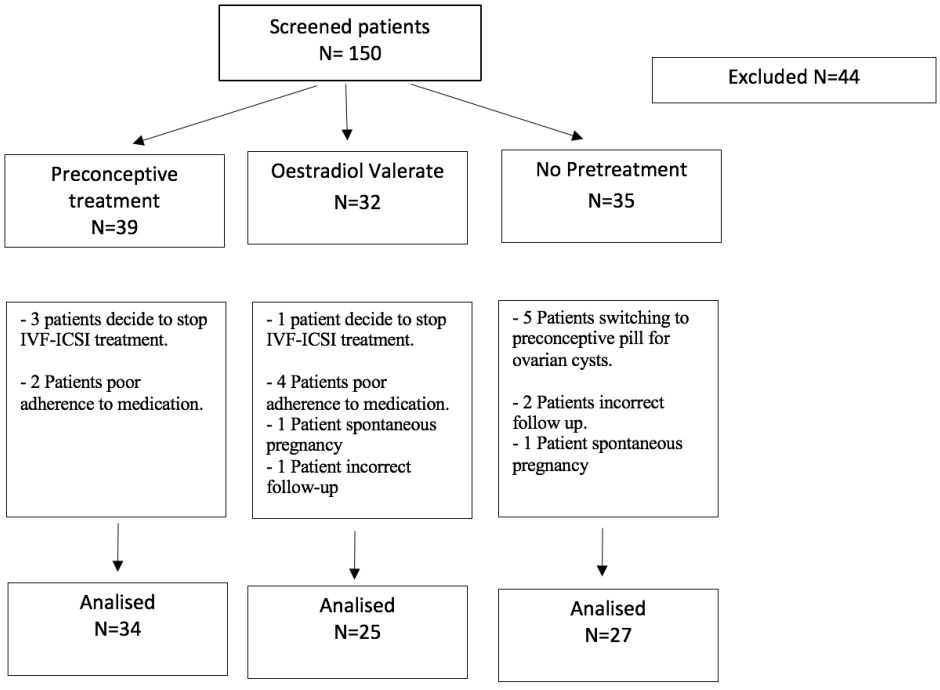


Figure 2. Patients Flow

Table 1 describes the dates of IC sign and screening and follow-up visit (last visit) for each patient.

Patient	IC sign date	Screening date	Follow up visit date	Discontinuation	Discontinuation reason
001	11/11/2015	11/11/2015	01/02/2016	No	.
002	26/11/2015	26/11/2015	26/02/2016	No	.
003	13/01/2016	13/01/2016	18/03/2016	No	.
004	13/01/2016	13/01/2016	29/04/2016	No	.
005	15/01/2016	15/01/2016	08/04/2016	No	.
006	28/01/2016	28/01/2016	05/04/2016	No	.
007	01/02/2016	01/02/2016	30/06/2016	No	.
008	01/02/2016	01/02/2016	16/03/2016	No	.
009	02/02/2016	02/02/2016	01/03/2016	No	.
010	02/02/2016	02/02/2016	31/03/2016	No	.
011	04/02/2016	04/02/2016	20/05/2016	No	.
012	15/02/2016	15/02/2016	13/06/2016	No	.
013	15/02/2016	15/02/2016	29/03/2016	No	.
014	18/02/2016	18/02/2016	28/07/2016	No	.
015	07/03/2016	07/03/2016	03/05/2016	Yes	Error treatment
016	15/03/2016	15/03/2016	17/11/2017	No	.
017	16/03/2016	16/03/2016	28/11/2016	No	.
018	18/03/2016	18/03/2016	12/05/2016	No	.
019	20/03/2016	20/03/2016	01/03/2017	No	.
020	28/03/2016	28/03/2016	24/06/2016	No	.
021	18/05/2016	18/05/2016	27/10/2016	No	.
022	02/06/2016	02/06/2016	17/10/2016	No	.
023	03/06/2016	03/06/2016	03/01/2017	No	.
024	08/06/2016	08/06/2016	05/08/2016	No	.
025	08/06/2016	08/06/2016	14/09/2016	No	.
026	09/06/2016	09/06/2016	06/09/2016	No	.
027	17/06/2016	17/06/2016	12/08/2016	No	.
028	11/07/2016	11/07/2016	02/12/2016	No	.
029	13/07/2016	13/07/2016	07/03/2017	No	.
030	17/08/2016	17/08/2016	24/10/2016	No	.
031	24/08/2016	24/08/2016	05/09/2016	No	.
032	26/08/2016	26/08/2016	12/11/2017	No	.
033	26/08/2016	26/08/2016	07/11/2016	No	.
034	20/09/2016	20/09/2016	14/11/2016	No	.
035	30/09/2016	30/09/2016	08/08/2017	No	.
036	03/10/2016	03/10/2016	01/11/2016	No	.
037	03/10/2016	03/10/2016	21/12/2016	No	.
038	07/10/2016	07/10/2016	29/06/2017	No	.
039	10/10/2016	10/10/2016	20/12/2016	No	.
040	25/10/2016	25/10/2016	16/06/2017	No	.
041	25/10/2016	25/10/2016	07/11/2016	Yes	Stop treatment

042	08/11/2016	08/11/2016	12/04/2017	No	.
043	23/11/2016	23/11/2016	20/02/2017	No	.
044	24/11/2016	24/11/2016	17/04/2017	No	.
045	12/12/2016	12/12/2016	23/03/2017	No	.
046	12/12/2016	12/12/2016	26/01/2017	No	.
047	14/12/2016	14/12/2016	08/03/2017	No	.
048	14/12/2016	14/02/2016	19/03/2018	No	.
049	14/12/2016	14/12/2016	12/04/2017	No	.
050	19/12/2016	19/12/2016	12/06/2017	No	.
051	21/12/2016	21/12/2016	13/07/2017	No	.
052	18/01/2017	18/01/2017	31/01/2018	No	.
053	19/01/2017	19/01/2017	27/10/2017	No	.
054	25/01/2017	25/01/2017	08/03/2017	No	.
055	31/01/2017	31/01/2017	05/07/2017	No	.
056	02/02/2017	02/02/2017	18/04/2017	Yes	Spontaneous Pregnancy
057	13/02/2017	13/02/2017	01/04/2017	No	.
058	22/02/2017	22/02/2017	11/06/2018	No	.
059	24/02/2017	24/02/2017	27/07/2017	No	.
060	27/02/2017	27/02/2017	09/02/2018	No	.
061	02/03/2017	02/03/2017	23/10/2017	No	.
062	23/03/2017	23/03/2017	21/08/2017	Yes	Change to OCP
063	28/03/2017	28/03/2017	19/07/2017	No	.
064	29/03/2017	29/03/2017	30/05/2017	No	.
065	24/05/2017	24/05/2017	23/10/2017	No	.
066	06/06/2017	06/06/2017	16/02/2018	No	.
067	12/06/2017	12/06/2017	05/01/2018	No	.
068	07/07/2017	07/07/2017	09/01/2018	No	.
069	09/07/2017	09/07/2017	13/12/2017	No	.
070	09/07/2017	09/07/2017		Yes	Error treatment
071	10/07/2017	10/17/2017		Yes	Incorrect follow up
072	10/07/2017	10/07/2017	22/01/2018	No	
073	18/07/2017	18/07/2017	23/07/2018	No	.
074	18/07/2017	18/07/2017	02/11/2017	No	.
075	19/07/2017	19/07/2017	29/11/2017	Yes	Change to OCP
076	28/08/2017	28/08/2017	17/01/2018	No	.
077	05/09/2017	05/09/2017	05/01/2018	No	.
078	13/09/2017	13/09/2017	11/12/2017	Yes	Spontaneous Pregnancy
079	08/11/2017	08/11/2017	31/01/2018	Yes	Stop treatment
080	10/11/2017	10/11/2017	11/12/2018	Yes	Change to OCP
081	10/11/2017	10/11/2017	12/02/2018	Yes	Stop treatment
082	11/11/2017	11/11/2017	27/02/2018	No	.
083	11/11/2017	11/11/2017	22/11/2017	No	.
084	15/11/2017	15/11/2017	24/01/2018	No	.
085	24/01/2018	24/01/2018	13/04/2018	No	.
086	24/01/2018	24/01/2018	18/07/2018	No	.

087	25/01/2018	25/01/2018	10/02/2018	Yes	Change to OCP
088	25/01/2018	25/01/2018	14/02/2018	Yes	Incorrect follow up
089	25/01/2018	25/01/2018	27/10/2018	Yes	Stop treatment
090	29/01/2018	29/01/2018	21/03/2018	Yes	Error treatment
091	31/01/2018	31/01/2018	07/05/2018	No	.
092	22/02/2018	22/02/2018	30/06/2018	No	.
093	22/02/2018	22/02/2018	09/04/2018	No	.
094	24/02/2018	24/02/2018	13/04/2018	Yes	Change to OCP
095	28/02/2018	28/02/2018	09/05/2018	No	.
096	05/03/2018	05/03/2018	10/05/2018	Yes	Error treatment
097	05/03/2018	05/03/2018	11/05/2018	No	.
098	05/03/2018	05/05/2017	14/06/2018	No	
099	06/03/2018	06/03/2018	15/06/2018	No	.
100	06/03/2018	06/03/2018	11/05/2018	No	.
101	07/03/2018	07/03/2018	24/04/2018	No	.
102	10/03/2018	10/03/2018	21/05/2018	Yes	Error treatment
103	11/03/2018	11/03/2018	22/05/2018	No	.
104	13/03/2018	13/03/2018	27/04/2018	Yes	Error treatment
105	10/04/2018	10/04/2018	11/04/2018	Yes	Incorrect follow up
106	11/05/2018	11/05/2018	20/07/2018	No	.

Table 1. Dates of IC screening, sign and last visit

8.1.1 PROCEDURES OF THE STUDY

The doctor who assessed the patient at the consultation prior to starting IVF-ICSI treatment, after verifying that they met all the inclusion criteria and no exclusion criteria, offered her participation in the study. Once the informed consent was signed, the patient was randomised into 3 possible groups, following a randomisation table provided by the biostatistics service of the Hospital La Paz. The randomisation groups were: taking oral contraceptives before starting ovarian stimulation, taking oral oestradiol before starting ovarian stimulation or no pre-treatment.

TREATMENT ARMS

Patients in the oral contraceptive treatment arm (levonorgestrel 150 mcg/ ethinylestradiol 0.3 mcg) will start treatment on the 1st-2nd day of menstruation of the previous cycle for at least 12 days, with 5 days of washout thereafter, after which controlled ovarian stimulation will be initiated.

Patients in the oral oestrogen (oestradiol valerate) treatment arm will start treatment 3 days before their expected period with a dose of 2 mg/12 hours until the day before the start of ovarian stimulation, which will begin between the 2nd-8th day of the cycle.

Patients belonging to the group without pre-treatment will start ovarian stimulation between the 2nd-3rd day of the cycle according to the usual pattern.

