Universitair Ziekenhuis Brussel

Clinical Trial Center

Study Protocol

Study Title: **Double trigger and ovum retrieval vs. conventional antagonist ovarian stimulation protocol in poor prognosis women undergoing IVF/ICSI: a randomized pilot study**
Study Acronym: **DUOPICK**
Phase of Development**: IV**
Protocol Number: **58335**
Protocol Version and Date: **version 3, 03/07/2019**
EudraCT Registry Number: **2019-000971-17**
ClinicTrials.gov Registry Number: **NCT03846544**
Indication: **fertility disorders**
Investigational product: **Elonva, Puregon**
Sponsor: **UZ Brussel**
Coordinating/Principal Investigator: **Prof. Dr. Christophe Blockeel**

PROTOCOL SIGNATURE PAGE

Protocol Version and date: version 3.0, 03/07/2019

Protocol Title: **Double trigger and ovum retrieval vs. conventional antagonist ovarian stimulation protocol in poor prognosis women undergoing IVF/ICSI: a randomized pilot study**

Sponsor: **UZ Brussel**

Principal Investigator: **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 Signature Date

1. Table of Contents

2. Trial Registration/Protocol Summary

Information	
EudraCT number: Date of registration:	2019-000971-17 20/02/2019
Clinical Trial Authorization (CTA) from FAMHP (FAGG) Date of approval:	14/06/2019
ClinicalTrials.gov:	NCT03846544
Official Title:	Double trigger and ovum retrieval vs. conventional antagonist ovarian stimulation protocol in poor prognosis women undergoing IVF/ICSI: a randomized pilot study
Study Phase/Type:	IV
Condition:	Fertility disorders
Objectives:	To compare the total number of metaphase II (MII) oocytes between the two groups
Investigational Product:	Elonva , Puregon
Interventions:	A single injection of 150mcg of corifollitropin alfa will be administrated at day 2/3 of the cycle, followed by 225IU of recombinant FSH (rFSH) in a fixed antagonist protocol. Cycle monitoring will be performed through serum estradiol (E2), progesterone and luteinizing hormone (LH) assessments, and serial transvaginal ultrasound examinations.The study will include exclusively patients with ≤9 follicles ≥11 mm on the day of triggering. Therefore, patients will be randomly allocated in two groups: Group A (control group) will undergo conventional triggering with hCG 5000 IU and a single oocyte retrieval (OR) will be performed 36 hours later; Group B (study group) will undergo triggering with GnRHa 0,2ml and a first OR will be performed 36 hours later. In both groups, triggering will be formed if two or more follicles ≥17 mm are observed and all follicles >10 mm will be aspirated, in line with current clinical practice (Nivet et al., 2016). In Group

	B (study group), 225 IU of rFSH will continue after the first trigger. Antagonist administration will be initiated when at least one follicle measuring ≥14 mm will be present in the ultrasound. If one or more follicles ≥17 mm are observed, patients will undergo a second triggering with hCG 5000 IU and a second OR will be performed. In case of patients with no follicular development following 13 additional days of rFSH, the cycle will be cancelled. Metaphase II (MII) oocytes will then be injected according to standard ICSI procedure. In the control group a fresh single/double embryo transfer will be performed according to clinical practice (day 3 or day 5). For the study group, a freeze-all strategy will be applied, followed by a transfer of a maximum two embryos in an artificially prepared frozen-thawed cycle.
Endpoints:	• Number of MII oocytes • Number of COCs • Number of preovulatory follicles • Number of oocytes fertilized • Number of available embryos and their quality • Duration of stimulation • Total dose of gonadotropins administered • Clinical pregnancy and live birth rates of the first frozen cycle • Cumulative live birth rates
Study population:	Infertile patients
Number of patients:	46
Overview of study design:	Single center randomized superiority pilot study
Statistical Considerations:	Our sample size of 46 patients (23 per group) has a power of at least 90% to detect superiority of the double trigger group (mean 5.4 MII) versus control group (mean 3.4 MII), standard deviation (SD=2), using a two-sided, t test, at significance level alpha of 0.05
Sponsor:	UZ Brussel
Country(ies) of Recruitment:	Belgium
Inclusion Criteria:	• Age ≥ 25 - ≤ 40 • BMI ≤ 35 and ≥ 19

