STATISTICAL ANALYSIS PLAN ENDOSCRATCH TRIAL NCT03108157

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Endometrial Scratch Effect on Pregnancy Rates in Patients Undergoing Egg-donation IVF

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Signatures

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Note: Add name, surname and date next to the signature.

SAP reviews

Protocol Version	SAP review	Section modified	Description and reason for modification	Date of modification

1 Introduction

1.1 Background

Embryo implantation remains one of the main challenges in assisted reproduction. Relevant improvements have been accomplished in reproductive medicine: different protocols for controlled ovarian stimulation and endometrial preparation, embryo culture with time-lapse technologies, embryo pre-implantational genetic testing and endometrial genetic assessment for implantation potential. Despite the fact that these changes have led to increasing pregnancy rates in the last few years, the implantation process is still inefficient, as it remains around 30% of all embryos replaced (1), and it is not yet totally understood.

1.2 Study justification

Several studies have tried to determine whether an endometrial injury performed in the cycle preceding the embryo transfer in in vitro fertilization (IVF) cycles could enhance embryo implantation. Barash et al (2) reported for the first time a two-fold increase in pregnancy rate in patients that had undergone multiple ES before the IVF cycle, compared to those patients who had no ES performed.

Since then, many authors have tried to determine ES effects after controlled ovarian stimulation (COS), but while some of them have found an increase in pregnancy rates (2–5), many others have been unable to find such differences (6–11). The main limitation in reaching a conclusion is that most of these are underpowered observational studies, with a low number of patients included, with differences in timing (luteal or follicular phase from the preceding or same cycle), number of ES (one, two or more procedures), type of catheter and different stimulation protocols. It is important to note that those studies that have found some positive effects of ES have included patients with implantation failures (5,12) whereas those that included patients in their first or second IVF cycle were unable to find any differences (8,9). It is also relevant that some studies included as control patients, those who had undergone a hysteroscopy prior to the IVF cycle and, even if an ES was not performed in these patients, we may assume that the endometrium was exposed to some "damage" as well (13). Another study included a cervical biopsy for those patients included in the control group, what cannot be really considered as "placebo" (10).

A systematic review conducted by Potdar et al (14) including 7 studies with 2062 patients, found a three-fold increase in pregnancy rates in those patients that received ES. Similar results were also found some time later by a Cochrane Review by Nastri et al (15) with moderate-quality evidence, signaling the need for well-designed trials without uterine instrumentation in the control group, stratification for implantation failure and the necessity to report live birth rates. This review also showed that endometrial injury on the day of oocyte retrieval decreased live birth (RR 0.31, 95% CI 0.14 to 0.69) and clinical pregnancy (RR 0.36, 95% CI 0.18 to 0.71).

All these studies were conducted after COS, but there is only one retrospective study in patients receiving embryos from donor eggs, and who have not undergone ovarian stimulation (14). When comparing egg donation cycles to other IVF treatments, we find two main differences: the first one is that embryo quality is presumably optimal, since all embryos come from donor eggs, avoiding the confusion factor of embryo quality according to maternal issues (age, BMI, polycystic ovaries, low ovarian reserve…) and the second difference is that all patients receive hormone replacement therapy with a homogeneous preparation of the endometrium, avoiding different hormonal environments caused by diverse responses to controlled ovarian stimulation in IVF.

1.3 Implantation Failure

Embry implantation is a complex process that requires successive coordinated steps to allow the embryo attach and invade the inner layers of the endometrium.

Implantation failure definition is very heterogeneous. Coughlan et al (17) considered implantation failure if 4 or more good quality embryos had been replaced in 2 or more embryo transfers.

When there is a suspicion of implantation failure, it is necessary to perform certain tests in order to exclude possible causes: embryo genetic testing, maternal coagulation and immune problems and window of implantation determination and thus endometrial receptivity to the embryo.

The endometrium is a dynamic tissue with a complex architecture that undergoes several changes during the menstrual cycle which have an important impact on embryo implantation. Endometrial scratching (ES) is a simple procedure aiming to create a mild

endometrial injury that has been proposed to improve the embryo-endometrium dialogue. Different authors have attributed this improvement to the effects of different cytokines and growth factors involved in an acute endometrial inflammatory process (18), the enhancement of new vascularization and decidualization (19), the improvement of endometrial maturation (13), and the promotion of endometrial gene expression that may lead to a better synchrony between the embryo and the endometrium (20).