The treatment distribution was as follows:

Patient	Group
001	Contraceptive
002	No treatment
003	No treatment
004	Oestrogen
005	Oestrogen
006	No treatment
007	No treatment
008	Contraceptive
009	Contraceptive
010	No treatment
011	Oestrogen
012	Contraceptive
013	No treatment
014	Contraceptive
015	Oestrogen
016	Contraceptive
017	No treatment
018	Contraceptive
019	Oestrogen
020	No treatment
021	No treatment
022	Oestrogen
023	Oestrogen
024	Oestrogen
025	Contraceptive
026	No treatment
027	No treatment
028	Oestrogen
029	Contraceptive
030	No treatment
031	No treatment
032	Oestrogen

033	Contraceptive
034	No treatment
035	No treatment
036	Contraceptive
037	Contraceptive
038	No treatment
039	No treatment
040	Contraceptive
041	Contraceptive
042	Contraceptive
043	Oestrogen
044	Oestrogen
045	No treatment
046	Contraceptive
047	Contraceptive
048	No treatment
049	No treatment
050	Contraceptive
051	No treatment
052	Contraceptive
053	Contraceptive
054	Contraceptive
055	Oestrogen
056	No treatment
057	Oestrogen
058	No treatment
059	Contraceptive
060	Oestrogen
061	Contraceptive
062	No treatment
063	Contraceptive
064	No treatment
065	No treatment
066	Oestrogen
067	Contraceptive
068	No treatment
069	No treatment
070	Contraceptive
071	Oestrogen
072	Oestrogen
073	Contraceptive
074	Oestrogen
075	No treatment
076	Oestrogen
077	Contraceptive
078	Oestrogen

079	Oestrogen
080	No treatment
081	Contraceptive
082	Contraceptive
083	Contraceptive
084	Contraceptive
085	Oestrogen
086	No treatment
087	No treatment
088	No treatment
089	Contraceptive
090	Contraceptive
091	Oestrogen
092	Oestrogen
093	Contraceptive
094	No treatment
095	Contraceptive
096	Oestrogen
097	Contraceptive
098	Oestrogen
099	Oestrogen
100	Oestrogen
101	Oestrogen
102	Oestrogen
103	Contraceptive
104	Oestrogen
105	No treatment
106	Contraceptive

Table 2. Patients distribution

8.1.2. METHODOLOGY AND FOLLOW-UP

After randomisation, the medication is given to the patient in the consultation room when it is appropriate and the guidelines for its use are explained.

Before starting controlled ovarian stimulation, all patients are scheduled to undergo a baseline transvaginal ultrasound to confirm that there is no follicular dominance and that they can therefore begin stimulation.

All patients start ovarian stimulation with HMG hp at a dose of 150-300 IU during the first 5 days of stimulation. The dose is re-evaluated thereafter based on ultrasound

response. Administration of the GnRH antagonist (Cetrorelix 0.25 mg/day or Ganirelix 0.25 mg/day) is performed when a dominant follicle of at least 13 mm is observed sonographically.

Ovulation is triggered with recombinant HCG 250 mg (Ovitrelle) or triptorelin 0.2 mg (Decapeptyl) when at least 2 follicles > 17 mm are observed. The cycle will be cancelled if < 2 dominant follicles are observed.

Follicular puncture is performed 36 hours later.

Embryo transfer is performed 3-5 days later in the same ovarian stimulation cycle or deferred depending on the patient's conditions: endometrial growth, safe hormonal values or risk of ovarian hyperstimulation.

Embryo transfer in the same ovarian stimulation cycle:

- Adequate endometrial growth with thickness of at least 7 mm and trilaminar proliferative appearance.
- Estradiol levels < 4000 pg/ml and serum progesterone on trigger day < 1.5 ng/ml.
- Less than 12 follicles developing on the day of the ovulatory trigger.

In this case, patients start with the administration of vaginal progesterone the same night of the follicular puncture 200 mg/12 hours until the outcome of BHCG; if it is positive, it will be maintained until week 12 of gestation.

The BHCG determination will be done 14 days after embryo transfer. If it is positive, the patient will be scheduled 2 weeks later for a gestational ultrasound and if it is negative and she has cryopreserved embryos from the same cycle, endometrial preparation will begin for a new transfer.

Frozen embryo transfer:

Endometrial preparation is performed in a substituted cycle with oral or transdermal oestrogens for 12-21 days. After this, ultrasound monitoring is performed to check for good endometrial growth. When a trilaminar aspect is observed with a thickness > 7 mm, the administration of vaginal progesterone 400 mg/12 hours is added. The embryo transfer is performed 3-5 days later depending on the evolutionary time of the cryopreserved embryo.

BHCG is determined 14 days after the embryo transfer. If it is positive, the patient is scheduled 2 weeks later for a gestational ultrasound and if it is negative and she has cryopreserved embryos from the same cycle, endometrial preparation for a new transfer will begin.

La ultima visita de seguimiento de las pacientes incluidas en el estudio fue tras alta con gestación evolutiva o tras confirmar ausencia de gestación y no disponer de mas embriones vitrificados del mismo ciclo de tratamiento.

8.2. SELECTION OF THE STUDY POPULATION

All patients included must meet all inclusion criteria and none exclusion criteria.

INCLUSION CRITERIA

- Patients from the Assisted Reproduction Service of the Hospital Universitario La Paz who started IVF-ICSI treatment with ovarian stimulation protocol with GnRH antagonists.

- Patients between 18-40 years of age, with previous primary infertility due to various causes such as mild-moderate male factor, tubal factor, grade I-II endometriosis, or primary infertility of unknown origin.

- Patients with a body mass index (BMI) < 30 kg/m2.

- Presence of regular ovulatory cycles (every 26-35 days).
- Less than 2 previous cycles of IVF.

- Patients with baseline hormonal values in the 1st phase of the cycle of FSH < 14 IU/ml and oestradiol < 80 pg/ml.

- Presence of both ovaries.

- Patients who give their written consent for inclusion after receiving the study information.

EXCLUSION CRITERIA

- Patients diagnosed with endometriosis grade III-IV.

- Patients with uterine malformations.

- Presence of previously unexcised hydrosalpinx.

- Severe male factor (< 100,000 MSR or testicular biopsy semen).

- Antral follicle count in 1st stage < 4 between both ovaries.

PARTICIPANTS EXCLUDED FROM THE STUDY

According to the recommendations for clinical studies and the evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient data confidentiality the patient could stop his/her participation in the study at any moment for any reason and it would involve no penalty or loss of benefits to which the participant is otherwise entitled. In case a patient decided to stop his/her participation in the study or withdraw their informed consent he/she could do it without an explanation of the reasons for his/her decision and received the best therapeutic option available.

Either if the patient decided to stop his/her participation or if the investigator decided the withdrawal of a patient under his/her judgement and criterion, the reasons, when known, where recorded in the CRF.

The following were withdrawal criteria:

- Withdrawal of informed consent or death of the patient.

- Under the investigator judgement.

- Spontaneous pregnancy before starting ovarian stimulation treatment.

- Need to change the treatment to which the patient has been randomized due to the absence of follicular rest prior to the start of ovarian stimulation.

In any case, the patient should conduct the follow-up visit, unless he/she expressed his/her opposition. Once the patients were not participating in the study they were attended following clinical practice.

8.3 TREATMENTS

Treatment administered

According to the information content in the "Guidance on Investigational Medicinal Products (IMPs) and other medicinal products used in Clinical Trials", Notice to Applicants, Volume 10, Clinical Trials, which intends to clarify and provide additional guidance on the definition of IMPs and to provide specific guidance about the use of non-investigational medicinal products (NIMP), the definition of an IMP is provided in Directive 2001/20/EC, Article 2 (d), as "a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form." Medicinal products with a marketing authorisation (MA) are classified as IMPs s when they are to be used as the test substance or reference substance in a clinical trial, provided they are used or

assembled (formulated or packaged) in a way different from the authorised form, or used for an unauthorised indication, or used to gain further information about the authorised form.

Products which are NIMPs as referred to in Art. 2(d) of Directive 2001/20/EC may be supplied to subjects participating in a trial and used in accordance with the protocol for preventive, diagnostic or therapeutic reasons and/or to ensure that adequate medical care is provided for the subject.

In this study the are defined as IMP the following products:

Investigational Medicinal Products (IMPs): Ovoplex, Meriestra and Progyluton. The study medication was provided and send to the Hospital Pharmacy free of charge.

The study medication was properly labeled and stored in a restriction access place following the Sponsor´s conditions.

1) OVOPLEX

PRODUCT NAME	DRUG COMPOSITION	MANUFACTURER	COUNTRY OF PURCHASE	PHARMACEUTICAL FORM	DOSE	BATCH NUMBER
OVOPLEX	150 mcg LEVONORGESTREL/30 MCG ETINILESTRADIOL	WYETH FARMA, S.A	SPAIN	TABLET	1 DAILY	A1888

2) MERIESTRA

PRODUCT NAME	DRUG COMPOSITION	MANUFACTURER	COUNTRY OF PURCHASE	PHARMACEUTICAL FORM	DOSE	BATCH NUMBER
MERIESTRA	2 MG VALERATO ESTRADIOL	NOVARTIS FARMACEUTICA, S.A.	SPAIN	TABLET	2 DAILY	B13899

3) PROGYLUTON

PRODUCT NAME	DRUG COMPOSITION	MANUFACTURER	COUNTRY OF PURCHASE	PHARMACEUTICAL FORM	DOSE	BATCH NUMBER
PROGYLUTON	2 MG VALERATO ESTRADIOL (WHITE TABLETS ONLY)	BAYER HISPANIA, S.L.	SPAIN	TABLET	2 DAILY	

Prior and concomitant therapy

All medications the patients were taking when they started the study were documented on the CRF.

All medications prescribed according to clinical practice were allowed in this study.

Method of assigning patients to treatment groups

Patients who met all inclusion and none exclusion criteria, who had signed informed consent, were randomized followed a table provided by the hospital´s biostatistics department.

Blinding

The clinical trial is openlabel.

Treatment compliance

In order to guarantee therapeutic compliance, the study medication was dispensed by the research staff during the patient's admission.

The researcher kept accurate records of the trial supplies received, stored and dispensed in documents intended for that purpose. All empty and partially empty containers of the study medication were stored until the accounting was monitored and then sent to the promoter. At the end of the study, all unused medication containers were also returned to the promoter.

8.4 EFFICACY AND SAFETY VARIABLES

8.4.1 Efficacy assessment

To evaluate the gestational outcomes (clinical gestation rate, miscarriage and live newborn) obtained in patients with a normoresponse profile, undergoing IVF-ICSI treatment in an antagonist protocol with pre-treatment in previous luteal phase (estradiol valerate or combined oral contraceptives) versus the results observed in patients without previous pre-treatment.

Secondary objectives have also been:

- To assess the cancellation rate due to insufficient response or absence of viable embryos observed in the different study groups.

- To evaluate the number of ovarian follicles observed ultrasonographically at the end of the stimulation, the number of oocytes obtained, the oocyte maturity rate and the number of embryos evolved in the different study groups.

- To evaluate the days necessary to complete the controlled ovarian stimulation and the doses of gonadotropins used in the different study groups.

- To establish a fixed treatment pattern in terms of doses and pre-treatment days, which will allow obtaining a homogeneous group and, therefore, an adequate statistical study.

