Universitair Ziekenhuis Brussel

Clinical Trial Center

Study Protocol

Study Title: Natural cycle versus hormone replacement therapy cycle for a frozen-thawed embryo transfer in PGT patients. A randomized trial.

Protocol Number: HRT_NC-FET

Protocol Version and Date: Version 4; Date, 26/03/2019

EudraCT Registry Number: 2018-003156-20

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Investigational product:

Sponsor:

Coordinating/Principal Investigator: Dr. Caroline Roelens, Prof. Dr. Christophe Blockeel

PROTOCOL SIGNATURE PAGE

Protocol Version and date: Version 1, 29/10/2018

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Sponsor:

Coordinating/Principal Investigator: Dr. Caroline Roelens, Prof. Dr. Christophe Blockeel

I agree:

- to assume responsibility for the proper conduct of this study
- to conduct the study in compliance with this protocol and any future amendments
- not to implement any deviations from or changes to the protocol without prior review and written approval from the Ethics Committee, except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements)
- that I am thoroughly familiar with the appropriate use of the investigational drug, as described in this protocol
- to ensure that all persons assisting me with the study are adequately informed about the investigational drug and their study-related duties and functions as described in the protocol
- that I am aware of and will comply with the current good clinical practice (GCP) guidelines and ethical principles outlined in the Declaration of Helsinki
- to conduct the study in accordance with all applicable laws and regulations

Printed name Caroline Roelens

Christophe Blockeel

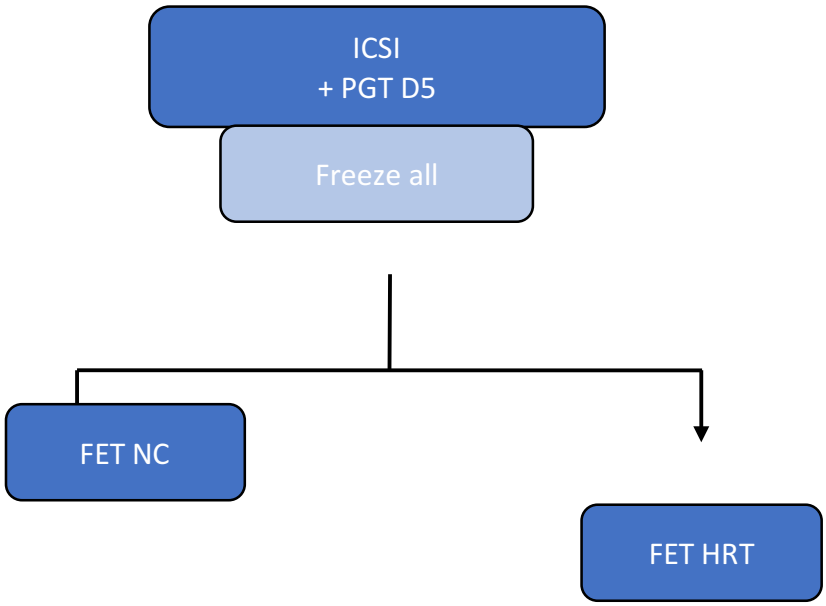
Signature Date

1. Introduction:

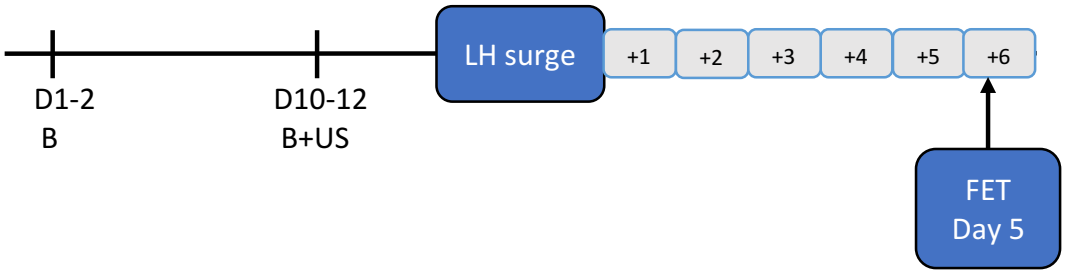
Cryopreservation of frozen-thawed embryos has become an indispensable procedure in reproductive medicine. Mainly, thanks to the development of the vitrification procedure and the improvement of embryo survival rates after thawing *(Rienzi et al., 2017; Loutradi et al. 2008),* pregnancy rates have substantially increased. Likewise, the implementation of a single embryo transfer policy to reduce multiple pregnancies without lowering cumulative delivery rates (*Peeraer et al., 2014)* has led to an increase in surplus embryos deriving from an ovarian stimulation cycle. Besides, freeze-all strategies have been adopted for an increasing number of indications, namely to avoid ovarian hyperstimulation syndrome (=OHSS), or for preimplantation genetic testing (=PGT) patients, or in case of late follicular progesterone (P) rise or in case of endometrium- embryo asynchrony *(Bosch et al., 2016; Roque et al., 2015; Healy et al., 2016; Devroey et al., 2011; Shapiro et al., 2008; Racca et al, 2018; Groenewoud et al., 2017).* These developments have led to an increase in the number of frozen-warmed embryo transfer (FET) cycles performed in fertility centres over the last decade.