2 Hypothesis, objective and aim of the clinical trial

2.1 Study hypothesis [level 2]

Patients that receive ES during the cycle preceding the embryo transfer, have increased endometrial receptivity and thus higher clinical pregnancy rates (CPR).

2.1.1 Operative hypothesis [level 3]

Null hypothesis: Clinical pregnancy rate in patients who have undergone an ES in the previous cycle is not different from those who have not received it.

Alternative hypothesis: Patients who receive an ES in the previous cycle have a 15% higher CPR than those who have not received it.

2.2 Main outcome [level 2]

The main objective of the ENDOSCRATCH trial is to determine if there are differences in pregnancy rates in egg donor IVF treatments when comparing patients receiving an ES before endometrial preparation for embryo transfer and those who will not receive any intervention. CPR will be determined by the visualization of a intrauterine gestational sac via vaginal ultrasound at approximately 6 weeks pregnancy.

2.2.1 Secondary outcomes [level 3]

2.2.1.1 Efficacy secondary outcomes [level 4]

1. Secondary endpoints are biochemical pregnancy rate (BPR), ongoing pregnancy rate (OPR), implantation rate (IR), miscarriage rate (MR), live birth rate (LBR) and cumulative pregnancy rate (CumPR).
    a. Biochemical pregnancy rate will be determined by the ratio between the number of patients with blood β-hCG levels over 10 mUI/ml 12 days after

the embryo transfer and the number of embryo transfers.

b. Ongoing pregnancy will be assessed via ultrasound beyond 12 weeks of pregnancy.

c. Implantation rate will be determined by the ratio between the number of gestational sacs and the number of replaced embryos.

d. Miscarriage rate will be determined by the ratio between the number of miscarriages and the total number of pregnancies.

Early miscarriage will be assessed if pregnancy stops before the 12^{th} week of pregnancy. Late miscarriage will be assessed if pregnancy stops between the 12^{th} and the 24^{th} week of pregnancy.

e. Live birth rate will be determined by the ratio between babies born and the number of embryo transfers.

f. Multiple pregnancy rate, determined by the ratio between the number of multiple pregnancies and the total number of pregnancies.

g. CumPR will be evaluated 12 months after randomization for all patients.

2. To determine the possible beneficial effect of ES in egg donor IVF recipients adjusted by: age, BMI, smoking habits.

3. To evaluate the CPR in both groups regarding the donor's age, male partner's age and sperm quality, number of eggs obtained and fertilized, total number of embryos obtained and quality and number of transferred embryos.

4. To determine the possible positive effect in specific treatment subgroups:

   a. Previous implantation failures from IVF with own eggs

   b. Previous implantation failures from IVF with donor eggs

   c. Previous miscarriages

   d. Previous live births

5. To evaluate the interference of ES with the endometrial preparation, in terms of:

   a. Endometrial thickness

   b. Stimulation duration

   c. Dose of medication needed

2.2.1.2 Safety secondary outcomes [level 4]

1. Obstetric outcome analysis to evaluate the possible association of this technique with:
    a. Early or late miscarriage
    b. Placentation anomalies
    c. Intrauterine growth restriction
    d. Preterm birth
    e. Premature membrane rupture
    f. Gestational diabetes
    g. Gestational hypertension
2. Secondary effects of ES:
    a. Pain
    b. Bleeding

3 Clinical trial design [level 1]

3.1 Clinical trial description [level 2]

This is a single-centre prospective RCT, fully conducted at ProcreaTec Fertility Clinic in Madrid, starting January 2017 to December 2019 to evaluate the effectiveness of an endometrial biopsy (scratching) before endometrial preparation, during the luteal phase of the previous cycle versus the conventional treatment protocol for egg donation IVF without endometrial biopsy.

3.1.1 Clinical trial summarized description [level 3]

1. Study: Interventional.
2. Assignment: Randomized.
3. Final classification: Efficacy study.
4. Intervention model: Parallel assignment.
5. Blinding: Not blinded.
6. Main outcome: The improvement in clinical pregnancy rate in egg donor IVF recipients.