- To evaluate the possible association of the exposure time to the different pretreatments with the reproductive results.

These objectives have been evaluated with the following variables:

- Clinical gestation rate by embryo transfer: No. of gestations clinically objectified by ultrasound, observing intrauterine gestational sac with embryo and positive heartbeat 4 weeks after embryo transfer, among the total number of embryo transfers.

- Rate of interrupted gestation: Number of gestations interrupted before 22 weeks of gestation among the total number of clinical gestations.

- Delivery rate per embryo transfer: Number of births among the total number of embryo transfers.

These variables will be determined independently, after embryo transfer in the same stimulation cycle, after deferred embryo transfer of vitrified embryos and as a cumulative rate (fresh transfer rate + deferred transfer rate).

Variables used to evaluate secondary objectives:

- Cycle cancellation rate: no. of cycles initiated cancelled due to absence of response or absence of evolving embryos/total cycles initiated.

- Duration of ovarian stimulation measured in days.

- Total doses of gonadotropins used during ovarian stimulation.

- Estradiol (pg/ml) and progesterone (ng/ml) levels on the day of the ovulatory trigger.

- Number of follicles > 16 mm observed on the day of the ovulatory trigger.

- Total number of oocytes obtained after follicular puncture.

- Number of mature oocytes obtained after follicular puncture.

- Total number of evolving embryos obtained per ovarian stimulation cycle.

- Cycle cancellation rate: Number of cycles initiated cancelled due to absence of response or absence of evolving embryos/total number of cycles initiated.

- Effect of exposure time to premedication on gestational results.

8.4.2. Safety assessment

Definitions

Adverse event: Any untoward medical occurrence in a clinical trial subject administered a medicinal product and which does not necessary have a causal relationship with this treatment.

Serious adverse event: Any untoward medical occurrence at any dose that:

- results in death
- is life-threatening*
- requires hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity or
- is a congenital anomaly or birth defect
- Important medical events** that may not result in death, be life-threatening or require hospitalization may be considered as SAE when, based upon appropriate medical judgment, they may require medical or surgical intervention.

* The term “life-theatening” in the definition of SAE refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.

** Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Unexpected adverse event: an "unexpected" adverse reaction is one, the nature or severity of which is not consistent with information in the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product).

Non-Serious Adverse Event: A non-serious AE is any AE which does not fulfill the definition of a serious AE.

Intensity Assessment Definitions:

- Mild – No or transient symptoms, no interference with the subject's daily activities.
- Moderate – Marked symptoms, moderate interference with the subject's daily activities.
- Severe – Considerable interference with the subject's daily activities, unacceptable.

Relationship to Trial Product Assessment Definitions

Assessment of the causal relationship	Definite	Probable	Possible	Conditional	Unrelated
Reasonable temporal sequence	Yes	Yes	Yes	Yes	Yes or No
Known response to the medicine	Yes	Yes	Yes	No	No
Reaction improvement when leaving medication	Yes	Yes	Yes or No	Yes or No	No
Reaction reappears with re-exposure	Yes	?	?	?	? or No
Alternative explanation to this reaction	No	No	Yes	No	Yes

Table 3. Karch and Lasagna algorithm

Definite:

- There is a plausible temporal sequence in relation to drug administration or with serum levels or tissue of it.
- The observe event match with the known adverse reactions scheme for the drug involved.
- Cannot be explained by concurrent disease or other drugs or chemicals.
- Response to withdrawal should be clinically plausible or improvement on discontinue the drug.
- It reappears upon re-administration.

Probable:

- There is a reasonable time sequence to drug exposure.
- The observe event match with the known adverse reactions scheme for the drug involved.
- It is unlikely attributable to undercurrent disease or other drugs or chemicals.
- After the drug be withdrawn following a reasonable clinical sequence.
- Improvement when discontinuing the drug.
- No re-exposure to complete this definition.

Possible:

- There is a reasonable time sequence to drug exposure.
- Agrees with the scheme known of adverse reactions.
- It may be due to patient's clinical condition or other drugs and chemicals administered concomitantly
- Information about the withdrawal may be absent or confused.

Conditional:

- A clinical event, including abnormalities in laboratory tests with a temporal relationship with respect to drug administration that makes unlikely the casualty

relationship and in which other drugs, chemicals or undercurrent disease provide plausible explanations.

Unrelated:

- Do not meet any of the above criteria.

Collection, Recording and Reporting of Adverse Events:

All events meeting the definition of an AE were collected and reported at each contact with the trial site (inclusion visit, hospitalization period, blood sampling for pharmacokinetics, follow-up visit…). During the clinical trial period volunteers were repeatedly asked about occurrence of adverse events.

All AEs, observed by the investigator or reported by the subjects, were recorded by the investigator and evaluated on the standard adverse event form.

No serious AEs were reported in this clinical trial.

As described in the protocol the investigator was responsible for reporting immediately the serious adverse events to the Sponsor. Any serious adverse event would have been reported to Pharmacovigilance Department of the Sponsor and to the study monitor.

Serious adverse events All serious adverse events should be reported within 24 hours of onset to the sponsor followed by a written confirmation according to the notification form.

The sponsor must notify the AEMPS, IECCR and CCAA involved in the trial all suspected and unexpected serious adverse reactions that could be related to the investigational drugs no later than 7 calendar days after the sponsor has first knowledge of it if fatal or life-threatening and not later than 15 calendar days if non fatal or non life-threating. This notification will be made within the abovementioned period even if all the information required on the form is not available. The form should be completed within 8 days.

All adverse events have been reported in the tabular form in this final clinical report.

Adverse events observed during the conduct of a clinical trial were recorded on the CRF of each participant in the study

It was recorded all adverse events regardless of causality attributed, in the form of description of adverse events.

This form is on the CRF of each participant in the study.

Depending on the nature of assessment, AE were classified as:

- Serious / not serious
- Unexpected / expected

The collection of adverse events were performed by the research team of trial, shall be specified by indicating the time of occurrence expressed as the minimum possible time unit, if it is serious or not serious and if expected or unexpected, its intensity (mild, moderate, severe), action taken with study drug (none, decreased, temporarily interrupted, permanently discontinued), and outcome (resolved, non resolved, resolved with squealed, death, unknown).

8.4.3 Clinical Laboratory Test

Blood samples for laboratory determinations taken at the screening and at the follow-up visit of the study were carried out in the local laboratory of each centre. The study and the methods used in the trial were carried out according to the Good Clinical Practice guidelines.

The following analytical determinations were performed in the screening, before each study drug administration and/or follow-up visits:

- Basal serum FSH (IU/l) in 1st cycle phase in cycle prior to start of treatment.

- Serum estradiol (pg/ml) in 1st phase of cycle in cycle prior to start of treatment.

- Antimullerian hormone (ng/ml) in cycle prior to start of treatment.

- Estradiol (pg/ml) and progesterone (ng/ml) levels on the day of the ovulatory trigger.

8.5 DATA QUALITY ASSURANCE

Suitable actions to guarantee the quality of data register were applied. This guarantees that these data are collected and processed in a truthful and correct way.

Concordance between the data collected and source documents has been checked by the study CRAs, who also verified compliance with the protocol, Standard Operating Procedures, GCP guidelines and Spanish laws.

8.6. STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

8.6.1. Statistical and analytical plans

The statistical analysis of the data collected in the study was performed with the collaboration of the Biostatistics Service of the Hospital Universitario La Paz, and the data were processed in Microsoft Excel format, which was later imported for statistical treatment in the SAS version 9.4 program. Statistically significant differences were considered to be those with a probability of error of less than 5% ($p<0.05$).95 The mean and standard deviation were used to describe continuous quantitative variables. Qualitative variables are described using absolute frequencies and relative frequencies expressed as percentages. When considered convenient, the descriptive analysis of the variables was represented graphically as a Box Plot. Comparisons of quantitative variables versus qualitative groups were made mainly by nonparametric tests, using the Kruskal-Wallis or Mann-Whitney U test. Frequency analysis between qualitative variables was performed using the c2 test or Fisher's exact test when necessary (in 2x2 tables if $N<20$, or if any value in the table of expected values is less than 5). The c2 was adjusted in all cases with the Yates correction.

8.6.2. Determination of sample size

The calculation of the sample size for our study was performed by the Biostatistics Service of the Hospital Universitario La Paz, taking into account the only previously published study comparing 2 pretreatment branches (combined oral contraceptives versus estrogens) for the programming of IVF-ICSI cycles in antagonist protocol. It was estimated that to detect differences with a statistical power of 80% in the test of the null hypothesis H0 of no difference in evolutionary gestation rate between groups, it was necessary to include at least 103 patients in each of the study arms, with an alpha error of 0.05. This was calculated using Fisher's Exact Test (2-tailed).

9. STUDY SUBJECTS

9.1. DISPOSITION OF SUBJECTS

The study was performed in 106 patients. 150 subjects were imformed, 106 patients signed the informed consent form and 106 were randomized to a treatmen group. Twenty randomized patients do not begin the treatment, so we analyzed the parameters of 86 individuals finally submitted to treatment.

Subjects included

The assigned medication was received by 106 patients according to the randomisation.

Demographic and other baseline characteristics

Demographic data of volunteers (age, smoking habit, previous reproductive treatments, cause of infertility and ovarian reserve parameters) were collected and tabulated and the arithmetic means, standard deviation, coefficient of variation, median, maximum and minimum were calculated. No other statistical analysis has been performed with the demographic data. Demographic characteristics are described in Table 4 and 5.