Despite this growing importance, little is known about the optimal endometrium preparation protocol prior to embryo transfer *(Ghobara et al, 2017; Groenewoud et al., 2017).* The two most common used treatment regimens to prepare the endometrium for embryo transfer are the so called natural cycle (NC) and the hormone replacement therapy (HRT) cycle. In a natural cycle the endometrium undergoes structural changes caused by consecutive exposure to estrogens and progesterone derived from the growing follicle. The moment of embryo transfer is determined by the occurrence of the natural ovulation, meaning on the fourth day after luteinizing hormone (LH) surge for cleavage stage embryos and for blastocysts on the sixth day (*Mackens et al., 2017*). HRT protocols are using exogenous administration of estrogens and progesterone in order to mimic a natural cycle and therefore endometrial development. The advantages of this latter protocol are the easy scheduling and minimal cycle monitoring. So far, no RCTs have been performed comparing a true NC (without hCG administration) and an HRT cycle for FET. However, concerns are raising about the high miscarriage rates reported in HRT cycles (Tomás et al., 2012; Hreinsson et al., 2016; van de Vijver et al., 2016; van de Vijver et al., 2017).

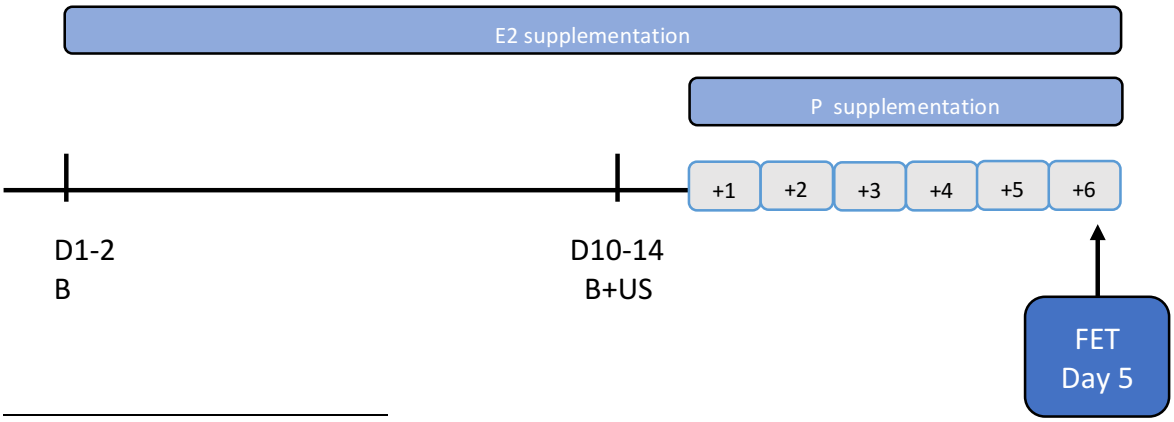
2. Study Flowchart and Design



Group A: NC (awaiting spontaneous LH surge)



Group B: HRT[1]



[1] Progesterone supplementation is started when the endometrium is considered adequate after E2 administration. E2 supplementation vary between 1 to 3 weeks depending on the adequate proliferation of the endometrium.

Legend: B: serum hormone analysis (estradiol (E2), P, LH, follicle stimulation hormone (FSH)); US: ultrasound scan; P: progesterone

3. Study objectives and endpoints

The aim of the current RCT is to compare miscarriage rates (before 8 weeks) between true NC (awaiting spontaneous LH surge) and HRT FET cycles in PGT patients, with biopsy on day 5 of embryonic development. The advantage of performing the study in PGT patients is the exclusion of aneuploidy as a cause of miscarriage.