3.2 Patient randomization [level 2]

Patients starting egg donor IVF cycles that fulfill inclusion criteria will be offered participation. If they agree they will be assigned to a treatment group according to the randomization chart, which will be obtained by a web-based randomization program using random blocks (randomization.com). Since patients in the study group will receive an intervention and those in the control group will not (no placebo intervention will be performed), blinding is not possible for patients nor for physicians. All patients will sign IC to be enrolled in the study.

1. Group A (176 patients): Intervention group. They will receive an ES during the luteal phase of the previous cycle to embryo transfer.
2. Group B (176 patients): Control group. They will undergo the conventional protocol for donor IVF treatment.

3.3 Sample size calculation [level 2]

The average CPR after embryo transfer in egg donor IVF cycles is 60% at our centre. Based on previous studies, where the difference in CPR for IVF cycles varied between 10 to 30%(2,4,5,12,14,21–23), we estimated that a 15% difference in CPR would be clinically relevant. According to that percentage, a total of 332 patients will be needed to detect a 15% difference between the two groups, with 80% statistical power and two-sided alpha of 0,05. Considering a 5% dropout rate, we will include 176 patients per study arm, 352 patients in total.

3.4 Interim analysis [level 2]

Non applicable.

3.5 Study setting [level 2]

We will collect data from patients undergoing an egg donor IVF cycle at ProcreaTec Fertility Centre in Madrid from the 13th of January 2017.

3.5.1 Eligible population [level 2]

Those patients undergoing an egg donor IVF treatment protocol will be eligible for this study.

3.5.2 Study population [level 2]

Study population will include those patients fulfilling the inclusion criteria who have accepted the study and signed the IC.

3.5.3 Inclusion and exclusion criteria

3.5.3.1 Inclusion criteria:

Patients will be included if they meet the following inclusion criteria:

- Age between 18 and 50 years
- Primary or secondary infertility
- Patients undergoing an IVF protocol with donor eggs
- Normal uterine cavity (transvaginal ultrasound scan)
- Patients with endometrial polyps can be included as long as polypectomy is performed at least two months before the treatment cycle

3.5.3.2 Exclusion criteria:

Patients will be excluded if:

- There is a severe male factor (less than 2 million sperms per ml)
- They have uterine anomalies such as uterine fibroids that impact the cavity, Mullerian malformations or severe adenomyosis
- They have unilateral or bilateral hydrosalpinx
- They have undergone a previous ES or hysteroscopy (at least one month before the randomization)
- Pre-implantation genetic testing cycles

3.6 Data collection

All study variables will be collected from patients included in the trial, from ProcreaTec clinical records, according to the information required in the data collection form (Annex I). Each doctor will include relevant information in the patients' clinical record and the principal investigator will be responsible for collecting and managing the information. Any adverse events will be reported by responsible doctors and managed by the principal investigator.

3.7 Ethical approval [level 2]

This study will be conducted after the authorization of the Ethical Committee of Princesa Hospital in Madrid (Registry nº 2934/12-01-2017). Clinical data will be treated confidentially following the Spanish data protection law (Spanish Organic Law 15/1999, 13thDec).

4 Statistical principles [level 1]

4.1 General considerations

Baseline characteristics of patients included will be analyzed as follows. Qualitative variables will be described using mean and standard deviation, non-normal variables will be summarized using median and 25% and 75% centiles. Qualitative variables will be described using frequency distribution.

The main outcome, CPR, and secondary outcomes, BPR, OPR, MR, IR, LBR and CumPR for each group will be analyzed with Chi-Squared test or Fisher's exact test. Efficacy of the treatment will be described as absolute and relative frequencies, together with the association strength by raw risk ratio (RR) with 95% confidence intervals. In addition, a general linear model, with a log link and binomial distribution, will be used to estimate the strength of association between primary and secondary outcomes adjusted by independent variables.

Results will be presented as RR and 95% confidence intervals. Statistical significance will be 0,05 (5% both sides α error) for all comparisons. Statistical analysis will be done using Stata 13 for Windows (StataCorp LP, Texas).

All analyses will be performed by the 9th version of SAS system.

4.2 Study population [level 2]

The main statistical analysis will be by intention to treat (ITT). Patients allocated to a determined treatment group will be followed and evaluated as members of that group, without regards to the fulfillment of the planned treatment.