	AGE	SMOKING HABIT	PREVIOUS REPRODUCTIVE TREATMENTS
001	35	NO	IVF
002	31	NO	NO
003	30	NO	AI
004	34	NO	AI
005	32	YES	IVF
006	31	NO	IVF
007	38	YES	IVF
008	30	YES	NO
009	37	YES	IVF
010	38	NO	NO
011	32	NO	AI
012	38	NO	IVF
013	39	YES	AI+IVF
014	35	NO	IVF
016	32	NO	AI
017	37	NO	IVF
018	37	NO	IVF
019	38	NO	NO
020	30	YES	AI
021	37	NO	AI
022	38	NO	AI
023	33	NO	NO
024	37	NO	IVF
025	37	NO	AI+IVF
026	38	NO	NO
027	38	NO	AI
028	34	NO	NO
029	37	YES	NO
030	35	YES	AI+IVF
031	37	YES	IVF
032	40	YES	NO
033	39	NO	NO
034	35	NO	AI

035	36	YES	NO
036	34	NO	IVF
037	38	YES	NO
038	30	YES	NO
039	33	NO	AI
040	32	YES	AI
042	31	NO	AI
043	39	YES	AI
044	39	NO	NO
045	34	YES	AI
046	32	YES	IVF
047	33	NO	AI
048	28	NO	IVF
049	37	NO	NO
050	30	NO	IVF
051	38	NO	IVF
052	38	NO	AI
053	36	YES	AI+IVF
054	38	YES	AI
055	38	NO	AI
057	38	NO	
058	36	NO	AI
059	38	NO	NO
060	38	NO	NO
061	32	YES	NO
063	39	YES	AI
064	32	YES	AI
065	31	YES	NO
066	33	NO	NO
067	34	NO	NO
068	40	NO	IVF
069	36	NO	NO
072	39	NO	NO
073	27	NO	NO
074	39	NO	AI
076	37	YES	NO
077	39	NO	IVF

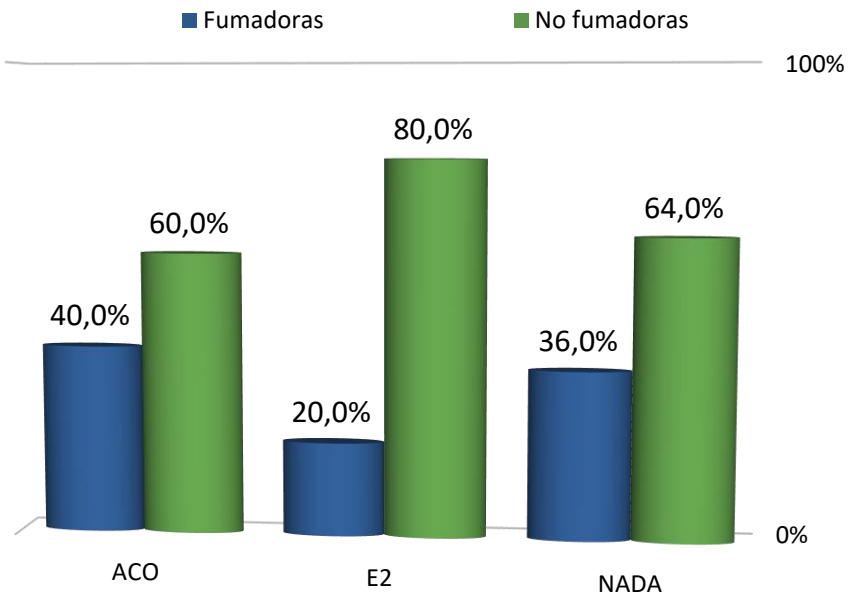
082	39	NO	NO
083	33	NO	NO
084	34	NO	AI
085	36	NO	IVF
086	32	NO	AI
091	37	NO	AI
092	35	NO	AI
093	36	YES	
095	35	NO	NO
097	36	YES	AI
098	37	NO	AI
099	35	NO	AI
100	36	YES	AI
101	35	NO	IVF
103	37	YES	NO
106	36	YES	NO

Table 4. Demographic characteristics

		N	Mean	Standard deviation (SD)	P value
AGE (y)	**ACO**	34	35,12	3,063	
	E2	25	36,36	2,361	
	NADA	27	34,70	3,383	P=0.162
	Total	86	35,35	3,028	

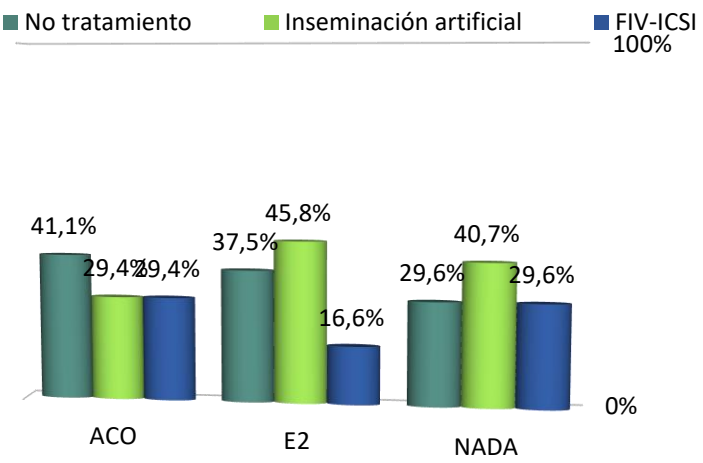
Table 5. Mean and Standar deviation of AGE.

The frequency of tobacco use in the different study groups is described in the following graph (Graph 1). No differences were observed between the different study groups (P=0.318)



Graph 1. Tobacco consumption in the different study groups

Another of the factors analyzed was the performance of previous reproductive treatments in the patients included in the study, one of the inclusion criteria being that they had not undergone more than 2 previous IVF-ICSI cycles. The data observed are shown in the graph below (Graph 2). No statistically significant differences were observed between groups (P= 0.653). Therefore, no statistical difference were found in demographic variables between groups, as shown in table 5 and graphs1-2.



Graph 2. Previous fertility treatments in the different study groups

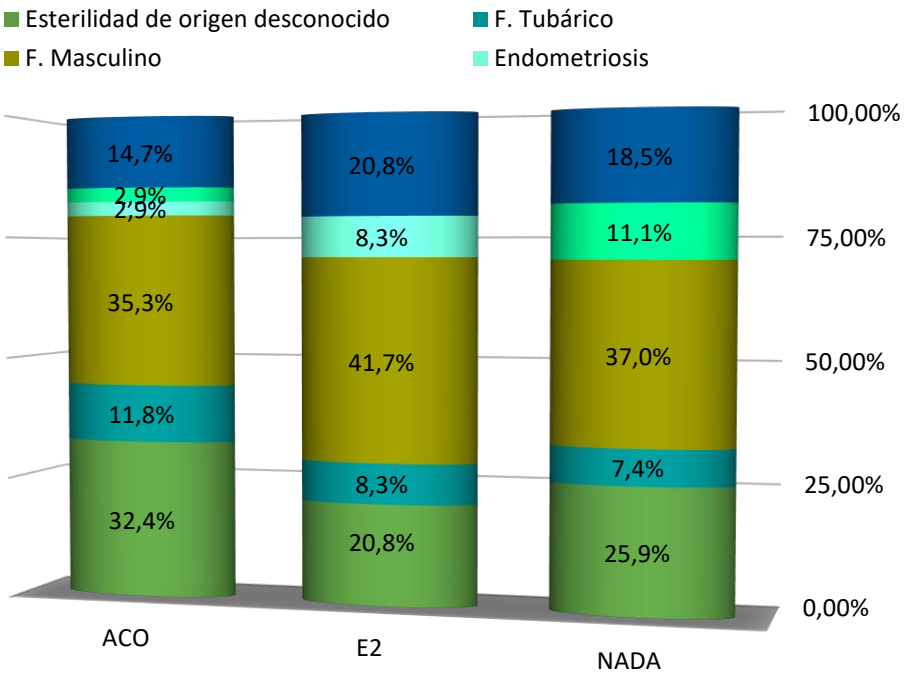
Cause of infertility and ovarian reserve parameters are recorded in the follow table:

	AMH	FSH BASAL	ESTRADIOL BASAL	CAUSE OF INFERTILITY
001	1.96	11.47	44	MIXED
002	2.19	8.46	31	MALE FACTOR
003	2.16	7	36	UNKNOWN
004	0.5	4.5	41	MALE FACTOR
005	3.85	7	43	MIXED
006	3.32	5.34	37	MALE FACTOR
007	2.04	10.21	58	MIXED
008	2.8	7	44	MALE FACTOR
009	2	2	45	TUBAL
010	1.67	9.32	52	MIXED
011	5.05	10.41	35	UNKNOWN
012	6.87	5	37	UNKNOWN
013	2.31	10	70	UNKNOWN
014	3.4	6	57	MALE FACTOR
016	1.65	8.75	28	MALE FACTOR
017	2.06	10.24	23	MALE FACTOR
018	6.64	7.5	20	UNKNOWN
019	0.78	7.02	57	MALE FACTOR

020	1.93	9.34	45	UNKNOWN
021	0.77	5.75	52	UNKNOWN
022	1.13	8.62	49	ENDOMETRIOSIS
023	6.78	6.07	72	MALE FACTOR
024	4.49	10.4	66	MALE FACTOR
025	7.86	5.25	45	UNKNOWN
026	3.22	5.73	33	MALE FACTOR
027	1.29	6.51	25	UNKNOWN
028	5.06	7.61	27	MALE FACTOR
029	2.33	9.52	46	MALE FACTOR
030	1.24	12.4	96	UNKNOWN
031	1.01	5.8	45	TUBAL
032	6.85	9.06	28	TUBAL
033	2.07	7.70	116	MALE FACTOR
034	5.20	5.19		MALE FACTOR
035	6.55	5.05	43	MALE FACTOR
036	1.72	5.15	43	MALE FACTOR
037	1.12	10.33	72	MALE FACTOR
038	1.4	6.3	70	MALE FACTOR
039	0.87	6.61	51	OVARIAN FACTOR
040	1	7	22	UNKNOWN
042	3.37	6.81	24	UNKNOWN
043	2	5.87	54	MALE FACTOR
044	2.9	6	78	ENDOMETRIOSIS
045	1.56	9.74	93	MIXED
046	1.98	10	45	UNKNOWN
047	1.95	6.85	51	ENDOMETRIOSIS
048	1.84	7.93	58	MALE FACTOR
049	4.92	8.22	52	MALE FACTOR
050	1.53	6.95	47	UNKNOWN
051	0.83	9.93	54	OVARIAN FACTOR
052	5.83	7.79	70	UNKNOWN
053	4.50	9.28	57	UNKNOWN
054	1.85	7.33	51	UNKNOWN
055	4.83	7.78	57	UNKNOWN

057	4.63	6.79	32	UNKNOWN
058	2.59	8.70	46	MIXED
059	5.48	8.72	38	MALE FACTOR
060	2.51	8.77	40	MALE FACTOR
061	2.3	7	47	MALE FACTOR
063	3.02	8.60	59	TUBAL
064	2.53	7.72	71	OVARIAN FACTOR
065	2.51	8.10	58	TUBAL
066	3.98	9.63	49	TUBAL
067	1.81			MIXED
068	2.61	5.85	29	MIXED
069	1.77	9.36	45	MALE FACTOR
072	6.39	6.88	23	MALE FACTOR
073	1.56	9.77	42	MALE FACTOR
074	1.98	7.11	39	MIXED
076	0.86	6.61	120	MIXED
077	1.99	5.27	59	OVARIAN FACTOR
082	1.33	6.67	60	MIX
083	2.35	7.25	35	TUBAL
084	1.49	9	65	MALE FACTOR
085	1.28	10.51	82	MALE FACTOR
086	1.31	7.03	59	UNKNOWN
091	2.5	6.2	57	UNKNOWN
092	1.90	8.40	81	MIXED
093				MALE FACTOR
095	2.07	9.14	39	TUBAL
097	5.84	6.5	40	UNKNOWN
098	5.94	8.78	56	UNKNOWN
099	1.90	8.4	81	UNKNOWN
100	2.49	7.99	37	MIXED
101	3.5	8	99	MALE FACTOR
103	1.06	16	40	MIXED
106	0.93	7.8	48	MIXED

Table 6. Cause of infertility and ovarian reserve parameters



Graph 3. Causes of infertility

		N	Mean	Standard deviation (SD)	P value
FSH basal	*ACO*	34	7,793	2,405	
	E2	25	7,776	1,550	
	NADA	27	7,845	1,940	P=0.968
	Total	86	7,805	2,009	
E2 basal	*ACO*	34	48,00	17,689	
	E2	25	56,12	24,020	P=0.494
	NADA	27	51,23	18,351	
	Total	86	51,46	20,028	
AMH basal	*ACO*	34	2,838	1,897	
	E2	25	3,363	1,957	P=0.159
	NADA	27	2,285	1,377	
	Total	86	2,816	1,798	

Table 7. Mean and Standar deviation of ovarian reserve parameters

No statistical difference were found in ovarian reserve parameters and cause of infertility between groups, as shown in table 7 and graph 3.