3.1 Primary outcome:

Miscarriage rate before 8 weeks of gestation, defined as a spontaneous loss of a clinical pregnancy before 8 weeks of gestational age, in which the embryo(s) is/are nonviable and is/are (not) spontaneously absorbed or expelled from the uterus (Zegers-Hochschild et al 2017).

3.2 Secondary outcomes:

Miscarriage rate after 8 weeks of gestation, defined as a spontaneous loss of a clinical pregnancy after 8 weeks but before 22 completed weeks of gestational age, in which the embryo(s) or fetus(es) is/are nonviable and is/are not spontaneously absorbed or expelled from the uterus (Zegers-Hochschild et al 2017).

Clinical pregnancy defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. In addition to intra-uterine pregnancy, it includes a clinically documented ectopic pregnancy (Zegers-Hochschild et al 2017).

Ongoing pregnancy rate defined as the number of pregnancies after 20 weeks of gestation.

4. Methodology

4.1 Study design

The current study will be an open label randomized trial using a two–arm design with 1:1 allocation ratio. The participating centre is the Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Belgium. PGT patients who fulfil the inclusion criteria of the study will be recruited from this centre. The aim of the current study is to investigate the effect of the above-mentioned treatment regimen (NC and HRT) in FET cycles on miscarriage rate.

4.2 Selection of study population

The study population consists of all PGT patients who fulfil the following inclusion criteria:

- Age between 18 and 42 years
- BMI under 35 kg/m^2
- Regular menstrual cycle pattern (i.e. 24-35 days cycle)
- First, second and third ICSI-PGT cycle
- First frozen embryo transfer cycle following a fresh ICSI-PGT attempt
- PGT with trophectoderm biopsy on day 5 of embryonic development
- Signed informed consent

The exclusion criteria involve:

- Oligo-amenorrhea
- Age above 43 years
- BMI above 35
- Contraindications for the use of hormonal replacement therapy

4.3 Screening

On the first consultation, the treating physician will inform the eligible PGT patients about the study. Patients willing to participate and fulfilling the inclusion criteria will be randomized to one of the two treatment arms following the fresh ICSI cycle if non-affected embryos are available. Informed consent forms will be signed either on the first day of the following menstrual cycle or on the moment of frozen-warmed embryo transfer.

4.4 Randomization

Patients will be randomized to either natural cycle frozen-thawed embryo transfer (group A), or hormonal replacement therapy cycle frozen-thawed embryo transfer (group B). As mentioned above, patients will only be randomized if they meet the complete eligibility criteria. Randomization will be ensured using a computer-generated randomization list and allocation concealment by the use of sealed opaque envelopes in the centre (physician will neither have access to the randomization list nor to the envelopes, which will be opened by study nurses involved in the study).

4.5 Interventions

- <u>Natural cycle FET (Group A)</u>

  Patients are asked to perform a blood sample, with evaluation of serum estradiol (E2), progesterone (P), luteinizing hormone (LH) and follicle stimulating hormone (FSH), on the first or second day of the menstrual cycle (day 1 is considered as the first day of fresh red blood loss before 12AM). If these serum hormonal values are considered basal for the beginning of the follicular phase, patients are asked to come back on day 10 to 12 of the cycle for blood sample and transvaginal ultrasound scan

in order to assess follicular growth. Depending on the values on day 1 or 2, the physician performing the daily monitoring can decide to perform an earlier examination if necessary.

The timing of ovulation is determined based on a combination of ultrasonography features (the presence of a dominant follicle and adequate endometrium) and endocrine hormonal values in serum blood samples. Ovulation is generally defined as an, at least, 180% increase of LH compared to the mean level in the previous 24h (Frydman et al., 1982).

Frozen-warmed embryo transfer will take place six days following the spontaneous LH surge. Another blood sample will be performed 12 days after embryo transfer measuring human chorionic gonadotrophin (hCG), progesterone and estradiol in order to assess the outcome of the FET cycle.