4.3 Patient flow diagram [level 2]

Patient diagram flow will be detailed with the CONSORT standards (fig 1).

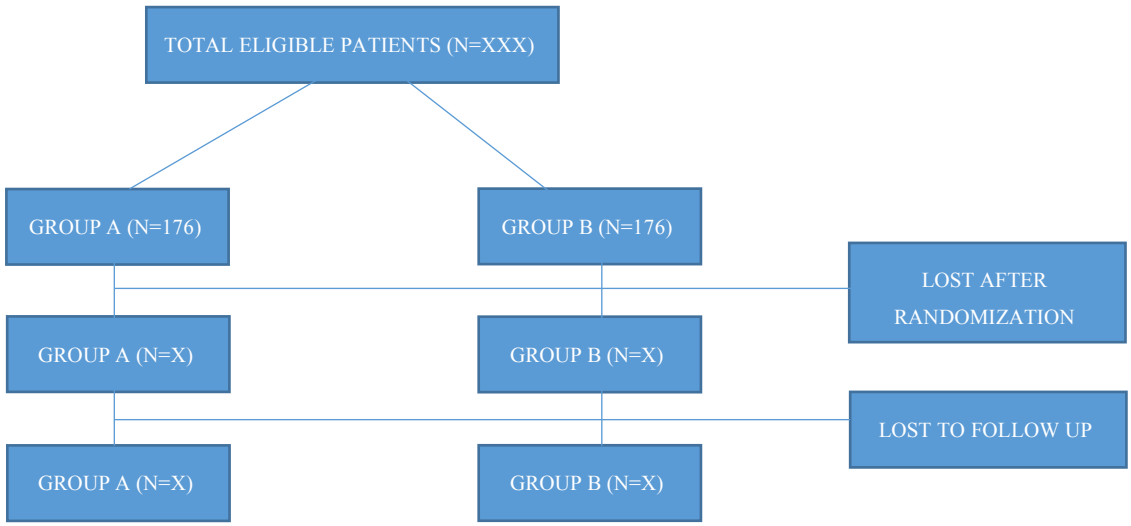

Figure 1. CONSORT patient flow diagram.

4.4 Outcome evaluation

4.4.1 Efficacy outcome

The primary study objective will be to evaluate if endometrial scratching improves CPR in patients undergoing egg-donation IVF versus patients without endometrial scratching. The primary study objective will be performed using the Chi-square statistic test at a 2-sided significance level of alpha = 0.05. The intervention effect will be quantified using the risk ratio (RR) with the 95% confidence interval and p-value (**¡Error! No se encuentra el origen de la referencia.**). The RR estimation will be realized using a generalized regression model with log link and binary outcome (clinical pregnancy equal to yes or no). The intervention RR will be adjusted by the following pre-treatment variables (Tabl): age, BMI, smoker, previous live birth, previous biochemical miscarriage, previous miscarriage, number of previous failed cycles with own eggs. Also, a multivariate model with the treatment will be performed to predict the CPR (Table 4). Finally, a predictive model will be generated with the treatment and the previous covariables.

	Group A (n=XXX)	Group B (n=XXX)	RR (IC 95%)	p-value
Biochemical pregnancy	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Clinical pregnancy	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Ongoing pregnancy	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Early miscarriage	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Late miscarriage	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Abortion	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Live birth	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Multiple pregnancy	NN (X%)	NN (X%)	XX (XX;XX)	P=XX
Cumulative clinical pregnancy rate (12 months)	NN (X%)	NN (X%)	XX (XX;XX)	P=XX

Table 1. Clinical outcome

	GROUP A (n=XXX)	GROUP B (n=XXX)
Race		
Arabian	XX (X%)	XX (X%)
Asian	XX (X%)	XX (X%)
Caucasian	XX (X%)	XX (X%)
Mixed	XX (X%)	XX (X%)
Hispanic	XX (X%)	XX (X%)
Mulatto	XX (X%)	XX (X%)
Black	XX (X%)	XX (X%)
Age	XX (XX)	XX (XX)
BMI	XX (XX)	XX (XX)
Smoking habit	XX (X%)	XX (X%)
Previous live birth	XX (X%)	XX (X%)
Previous miscarriage	XX (X%)	XX (X%)
Previous biochemical miscarriage	XX (X%)	XX (X%)
Previous ectopic pregnancy	XX (X%)	XX (X%)
Previous abortion	XX (X%)	XX (X%)
Number of previous failed cycles with own eggs		
0	XX (X%)	XX (X%)
1	XX (X%)	XX (X%)
2	XX (X%)	XX (X%)
+2	XX (X%)	XX (X%)
Number of previous failed cycles with donor eggs		
0	XX (X%)	XX (X%)
1	XX (X%)	XX (X%)
2	XX (X%)	XX (X%)
+2	XX (X%)	XX (X%)