	• Antimüllerian hormone (AMH) level of ≤1.5 ng/mL or antral follicular count of ≤6 follicles or ≤5 oocytes retrieved in a previous cycle following standard conventional ovarian stimulation. • Normal basal ultrasound < 6 months before the start of the treatment (presence of uterus and two ovaries without proof of abnormalities) • Signed informed consent
Exclusion Criteria:	• Testicular sperm extraction • History of > 3 three consecutive previous unsuccessful IVF cycles • Use of oral contraceptives <3 months before start of the treatment • PCOS according to the Rotterdam criteria • Ovarian stimulation for pre-implantation genetic testing (PGT-A/M) • Medical/social freezing • History of untreated autoimmune, endocrine or metabolic disorders • Ovarian cystectomy or oophorectomy • Patients will be allowed to participate in the study only once.
Date of first enrolment:	September 2019
Target sample size:	46

3. Protocol Version History

Version No.	Approval Date Lead EC	Release Date
1.0		

4. Sponsor/Coordinating Investigator Information

Coordinating Investigator: Prof. Dr. Christophe Blockeel

Sponsor: UZ Brussel

Principal Investigator: Prof. Dr. Christophe Blockeel

Co-investigators: Prof. Dr. Panagiotis Drakopoulos, Dr. Liese Boudry

Statistician: Prof. Dr. Panagiotis Drakopoulos

Laboratory: UZ Brussel

Pharmacy: UZ Brussel

Study Coordinator: Elsie Nulens, CRG UZ Brussel

Study sites and co-investigators: CRG-UZ Brussel

5. List of Abbreviations

ADR	Adverse drug reaction
AMH	Anti-müllerian hormone
ART	Assisted reproductive technologies
BMI	Body mass index
CI 95%	Confidence Interval 95%
CC	Cumulus cells

COC	Cumulus oocyte complexes
COH	Controlled ovarian hyperstimulation
E2	Oestradiol
hCG	Human chorionic gonadotropin
FF	Follicular fluid
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
IU	International units
Infertility	The incapacity to conceive after 12 months of unprotected intercourse
IVF/ICSI	In vitro fertilization/Intra-cytoplasmic sperm injection
IVM	In vitro maturation
LH	Luteinizing hormone
MII	Metaphase II oocytes
OHSS	Ovarian hyperstimulation syndrome
OR/OPU	Oocyte retrieval/ovum pick up
PGT-A/M	Pre-implantation genetic testing for aneuploidy/monogenic disorders
RCT	Randomised Controlled Trial
rFSH	Recombinant follicle stimulating hormone

6. Introduction

Controlled ovarian hyperstimulation (COH) has been established as a prerequisite in assisted reproduction technologies (ART), as it induces multifollicular development, resulting in a higher number of oocytes retrieved. The rationale is that an increase in the number of oocytes harvested could yield more available embryos, providing higher cumulative live birth rates in infertile patients (Drakopoulos et al., 2016).

On the other hand, multiple major and minor waves in follicular development have been described during the intraovulatory period of healthy women (Baerwald et al., 2003). It seems plausible that ovarian stimulation does not take full advantage of the whole follicular cohort as even small folli

cles (10-14 mm in the study by Nivet et al (Nivet et al., 2016); 8-12 mm in the study by Wirleitner et al (Wirleitner et al., 2018) have been shown to produce transferable embryos and viable pregnancies (Nivet et al., 2016; Wirleitner et al., 2018).
However, aspiration of small follicles may not be the optimal strategy to take full advantage of the whole follicular cohort: in fact, aspiration of small follicles yields significantly lower oocyte recovery rates and proportion of transferable embryos compared with medium and large follicles (Nivet et al., 2016).
Thus, prolonging COH after the first ovarian aspiration and performing a second egg retrieval when small follicles have reached a medium/large size might increase the number of oocytes harvested and provide more available embryos.

7. Study Schematic and Schedule of Activities

7.1 Study Schematic

This is a prospective superiority randomized pilot study which will be performed at UZ Brussel

7.2 Study Activities

The total duration of the study will be approximately 1.5 years
The duration of treatments is per investigators' judgment.
Number of patients included: A total number of 46 women will be analyzed

8. Study Objectives and endpoints

8.1 Primary Objective

The primary objective measure will be to compare the total number of metaphase II (MII) oocytes between the two groups.

8.2 Secondary Objectives

Secondary objectives will be to compare the total number of available embryos, number of cumulus oocyte complexes (COCs) retrieved, number of oocytes fertilized, duration of stimulation, total dose of gonadotropins administered, clinical pregnancy and live birth rates of the first frozen cycle and cumulative live birth rates (defined as the first live birth following the transfer of fresh and subsequent frozen-thawed embryos) between the two groups. A cost-effectiveness analysis will be performed in case of superiority of the double trigger group.