- Hormonal replacement therapy cycle FET (Group B)

Patients are asked to perform a blood sample, with evaluation of serum estradiol (E2), progesterone (P), luteinizing hormone (LH) and follicle stimulating hormone (FSH) on the first or second day of the menstrual cycle (day 1 is considered as the first day of fresh red blood loss before 12AM). If these values are considered basal for the beginning of the follicular phase, estrogen supplementation (Estradiol valerate, Progynova® 3x2mg/day) is started to induce proliferation of the endometrium. Blood sample and transvaginal ultrasound are thereafter performed ten to fourteen days later. If the endometrium is considered adequate (generally considered if triple line and above 6,5 mm thickness)(Liu et al., 2018), embryo transfer is scheduled on the sixth day of progesterone (vaginal micronized progesterone, Utrogestan® 2x200mg twice a day) supplementation.

If the endometrium remains inadequate on day 10-14, estrogen priming will be prolonged with a maximum of 14 days of additional estrogen supplementation. If hereafter the endometrium stays below 6,5mm the cycle will be cancelled.

Another blood sample will be performed 12 days after embryo transfer measuring hCG, P and E2 in order to assess the outcome of the FET cycle.

In case of a positive pregnancy test, estrogen and progesterone supplementation will be continued until 8 weeks of gestation (in line with the onset of the luteo-placental shift) (Scott et al., 1991).

In case of escape spontaneous ovulation embryo transfer will be performed considering the presumable time of ovulation.

5. Treatment

- Group A: NC FET

In this group, no medication will be administered to the patients. The aim of this protocol is to detect the occurrence of a spontaneous LH surge in order to calculate the day of embryo transfer.

- Group B: HRT FRET

Estradiol valerate (Progynova®, Bayer-Schering Pharma AG, Berlin, Germany) will be started on the first or second day of the menstrual cycle, in case of basal hormonal conditions, at a regimen of 2mg thrice a day. After ten to fourteen days of estrogen supplementation a check-up is planned with a blood sample and transvaginal ultrasound. If the endometrium is adequate (as above mentioned) transfer of the frozen-thawed embryo is planned on the sixth day of progesterone supplementation. We will use micronized progesterone vaginally (Utrogestan®, Besins International) two capsules of 200 mg twice a day.

HRT cycles, using a consecutive administration of estrogen and progesterone, are used worldwide especially due to the advantage of easy and flexible scheduling and minimal monitoring which reduces the visits to the hospital compared to NC FRET. Although the universally use of HRT protocols in fertility centres no consensus is yet established concerning the ideal duration, route of administration or type of drugs used in order to prepare the endometrium for embryo transfer (Glujovsky et al., 2010).

We will adopt the above-mentioned protocol which is in line with the local protocol applied in the Centre of Reproductive Medicine in Brussels.

6. Data collection and Management

5.1 Statistical analysis

Continuous variables will be analyzed using the independent *t*-test or Mann–Whitney *U*-test depending on the normality of the distribution. Normality will be examined by the use of the Shapiro–Wilk test. Categorical variables will be analyzed by Pearson's chi-squared test or Fisher's exact test, as appropriate. To identify characteristics that may be related with the miscarriage rate, multivariate logistic regression analysis will be performed with the miscarriage rate as the dependent variable and transfer policy (NC versus HRT) as the main independent variable. Candidate variables considered for the analysis are: age, BMI, indication of infertility, percentage of patients with at least one top quality embryo and outcome of the previous fresh attempt. All candidate variables will be simultaneously entered into the logistic regression model. All tests will be two-sided with a level of significance set at $p < 0.05$. Analyses will be performed using STATA 15.0.

5.2 Sample size calculation

Sample size calculation was performed using internal data of our centre considering early miscarriage rates (defined as miscarriage before 8 weeks of gestation). We observed in our PGT population in 2017 an early miscarriage rate per started FET cycle of 9,9% in the natural cycle group compared to 18,4% in the group using HRT.

Based on these results, 522 patients are required (261 in each treatment arm) to have an 80% chance of detecting, as significant at the 5% level, an increase in the primary outcome measure (early pregnancy loss) from 9,9% in the NC group to 18,4% in the HRT group.

7. Ethical consideration

The protocol will be submitted to the local Ethics Committee of UZ Brussel. Strict confidentiality of all personal and research data will be ensured. The recruiting centre will be required to sign a clinical trial agreement document detailing their commitment towards complying with the relevant laws, regulations, codes of practice and obligations to publication. There are no conflicts of interest.
No specific reimbursement will be given to patients participating in the study.

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