279 *Table 2. Baseline characteristics*

	Group A (n=XXX)	Group B (n=XXX)	p-value
Donor's age	NN (X%)	NN (X%)	P=XX
Male partner's age	NN (X%)	NN (X%)	P=XX
Fresh sperm	NN (X%)	NN (X%)	P=XX
Frozen sperm	NN (X%)	NN (X%)	P=XX
Donor sperm	NN (X%)	NN (X%)	P=XX
Total sperm count	NN (X%)	NN (X%)	P=XX
Number of MII obtained	NN (X%)	NN (X%)	P=XX
Number of fertilized eggs	NN (X%)	NN (X%)	P=XX
Total number of embryos obtained	NN (X%)	NN (X%)	P=XX
Number of replaced embryos	NN (X%)	NN (X%)	P=XX
Quality of replaced embryos			
HATCHED	NN (X%)	NN (X%)	P=XX
HATCHING	NN (X%)	NN (X%)	P=XX
EXPANDED	NN (X%)	NN (X%)	P=XX
EARLY BLASTOCYST	NN (X%)	NN (X%)	P=XX
MORULA	NN (X%)	NN (X%)	P=XX
CLEAVAGE STAGE	NN (X%)	NN (X%)	P=XX

Table 3. Cycle characteristics

Adjusted model		
	RR (CI 95%)	**p-value**
Group A vs Group B	XX (XX;XX)	P=XX
Age	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
BMI	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
Smoking habit	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
Previous live births	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
Previous biochemical miscarriage	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
Previous miscarriage	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
Previous IVF own-eggs	XX (XX;XX)	P=XX
Group A vs Group B	XX (XX;XX)	P=XX
Previous IVF donor eggs	XX (XX;XX)	P=XX

Table 4. Model to evaluate the CPR and LBR according with the treatment and adjusted by age, BMI, smoking habit, previous live birth, previous biochemical miscarriage, previous miscarriage, previous IVF own eggs or previous IVF donor eggs failures.

Multivariate model		
	RR (CI 95%)	**p-value**
Endometrial scratching vs no intervention	XX (XX;XX)	P=XX
Age	XX (XX;XX)	P=XX
BMI	XX (XX;XX)	P=XX
Smoking habit	XX (XX;XX)	P=XX
Previous live birth	XX (XX;XX)	P=XX
Previous biochemical pregnancies	XX (XX;XX)	P=XX
Previous miscarriages	XX (XX;XX)	P=XX
Previous failed own eggs IVF embryo transfers	XX (XX;XX)	P=XX
Previous failed egg-donor IVF embryo transfers	XX (XX;XX)	P=XX

Table 5. Multivariate model to evaluate the CPR with the treatment adjusted by covariables

The following subgroups for the CPR will be defined (Table 6): previous implantation failures with own eggs, previous implantation failures with donor eggs, previous

miscarriage, previous live births. In each group, the effect of endometrial scratching (RR) for the CPR will be estimated using a generalized lineal model with log link and binary outcome (CPR equal yes or no). The comparison between groups will be realized using interaction between treatment and subgroup in the generalized linear model.

	GROUP A		GROUP B		
	CPR	**Total**	**CPR**	**Total**	**RR (IC 95%)**
Previous implantation failures with own eggs (p=XX)					
Yes	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
No	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
Previous implantation failures with donor eggs (p=XX)					
Yes	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
No	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
Previous miscarriages (p=XX)					
Yes	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
No	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
Previous live births (p=XX)					
Yes	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)
No	XX (XX%)	XX	XX (XX%)	XX	XX (XX;XX)

Table 6. Effect of endometrial scratching on CPR by subgroups

The effect of endometrial scratching on secondary study objectives, LBR and CumPR, will be evaluate using the Chi-square statistic test at a 2-sided significance level of alpha = 0.05. The intervention effect will be quantified using the risk ratio (RR) with the 95% confidence interval and p-value (**¡Error! No se encuentra el origen de la referencia.**). The RR estimation will be realized using a generalized regression model with log link and binary outcome (clinical pregnancy equal to yes or no). The intervention RR will be adjusted by the following pre-treatment variables (Tabl): age, BMI, smoker, previous live

birth, previous biochemical miscarriage, previous miscarriage, number of previous failed cycles with own eggs and donor eggs.