8.3 Endpoints

- Number of MIIs
- Number of COCs
- Number of available embryos
- Number of oocytes fertilized
- Duration of stimulation
- Total dose of gonadotropins administered
- Clinical pregnancy and live birth rates of the first frozen cycle
- Cumulative live birth rates

9. Investigational Plan

9.1 Overall Study Design

Single center randomized pilot study

9.2 Study Duration for Subjects

9.2.1 Screening

Following first infertility consultation

9.2.2 Treatment Period

Approximately 2-3 weeks

9.2.3 Unscheduled Visit(s)

N/A

9.2.4 Early Study Termination

N/A

9.2.5 End of Study

July 2020 (anticipated)

10. Selection of Subjects

10.1 Selection of Study Population

The study will include all consecutive infertile women who attend our center, fulfill the inclusion criteria and consent to participate.

10.2 Inclusion Criteria

The study will include normo-ovulatory patients with the following criteria:

- Age ≥ 25 and ≤ 40 age
- BMI ≤ 35 and ≥ 19
- Antimüllerian hormone (AMH) level of ≤1.5 ng/mL or antral follicular count of ≤6 follicles or ≤5 oocytes retrieved in a previous cycle following standard conventional ovarian stimulation.
- Normal basal ultrasound < 6 months before the start of the treatment (presence of uterus and two ovaries without proof of abnormalities)
- Signed informed consent

10.3 Exclusion Criteria

The exclusion criteria will be:

- Testicular sperm extraction
- History of more than three consecutive previous unsuccessful IVF/ICSI cycles
- Use of oral contraceptives <3 months before start of the treatment
- PCOS according to the Rotterdam criteria
- Ovarian stimulation for pre-implantation genetic testing (PGT-A/M)
- Medical/social freezing
- History of untreated autoimmune, endocrine or metabolic disorders,
- Ovarian cystectomy or oophorectomy
- Patients will be allowed to participate in the study only once.

10.4 Contraception/Pregnancy Avoidance

N/A

11. Screening and Randomization

11.1 Screening and Enrollment

A responsible investigator will identify potential eligible women for this study. They will be screened and documented as eligible submitted to inclusion criteria. The investigator will obtain informed consents for participation in the study using local IRB approved informed consent forms. Consent will be obtained at the follow up consultation

11.2 Randomization

This is a prospective superiority randomized pilot study. Patients will be randomized to either control group (Group A) or double trigger group (Group B), on the day of triggering, only after patient eligibility has been established and patient consent has been obtained. Randomization sequence and allocation will be created using a computer-generated randomization list, using 1:1 allocation.

11.3 Blinding Procedures

Clinicians who will perform the oocyte retrievals and embryologists will be blinded.

12. Interventions/Treatment

12.1 Treatments Administered

A single injection of 150mcg of corifollitropin alfa will be administrated at day 2/3 of the cycle, followed by 225IU of recombinant FSH (rFSH) in a fixed antagonist protocol. Cycle monitoring will be performed through serum estradiol (E2), progesterone and luteinizing hormone (LH) assessments, and serial transvaginal ultrasound examinations. The study will include exclusively patients with ≤9 follicles ≥11 mm on the day of triggering. Therefore, patients will be randomly allocated in two groups: Group A (control group) will undergo conventional triggering with hCG 5000 IU and a single oocyte retrieval (OR) will be performed 36 hours later; Group B (study group) will undergo triggering with GnRHa 0,2ml and a first OR will be performed 36 hours later. In both groups, triggering will be performed if two or more follicles ≥17 mm are observed and all follicles >10 mm will be aspirated, in line with current clinical practice (Nivet et al., 2016). In Group B (study group), 225 IU of rFSH will continue after the first trigger. Antagonist administration will be initiated when at least one follicle measuring ≥14 mm will be present in the ultrasound. If one or more follicles ≥17 mm are observed, patients will undergo a second triggering with hCG 5000 IU and a second OR will be performed. In case of patients with no follicular development following 13 additional days of rFSH, the cycle will be cancelled. Metaphase II (MII) oocytes will then be injected according to standard ICSI procedure. In the control group a fresh single/double embryo transfer will be performed

according to clinical practice (day 3 or day 5). For the study group, a freeze-all strategy will be applied, followed by a transfer of a maximum two embryos in an artificially prepared frozen-thawed cycle (overview of all procedures: tables and figures, fig.2).