9.2. PROTOCOL DEVIATIONS

In November 2017, the drug Meriestra (2mg valerate oestradiol) was withdrawn from the market due to stock out. We requested authorisation from the competent authority AEMPS (Spanish Agency of Medicines and Medical Devices) to exchange it for another drug with the same active ingredient, Progyluton (only white tables, 2 mg valerate oestradiol), which was authorised on 24th January 2018.
Recruitment was therefore halted from November 2017 to January 2018. For this reason, we also requested an extension of the study until July 2018.

10. EFFICACY EVALUATION

10.1 EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL PATIENT DATA

We performed an analysis by protocol of the data obtained, which are shown in the following tables.

10.1.1 PRIMARY EFFICACY VARIABLES

- *PREGNANCY RATES*

The main objective of this study was to observe the effects of the use of steroid pretreatments prior to the start of ovarian stimulation in an IVF-ICSI cycle on the gestational rates of the patients.

Due to the need for embryo vitrification in a significant percentage of the cycles performed daily, we decided to evaluate these rates separately in the cycles with transfer in the same stimulation cycle with respect to those cases with deferred embryo transfer.

Transfer was performed in the same stimulation cycle (fresh) in 51 patients included in the study, which represented 59.30% of the cycles, and deferred transfer in 23 patients, which represented 26.74% of the cycles. In the remaining 12 patients, no transfer was performed due to cancellation of the cycle, accounting for 13.95% of the cycles.

However, in the calculation of the gestational rates with delayed embryo transfer, patients with direct delayed embryo transfer (23 patients) and patients with negative fresh transfer who underwent a subsequent embryo transfer after endometrial preparation (7 patients) were included.
This was done to avoid possible biases due to the effect of stimulation and pretreatment on endometrial conditions and to be able to assess the direct effect on embryo implantation capacity in these patients.

Vitrified embryo transfers were performed in 30 of the patients included in the study: 14 in the group pre-treated with contraceptives, 8 in the study group with estrogens and 8 in the group without treatment in the luteal phase prior to stimulation.

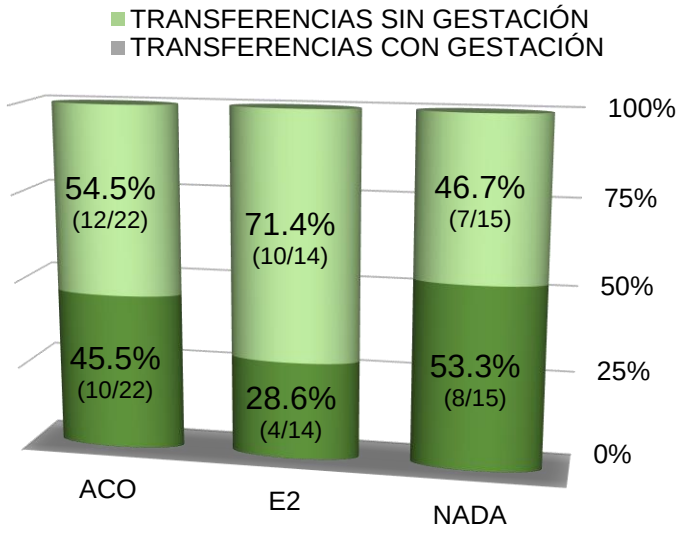
Deferred embryo transfers were not performed in the other 44 patients who achieved embryo transfer (17 in the group pretreated with OCP, 14 in the group pretreated with E2 and 13 in the group without pretreatment), taking into account that 12 of the 86 patients included in the study were cancelled cycles. Deferred transfer was not performed in patients who achieved gestation after fresh transfer or in patients who did not have vitrified embryos. No significant differences were observed between groups in the percentage of deferred transfers (P=0.784).

10.1.1.1. CLINICAL PREGNANCY RATES

- *CLINICAL PREGNANCY RATE AFTER FRESH EMBRYO TRANSFER*

Among the 51 fresh transfers, 22 clinical gestations were obtained, representing a clinical gestation rate of 43.1% among the 3 groups under study, compared to an absence of pregnancy in the other 29 transfers.

Regarding the data observed in the different study groups, in the group treated with oral contraceptives, 22 fresh transfers were performed, of which 10 showed clinical gestation (45.5%), in the group treated with estrogens, 14 fresh transfers were performed, of which 4 showed clinical gestation (28.6%) and in the group without previous treatment, 15 fresh transfers were performed, of which 8 showed clinical gestation (53.3%). However, the differences observed between groups did not reach statistical significance (P=0.388).

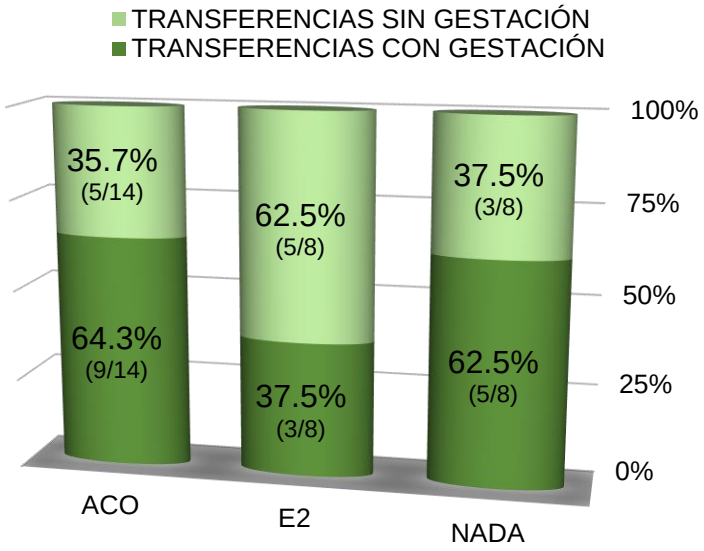


Graph 4. Clinical pregnancy rate after Fresh embryo transfer

- *CLINICAL PREGNANCY RATE AFTER FROZEN EMBRYO TRANSFER*

Of the 30 patients with deferred transfer included in the study (of whom 23 had direct deferred transfer and 7 after failure of fresh transfer), 17 clinical gestations were obtained, giving an overall clinical gestation rate among the 3 groups of 56.66%.

Regarding the data observed in the different groups under study, in the group treated with oral contraceptives, 14 deferred transfers were performed, of which 9 showed clinical gestation (64.3%), in the group treated with estrogens 8 deferred transfers were performed, of which 3 had clinical gestation (37.5%) and in the group without previous treatment 8 deferred transfers were performed, of which 5 had clinical gestation (62.5%). However, the differences observed between groups did not reach statistical significance (P=0.441).



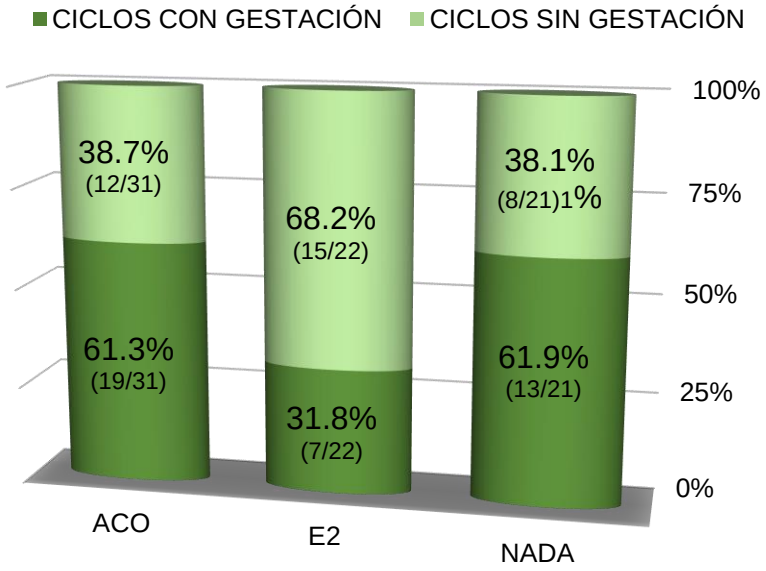
Graph 5. Clinical pregnancy rate after Frozen embryo transfer

- *CUMULATIVE PREGNANCY RATE*

To calculate this rate, all the patients in whom transfer was performed were included, which were 74 (31 patients had embryo transfer in the group pretreated with contraceptives, 22 patients in the group pretreated with estrogens and 21 patients in the group without pretreatment), with 39 clinical gestations obtained. The overall cumulative clinical gestation rate between groups was 52.70%.

Of the 86 patients included in the study, 12 were cancelled cycles that did not reach embryo transfer, so they were not included in the analysis.

Thus, in the group pretreated with contraceptives, 10 gestations were obtained after fresh transfer + 9 gestations after deferred transfer, with a cumulative gestation rate of 61.29% (19/31), in the group pretreated with estrogens 4 gestations were obtained after fresh transfer + 3 gestations after deferred transfer, with a cumulative gestation rate of 31. 81% (7/22) and finally, in the group without pretreatment, 8 gestations were obtained after fresh transfer + 5 gestations after delayed transfer, with a cumulative gestation rate of 61.9% (13/21). Again, statistical significance is not reached (P=0.105).



Graph 6. Cumulative clinical pregnancy rate

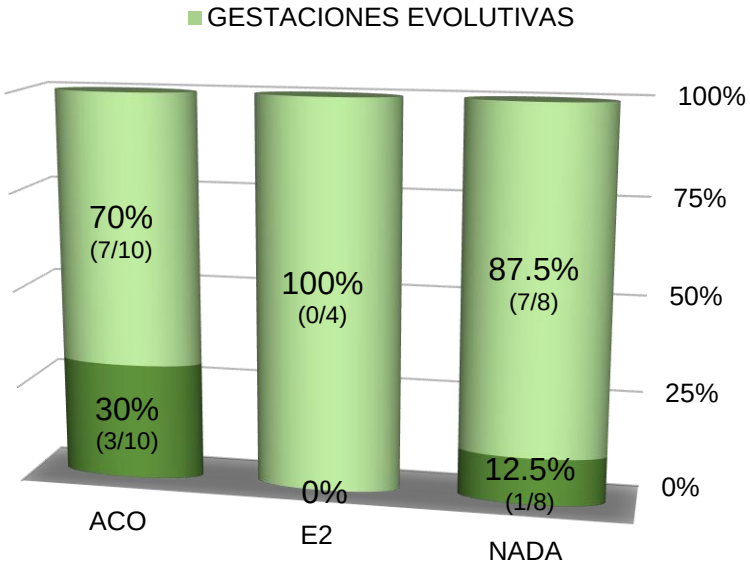
10.1.1.2. MISCARRIAGE RATES

Once again, we performed an analysis of the pregnancies interrupted in the different groups under study after transfer in the same stimulation cycle, after delayed transfer and finally, the cumulative rate between the two.