The effect of endometrial scratching on stimulation duration, endometrial thickness and total dose required will be evaluate using the Student´s t statistic test at a 2-sided significance level of alpha = 0.05. If the normality hypothesis is not rejected with the Shapiro-Wilk test, the objective variables will be presented using the mean and the standard deviation. If the normality hypothesis is rejected, the variables will be analyzed with no-parametric test of Mann-Whitney and summarized with the median and range interquartile, 25 and 75 percentile. The data will be described according to the Table 7.

	GROUP A (n=XXX)	**GROUP B (n=XXX)**	**p-value**
Stimulation duration	XX (XX)	XX (XX)	P=XX
Endometrial thickness	XX (XX)	XX (XX)	P=XX
Total dose required	XX (XX)	XX (XX)	P=XX

Table 7. Results of endometrial preparation

4.4.2 Safety outcomes

The effect of endometrial scratching on pregnancy complications: miscarriage, placentation anomalies, intrauterine growth restriction, preterm birth, premature membrane rupture, gestational diabetes and gestational hypertension, will be evaluated using the Chi-square statistic test at a 2-sided significance level of alpha = 0.05. The intervention effect will be quantified for each safety outcome using the risk ratio (RR) with the 95% confidence interval and p-value (**¡Error! No se encuentra el origen de la referencia.**). The RR estimation will be realized using a generalized regression model with log link and binary outcome (yes or no). The intervention RR will be summarized according to Table 8.

	GROUP A (n=XXX)	GROUP B (n=XXX)	RR (IC 95%)	p-value
Miscarriage	XX (X%)	XX (X%)	XX (XX;XX)	P=XX
Placentation abnomalies	XX (X%)	XX (X%)	XX (XX;XX)	P=XX
Intrauterine growth restriction	XX (X%)	XX (X%)	XX (XX;XX)	P=XX
Preterm birth	XX (X%)	XX (X%)	XX (XX;XX)	P=XX
Premature membrane rupture	XX (X%)	XX (X%)	XX (XX;XX)	P=XX
Gestational diabetes	XX (X%)	XX (X%)	XX (XX;XX)	P=XX
Gestational hypertension				

Table 8. Obstetrical outcome.

The absolute and relative frequencies of the pain and bleeding will be summarized by the patient with endometrial scratching. The endometrial preparation time will be described with the median, range interquartile, 25 and 75 percentile.

4.5 Baseline characteristics

The baseline characteristics: demographics, clinical, laboratory and relationship with the treatments will be presented with the mean and standard deviation values or absolute and relative frequency according to the treatment. If the distribution of continuous variable is not normal (Kolmogorov-Smirnov test), the information will be summarized with median and range interquartile, 25 and 75 percentile.

4.6 Evaluation of lost or unknown data.

The outlier data will be described with the maximum and minimum values. The outlier values will be revised with the CRD and corrected it. As loss to follow-up is expected to be minimal (i.e. less than one percent missing data on primary and secondary outcomes), missing values will not be imputed.

4.7 Additional considerations

When the fifty percent of the cells have expected counts less than five, the chi-square tests will be replaced by Fisher's exact test.

The protocol or statistical analysis plan deviation will be described and justified in the deviation documents.

5 GLOSSARY

BMI: body mass index

BPR: Biochemical pregnancy rate

CI: confidence interval

COS: Controlled ovarian stimulation

CPR: Clinical pregnancy rate

CumPR: Cumulative pregnancy rate

ES: Endometrial scratching

IC: Informed consent

CI: Confidence Interval

IR: implantation rate

IUI: Intrauterine insemination

IVF: In vitro Fertilization

LBR: Live birth rate

MR: miscarriage rate

OPR: ongoing pregnancy rate

OR: Odds ratio

RR: Risk ratio

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