- *MISCARRIAGE RATE AFTER FRESH EMBRYO TRANSFER*

A total of 22 clinical gestations were obtained after embryo transfer in the same stimulation cycle, of which 4 were non-evolutionary, with an overall interrupted gestation rate of 18.18%. The terminations were distributed as follows: 3 in the group pretreated with contraceptives, none in the group pretreated with estrogens and 1 in the group without pretreatment.

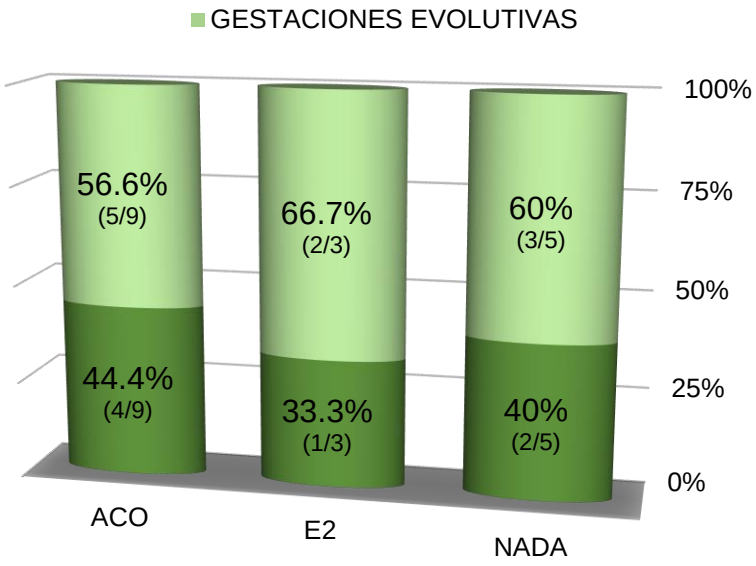
Among the pregnancies interrupted only 1 was above 12 weeks of gestation, being from the group treated with contraceptives, the rest were interruptions below 12 weeks of gestation. Once again, the differences observed between groups did not reach statistical significance (P=0.368).



Graph 7. Miscarriage rate after fresh embryo transfer

- *MISCARRIAGE RATE AFTER FROZEN EMBRYO TRANSFER*

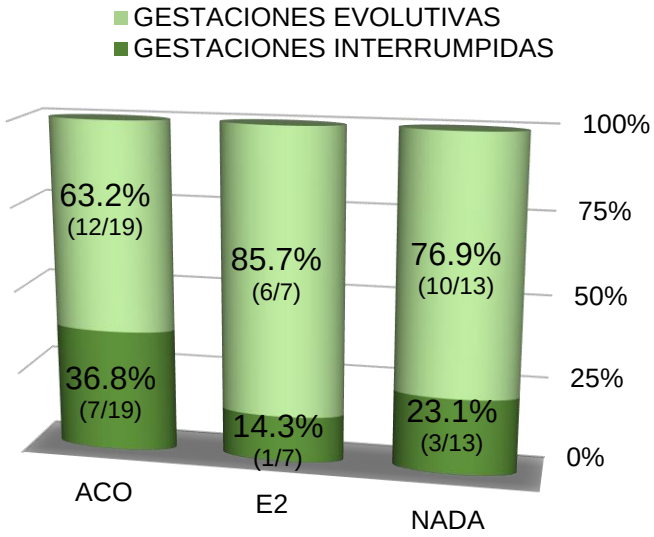
Seventeen gestations were obtained after delayed embryo transfer among the 3 study groups, with 7 gestational interruptions, giving an overall interrupted gestation rate of 41.17%. The terminations were distributed as follows: 4 in the group pretreated with contraceptives, one in the group pretreated with estrogens and 2 in the group without pretreatment. In all cases there were abortions below 12 weeks. In the comparative analysis between the study groups, no significant differences were detected (P=0.942)



Graph 8. Miscarriage after frozen embryo transfer

- *CUMULATIVE MISCARRIAGE RATE*

A total of 39 gestations were obtained after fresh transfer + delayed transfer, with 11 aborted gestations, giving a cumulative aborted gestation rate of 28.20%. The abortions were distributed as follows: 7 in the group pretreated with contraceptives, one in the group pretreated with estrogens and 3 in the group without pretreatment. After the comparative analysis between groups, no statistically significant differences were detected (P=0.463)



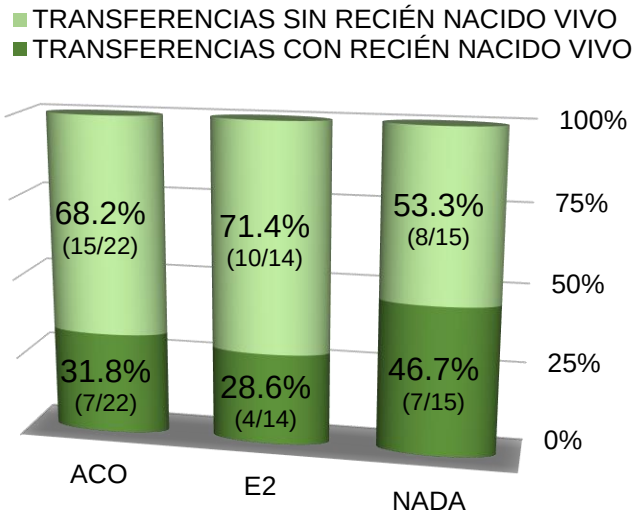
Graph 9. Cumulative miscarriage rate

10.1.1.3. LIVE BITH RATE PER EMBRYO TRANSFER

We analyzed this rate once again, taking into account the newborns after fresh transfer, after delayed transfer and the cumulative rate between the two.

- *LIVE BIRTH RATE AFTER FRESH EMBRYO TRANSFER*

Eighteen evolutionary gestations with live newborn were obtained after 51 fresh transfers, only one of them being a twin gestation. This multiple gestation was obtained in the group pretreated with contraceptives. The distribution of pregnancies by study group is shown below. The overall live birth rate in the study population was 35.29%. In the comparative analysis between groups, no statistically significant differences were detected (P=0.537).

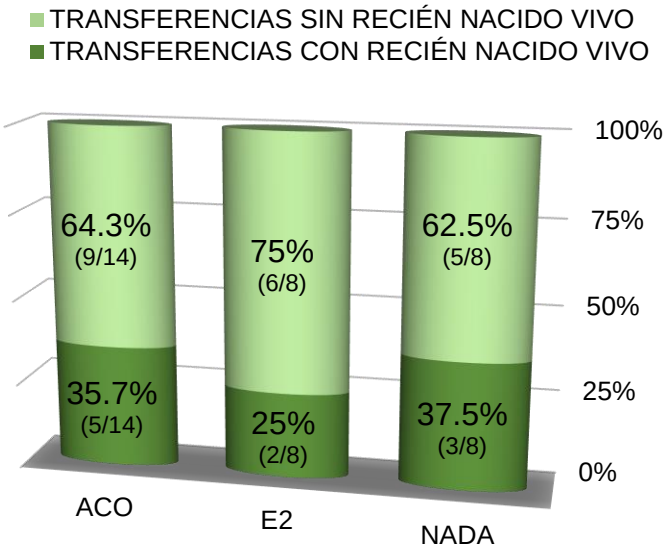


Graph 10. Live birth rate after fresh embryo transfer

- *LIVE BIRTH RATE AFTER FROZEN EMBRYO TRANSFER*

Ten evolutionary gestations with live newborn were obtained after 30 frozen embryo transfers, only one of them being a twin gestation. This multiple gestation was obtained in the group pretreated with contraceptives once again. The distribution of pregnancies by study group is shown below. The overall live birth rate in the study population after

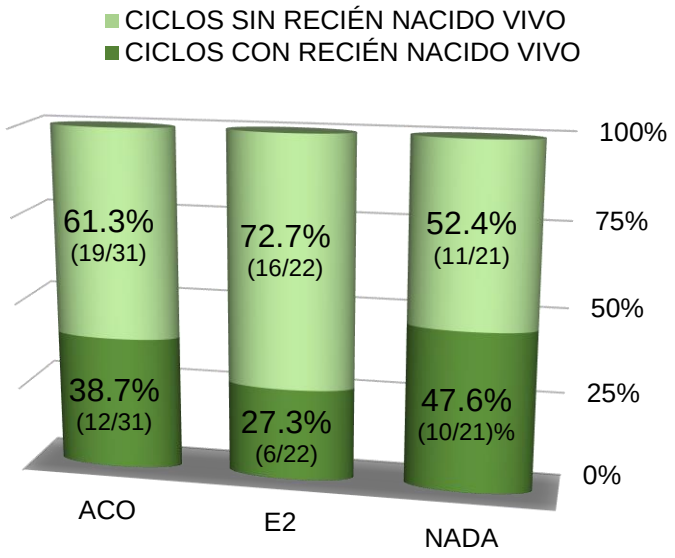
delayed embryo transfer was 33.33%. In the comparative analysis between groups, no statistically significant differences were detected (P=0.872).



Graph 11. Live birth rate afther frozen embryo transfer

- *CUMULATIVE LIVE BIRTH RATE*

A total of 28 evolutionary gestations with live birth were obtained in the 74 patients included in the study who went on to have embryo transfer, representing an overall rate of delivery with live birth of 37.83%. Only 2 of these gestations were multiple gestations as previously indicated with a total of 30 live newborns. The distribution of the pregnancies by study group is shown below. In the comparative analysis between groups, despite the differences observed, statistical significance was not reached (P=0.443).



Graph 12. Cumulative live bith rate

	CLINICAL PREGNANCY RATE (FRESH ET)	MISCARRIAGE AFTER FRESH ET	LIVE BIRTH RATE (FRESH ET)	CLINICAL PREGNANCY RATE (FROZEN ET)	MISCARRIAGE AFTER FROZEN ET	LIVE BIRTH RATE (FROZEN ET)
001	0		0	NO ET		
002	NO ET			NO ET		
003	1	NO	1	NO ET		
004	0		0	NO ET		
005	1	NO	1	NO ET		
006	1	NO	1	NO ET		
007	0		0	0		0
008	1	NO	1	NO ET		
009	NO ET			NO ET		
010	1	NO	1	NO ET		
011	0		0	NO ET		
012	0		0	NO ET		
013	0		0	NO ET		
014	0		0	1	YES	0

016	1	YES	0	NO ET		
017	NO ET			0		0
018	NO ET			NO ET		
019	NO ET			NO ET		
020	NO ET			NO ET		
021	1	YES	0	NO ET		
022	0		0	0		0
023	0		0	NO ET		
024	0		0	NO ET		
025	0		0	NO ET		
026	0		0	NO ET		
027	0		0	NO ET		
028	1	NO	1	NO ET		
029	0		0	0		0
030	0		0	NO ET		
031	NO ET			NO ET		
032	NO ET			0		0
033	0		0	NO ET		
034	NO ET			NO ET		
035	NO ET			1	NO	1
036	0		0	NO ET		
037	1	NO	1	NO ET		
038	NO ET			1	NO	1
039	1	NO	1	NO ET		
040	NO ET			NO ET		
042	NO ET			1	NO	1
043	1	NO	1	NO ET		
044	0		0	NO ET		
045	1	NO	1	NO ET		
046	1	NO	1	NO ET		
047	1	NO	1	NO ET		
048	1	NO	1	NO ET		
049	1	NO	1	NO ET		
050	0		0	1	YES	0
051	0		0	NO ET		
052	NO ET			1	YES	0
053	NO ET			1	NO	1

054	1	NO	1	NO ET		
055	NO ET			0		0
057	0		0	NO ET		
058	NO ET			1	YES	0
059	NO ET			0		0
060	NO ET			0		0
061	0		0	NO ET		
063	0		0	0		0
064	NO ET			NO ET		
065	NO ET			1	NO	1
066	NO ET			1	NO	1
067	1	YES	0	0		0
068	NO ET			NO ET		
069	0		0	1	YES	0
072	NO ET			0		0
073	NO ET			1	YES	0
074	NO ET			NO ET		
076	NO ET			NO ET		
077	NO ET			1	NO	1
082	0		0	0		0
083	NO ET			NO ET		
084	1	YES	0	NO ET		
085	1	NO	1	NO ET		
086	NO ET			0		0
091	NO ET			NO ET		
092	0		0	NO ET		
093	NO ET			1	NO	1
095	1	NO	1	NO ET		
097	NO ET			1	NO	1
098	NO ET			1	YES	0
099	0		0	NO ET		
100	0		0	NO ET		
101	NO ET			1	NO	1
103	1	NO	1	NO ET		
106	0		0	NO ET		

Table 8. Gestational outcomes

10.1.2. OTHER OBJECTIVES OF PRIMARY INTEREST

- *EFFECT OF EXPOSURE TIME TO PRETREATMENTS ON GESTATIONAL OUTCOMES.*

The following results were observed for the two groups that received treatment, according to the possibility of gestation based on this time of exposure:

- GROUP PRETREATED WITH CONTRACEPTIVES

Of the 34 patients included in this study group, 19 achieved gestation in fresh transfer or after delayed transfer, compared to 15 patients who did not achieve gestation. The mean exposure time between the two groups did not show statistically significant differences (P=0.941).

	N	Mean	Standard deviation (SD)
GESTATION	19	24,65	7,607
NO GESTATION	15	25,93	8,740

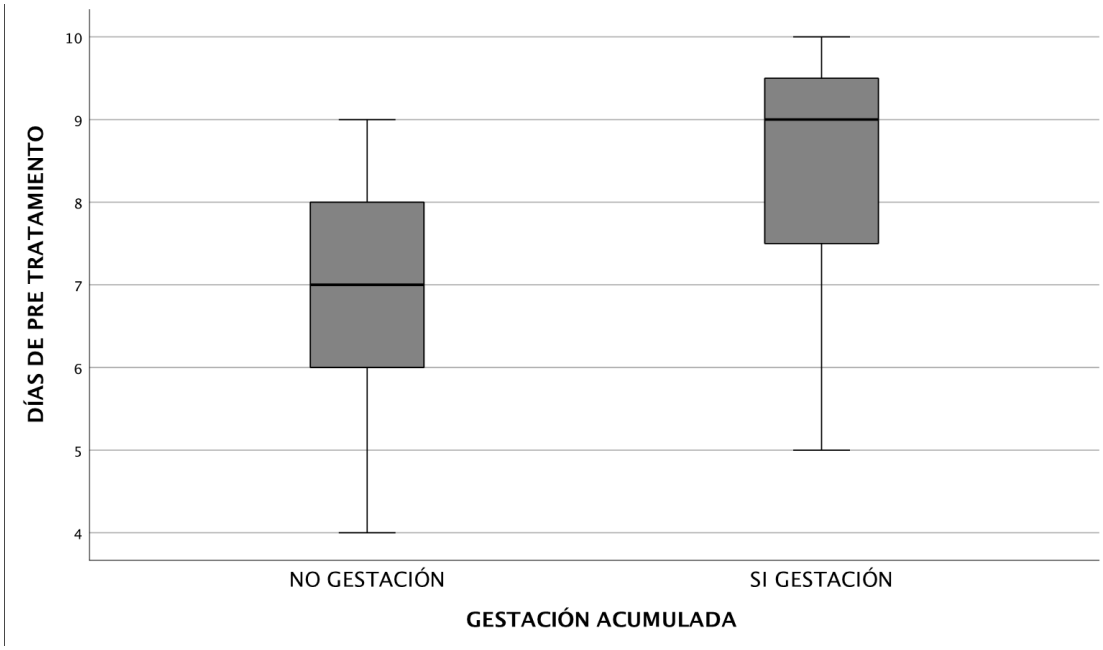
Table 9. Pregnancy outcomes Contraceptive group

- GROUP PRETREATED WITH OESTROGENS

Of the 25 patients included in this group, 7 achieved gestation in fresh or delayed transfer, compared to 18 patients who did not. The mean exposure time between the two groups did not reach statistical significance, but was close (P=0.078).

	N	Mean	Standard deviation (SD)
GESTATION	7	8,29	1,976
NO GESTATION	18	6,80	1,424

Table 10. Pregnancy outcomes Oestrogen group



Ilustration 1. Pregnancy outcomes in the oestrogen group related to days of exposure

10.1.3. SECONDARY OBJECTIVES

- *OVARIAN STIMULATION PARAMETERS*

Among the secondary objectives of the study was to evaluate different parameters directly related to stimulation and the response of these patients according to the treatment received in the luteal phase prior to the start of the cycle. The following is a breakdown of the different factors analyzed in this regard.

- The first parameter analyzed was the **TOTAL DOSAGE of gonadotropins** required during stimulation with HMG-HP (Menopur®) in the different study groups, as shown in the following table. No statistically significant differences were observed (P=0.670).

	N	Mean	Standard deviation (SD)
DOSE HMG (UI) ACO	34	2303,56	603,06
E2	25	2215,08	553,03
NADA	27	2206,11	698,27
Total	86	2247,24	615,47

Table 11. Dose of gonadotropines in each study group

- Another factor analyzed was the **DURATION of stimulation** measured in days in the different groups under study. These times are shown in the following table. Once again, no statistically significant differences were observed (P=0.188).

	N	Mean	Standard deviation (DS)
TIME(DAYS)) ACO	34	10,65	1,704
E2	25	10,96	1,241
NADA	27	10,30	1,409
Total	86	10,63	1,495

Table 12. Duration of stimulation in each study group

- Among the parameters evaluated, we included **hormonal determinations on the day of the ovulatory trigger of PROGESTERONE (ng/ml) and ESTRADIOL (pg/ml) levels,** to evaluate the effect of the pre-medications used in the study on hormonal changes and the possibility of embryo transfer in an ongoing versus deferred cycle. In both determinations we did not find statistically significant differences between the different groups under study (P=0.485 in the comparison of Progesterone levels and P=0.852 in the comparison of Estradiol levels). We see the values observed by groups in the tables below.

		N	Mean	Standard deviation (SD)
Progesterone	*ACO*	34	0,861	0,355
	E2	25	0,824	0,382
	NADA	27	0,972	1,021
	Total	86	0,893	0,642

Table 13. Progesterone level on trigger day

		N	Mean	Standard deviation (SD)
Estradiol	*ACO*	34	2194,44	1007,17
	E2	25	2605,32	1832,94
	NADA	27	2452,44	1152,71
	Total	86	2394,88	1333,80

Table 14. Oestradiol level on trigger day

Finally, we have recorded the ovarian response to stimulation in the different study groups, assessing different parameters:

I. **NUMBER OF FOLLICLES > 16 mm on the day of the ovulatory trigger.** The measurement was performed by transvaginal ultrasound, taking the mean of both major diameters in each of the follicles. The data observed are reflected in the following table. No statistically significant differences were observed between groups (P=0.564).

		N	Mean	Standard deviation (SD)
Follicles	*ACO*	34	9,00	5,371
	E2	25	8,36	4,386
	NADA	27	9,81	5,791
	Total	86	9,07	5,217

Table 15. Number of follicles > 16 mm on trigger day

II. **NUMBER OF CUMULUS-OVOCITARY COMPLEXES** obtained in the follicular puncture. The data collected are shown in the following table. No significant differences were observed between the groups to be compared (P=0.912).

		N	Media	Desviación estándar (DS)	Mínimo	Máximo
Complejos C-O	*ACO*	34	7,56	5,378	0	23
	E2	25	7,52	3,906	2	18
	NADA	27	7,63	5,924	0	25
	Total	86	7,57	5,126	0	25

Table 16. Number of complexes obtained in each group

III. **NUMBER OF MII**, or mature, oocytes that can be used for fertilization. As in the previously used parameters, we can see the data collected in the following table. No statistically significant differences were observed in the comparison between the study groups (P=0.972).

		N	Media	Desviación estándar (DS)	Mínimo	Máximo
Ovocitos MII	*ACO*	34	6,32	5,168	0	21
	E2	25	5,76	3,677	2	15
	NADA	27	6,15	4,680	0	18
	Total	86	6,10	4,576	0	21

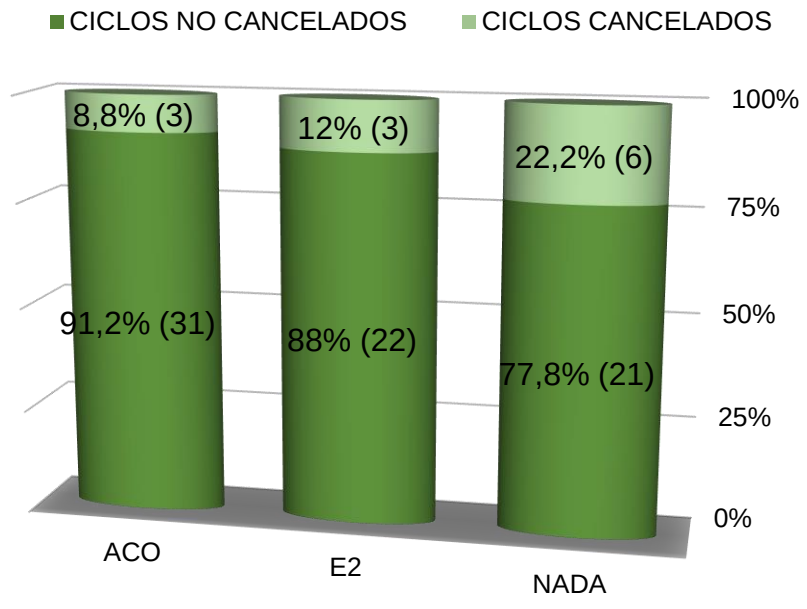
Table 17. Number of mature oocytes obtained in each group

IV. Another of the parameters analysed in relation to stimulation was the number of transferable embryos obtained in the different groups to be compared. Again we did not observe statistically significant differences between the groups compared (P=0.976). We see the outcomes obtained in the following table.

		N	Media	Desviación estándar (DS)	Mínimo	Máximo
Nº Embriones	*ACO*	34	2,44	1,599	0	6
	E2	25	2,36	1,655	0	6
	NADA	27	2,70	2,880	0	14
	Total	86	2,50	2,079	0	14

Table 18. Number of transfereable embryos in each group

V. The last of the parameters analysed in terms of the outcomes of stimulation was the cycle cancellation rate observed in the different study groups. We see the data observed in the graph below, with no statistically significant differences detected between the study groups (P=0.307).



Graph 13. Cancelation rate in each study group

Regarding the causes of cycle cancellation:

- In the contraceptive treatment group, 1 cycle was cancelled due to low response (< 3 developing follicles) and 2 due to absence of evolutive embryos for transfer on day 3 of development.
- In the oestrogen treatment group, the 3 cycles cancelled were due to failure to obtain transferable developing embryos.
- In the no pre-treatment group, 2 cycles were cancelled due to low response, 1 cycle was cancelled due to failure to obtain oocytes in the follicular puncture and in 3 cycles no transferable embryos were obtained.

	TOTAL DOSAGE (UI)	DURATION STIMULATION (Days)	E2 TRIGGER DAY (pg/ml)	PROGESTERONE TRIGGER DAY (Ng/ml)	Nº FOL > 16 mm	Nº COC	Nº MII	Nº Embryos	Cancellation rate
001	2358	9	2085	1.14	5	5	5	1	0
002	1385	7	274	0.73	3	0	0	0	CANCEL
003	1500	10	1456	0.55	9	12	9	4	0
004	2700	9	935	0.67	4	4	4	2	0
005	1200	12	692	0.86	4	6	2	2	0
006	1125	9	4249	0.86	15	8	6	5	0
007	1684	10	1611	0.67	8	5	5	4	0
008	1234	10	1363	0.74	9	5	4	4	0
009	2075	10	1015	0.74	3	3	1	0	CANCEL
010	2250	10	2740	0.85	10	10	8	2	0
011	1446	12	1521	1.07	10	9	9	6	0
012	1750	9	1807	0.52	9	8	0	1	0
013	1646	9	2260	0.41	6	5	3	2	0
014	1500	10	1481	0.85	8	8	8	7	0
016	2250	11	723	0.83	11	9	8	4	0
017	2475	11	3623	1.64	11	13	11	3	0
018	1469	14	488	0.76	2	0	0	0	CANCEL
019	2472	10	2199	0.61	7	5	2	2	0
020	2225	9	2284	1.16	14	8	4	0	CANCEL

021	2246	9	2063	1.10	6	2	2	2	0
022	3072	11	782	1.03	8	8	7	4	0
023	1650	11	2874	0.63	10	9	7	6	0
024	2472	15	1423	0.82	4	2	2	1	0
025	1568	14	2213	0.46	11	7	7	5	0
026	2136	10	3702	1.37	10	9	9	3	0
027	2550	12	1253	0.68	10	10	9	2	0
028	1549	11	1898	0.49	7	7	3	2	0
029	3558	13	1468	0.9	4	3	3	3	0
030	2375	11	1576	0.64	5	1	1	1	0
031	2358	9	1952	5.67	5	0	0	0	CANCEL
032	1500	10	4467	1.16	14	13	13	4	0
033	2734	11	2879	1.30	7	7	2	2	0
034	1120	10	512	0.30	0	0	0	0	CANCEL
035	1796	10	4719	0.93	30	25	18	14	0
036	3558	13	1742	0.56	6	6	5	1	0
037	2882	11	1478	0.27	7	7	6	2	0
038	1273	11	4127	1.82	16	12	12	6	0
039	4158	14	2367	0.46	6	2	2	1	0
040	2700	9	1487	1.12	7	7	6	2	0
042	2431	13	2663	0.64	13	10	8	6	0
043	2250	10	1518	0.67	4	5	3	2	0
044	2025	9	1752	0.53	3	3	3	2	0
045	2585	10	3389	0.80	10	10	8	2	0
046	1950	9	3282	0.76	5	3	3	3	0
047	1687	10	2843	1.03	12	12	11	4	0
048	1998	9	2601	0.68	15	15	13	6	0
049	2335	12	2079	0.16	8	6	4	2	0
050	2320	12	2693	1.71	10	9	8	3	0
051	3000	10	1952	0.74	4	3	3	2	0
052	1722	10	3476	1.16	25	23	21	5	0
053	2475	11	4366	0.77	13	6	5	4	0
054	1800	8	1120	0.37	3	1	1	1	0
055	1650	11	2059	0.47	8	7	6	2	0
057	1875	12	640	0.64	3	3	3	2	0
058	2000	10	2060	0.69	18	18	13	4	0
059	2100	14	2197	0.23	7	6	5	2	0

060	2300	10	7369	1.24	10	7	7	4	0
061	1650	11	2153	1.44	9	7	7	1	0
063	2025	9	1669	0.80	7	5	5	4	0
064	3300	12	1284	0.39	8	3	1	0	CANCEL
065	1870	10	4165	0.61	11	11	9	4	0
066	2244	12	7266	2.1	20	18	15	5	0
067	2800	12	3773	0.90	9	9	9	3	0
068	2700	12	2917	1.02	10	5	4	0	CANCEL
069	2250	10	1632	0.15	7	6	5	2	0
072	1983	11	1878	0.45	11	9	7	1	0
073	2475	11	3377	1.02	20	20	20	5	0
074	3300	11	1515	0.50	5	3	2	0	CANCEL
076	3150	12	4360	0.76	13	13	6	0	CANCEL
077	1800	8	2311	0.86	10	10	6	2	0
082	3000	10	3500	1.09	6	4	4	2	0
083	2325	8	1537	0.71	9	9	7	0	CANCEL
084	3300	11	1117	1.16	3	3	2	1	0
085	3035	12	2531	0.84	10	10	9	3	0
086	3225	12	3369	1.17	10	7	7	2	0
091	2057	11	2964	0.9	10	10	5	0	CANCEL
092	2250	10	1762	0.46	4	4	3	2	0
093	2550	9	3073	0.69	13	13	8	2	0
095	2475	11	1919	0.91	8	2	2	1	0
097	2250	10	4234	1.59	24	22	20	2	0
098	2450	11	2926	1.09	13	13	13	1	0
099	2250	10	1762	0.46	4	4	3	2	0
100	2250	10	2768	0.7	8	7	5	2	0
101	2247	11	5272	1.46	16	9	5	2	0
103	3300	11	2040	0,51	7	5	5	1	0
106	2250	10	1039	1.42	4	3	3	2	0

Table 19. Summary of ovarian stimulation parameters data in each group

10.2. EFFICACY RESULTS CONCLUSIONS

In our study, no statistically significant differences were detected in the rates of clinical gestation, abortion or delivery with live newborn between the different study groups, neither in fresh transfer, nor delayed or per cycle initiated. However, there was a trend towards worse gestational outcomes in the oestrogen pre-treated group in terms of clinical gestation rate and live birth in the three situations described above.

- No statistically significant differences were detected in any of the parameters evaluated during the stimulation cycle: days of stimulation, dose of gonadotrophins used, levels of oestradiol or progesterone on the day of the ovulatory trigger, number of follicles developed, number of oocytes obtained, number of mature oocytes obtained, number of embryos evolved or cycle cancellation rate.

- No differences were observed in the possibility of achieving gestation according to the time of exposure to oral contraceptives in the patients included in our study.

- No statistically significant differences were detected between the time of exposure to oestrogens in patients belonging to this study group, between patients who did or did not achieve pregnancy. However, there are differences close to reaching this significance, noting that a longer exposure time seems to be associated with a higher probability of achieving pregnancy.

- The use of oestrogens in normorresponding patients is likely to be a comparable strategy in terms of gestational outcomes with optimised administration schedules, taking into account the studies published to date.

11. SAFETY EVALUATION

No adverse effects were detected in the patients included in the study. There were no deaths, other serious adverse events or other significant adverse events during the study.

12. DISCUSSION AND OVERALL CONCLUSIONS

At the present time, there is an increasing demand for reproductive treatments in our society, mainly due to delayed childbearing and changes in both society and lifestyle that lead to an increase in infertility rates. This means an increase in the workload in human reproduction units, making it necessary to organise the activity in an efficient way that allows the available resources to be used and avoids the periodic overload of activity.

With this objective in mind, the programming of the start of IVF-ICSI cycles in an antagonist protocol with different steroid treatments has been proposed. This allows for a more flexible start and, therefore, an equitable distribution of the workload. Among the drugs proposed for this purpose are oral contraceptives, oestrogens or gestagens. These 3 strategies have been shown to be useful in organising activity, although their impact on gestational rates is controversial due to the different published conclusions. For this reason, the idea of conducting this study arose in order to add new data to the available evidence.

We conducted a prospective randomised controlled study, including patients with a normorresponding profile who were going to start IVF-ICSI treatment in an antagonist protocol and met all the inclusion criteria and none of the exclusion criteria. Randomisation was performed using a randomisation table developed by the statistical

service of our hospital. Although the initial aim was to have a larger sample size, this was not possible due to several limitations in our working environment, which will be discussed later. 106 patients were randomised between the 3 study arms: treatment with oral contraceptives prior to the start of ovarian stimulation, treatment with oestradiol valerate prior to the start of ovarian stimulation or no pre-treatment. Finally, 86 patients successfully completed treatment and subsequent follow-up, and data from these patients were included in the analysis of the outcomes of the study. The statistical analysis was carried out by the Biostatistics Service of the Hospital La Paz. A comparative analysis between groups was performed, based on the null hypothesis of the absence of statistically significant differences in gestational outcomes between groups.

No statistically significant differences were detected between groups in relation to the baseline characteristics of the patients included, such as age, BMI, smoking habit or cause of infertility.

No statistically significant differences were found in ovarian reserve parameters (AMH, baseline FSH, baseline E2) when comparing the different study groups.

No statistically significant differences were found in the cycle response parameters between the different study groups (days of stimulation, gonadotropin dose, E2 or progesterone levels on the day of the ovulatory trigger, total number of oocytes obtained, number of mature oocytes, number of embryos obtained or cancellation rate).

Finally, after analysing the clinical gestation rate, miscarriage rate and live birth rate, after fresh transfer, delayed transfer and per cycle in the different study groups, no statistically significant differences were detected, although there was a tendency towards worse outcomes in the group pretreated with oestrogens.
These outcomes could be related to the treatment regimen selected in our study and the time of exposure to oestradiol valerate in this study group.
After analysing this last aspect, we observed that higher gestation rates were obtained in patients with more days of oestrogen exposure.

CONCLUSIONS

In our study we have not detected statistically significant differences in gestational rates between the different study groups. We have analysed these rates both after transfer in the same stimulation cycle and with delayed embryo transfer to assess the possible effect that these medications could have on implantation. However, the outcomes observed are comparable in both scenarios. Finally, we also observed no statistically significant differences in cumulative gestational rates, although there is a tendency for worse outcomes in the group pretreated with oestrogens in all cases.

In this subgroup, it appears that longer exposure to estrogen pre-treatment is associated with better gestational outcomes.

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