12.2 Product characteristics

Elonva (corifollitropin alfa) 150 µg

Each pre-filled syringe contains 150 micrograms of corifollitropin alfa in 0.5 mL solution for injection. Elonva is indicated for Controlled Ovarian Stimulation (COS) in combination with a Gonadotropin Releasing Hormone (GnRH) antagonist for the development of multiple follicles in women participating in an Assisted Reproductive Technology (ART) program

Puregon (follitropine beta) 225 IU

One vial contains 225 IU of Puregon. The solution for injection contains the active substance follitropin beta. Puregon is indicated for the treatment of female infertility in the following clinical situations:

- Anovulation (including polycystic ovarian syndrome, PCOS) in women who have been unresponsive to treatment with clomifene citrate.
- Controlled ovarian hyperstimulation to induce the development of multiple follicles in medically assisted reproduction programs [e.g. in vitro fertilisation/embryo transfer (IVF/ET), gamete intra-fallopian transfer (GIFT) and intracytoplasmic sperm injection (ICSI)].

12.3 Randomization and Stratification

Patients will be randomized to either control group (Group A) or double trigger group (Group B), on the day of triggering, only after patient eligibility has been established and patient consent has been obtained. Only patients with ≤ 9 follicles of ≥ 11mm will be included. Randomization sequence and allocation will be created using a computer generated randomization list, using 1:1 allocation.

12.4 Direction of Administration

Elonva (corifollitropine alfa) and Puregon (follitropine beta)

To prevent painful injections and minimize leakage from the injection site the medication should be slowly administered subcutaneously (or intramuscularly). The subcutaneous injection site should be alternated to prevent lipoatrophy. Any unused solution should be discarded.

Subcutaneous injections may be carried out by patient or partner, provided that proper instructions are given by the physician. Self-administration should only be performed by patients who are well-motivated, adequately trained and with access to expert advice.

12.5 Dosing Regimen

Dosage of Elonva (corifollitropine alfa) is 150 µg and is fixed. Puregon (follitropine beta) 225 IU is started after the first week of stimulation.

12.6 Treatment Compliance

Self-administration will be performed after adequate training of the patient.

12.7 Known Undesirable Effects of Study Drug

Elonva® and Puregon® have the following side effects:
OHSS (ovarian hyperstimulation syndrome)
OHSS is a medical event distinct from uncomplicated ovarian enlargement. Clinical signs and symptoms of mild and moderate OHSS are abdominal pain, nausea, diarrhoea, mild to moderate enlargement of ovaries and ovarian cysts. Severe OHSS may be life-threatening. Clinical signs and symptoms of severe OHSS are large ovarian cysts, acute abdominal pain, ascites, pleural effusion, hydrothorax, dyspnoea, oliguria, haematological abnormalities and weight gain. In rare instances, venous or arterial thromboembolism may occur in association with OHSS. Transient liver function test abnormalities suggestive of hepatic dysfunction with or without morphologic changes on liver biopsy have also been reported in association with OHSS.
Women with known risk factors for a high ovarian response may be especially prone to the development of OHSS during or following treatment with Puregeon. For women having their first cycle of ovarian stimulation, for whom risk factors are only partially known, close observation for early signs and symptoms of OHSS is recommended.
To reduce the risk of OHSS, ultrasonographic assessments of follicular development should be performed prior to treatment and at regular intervals during treatment. The concurrent determination of serum oestradiol levels may also be useful. In ART there is an increased risk of OHSS with 18 or more follicles of 11 mm or more in diameter. When there are 30 or more follicles in total it is advised to withhold hCG administration.
The chance of having a side effect is described by the following categories:
Common (may affect up to 1 in 10 women)

- Ovarian hyperstimulation syndrome (OHSS)
- Pelvic pain
- Feeling sick (nausea)
- Headache
- Tiredness (fatigue)
- Pelvic discomfort

- Breast

Uncommon (may affect up to 1 in 100 women)

- Ovarian torsion (twisting of the ovary resulting in extreme lower stomach pain)
- Liver enzyme increases
- Miscarriage
- Pain after oocyte retrieval
- Procedural pain
- Releasing an egg too early (premature ovulation)
- Abdominal distension
- Vomiting
- Diarrhoea
- Constipation
- Back pain
- Breast pain
- Bruising or pain at the injection site
- Irritability
- Mood swings
- Dizziness
- Hot flush

Not known (cannot be estimated from available data)

- Allergic reactions (hypersensitivity reactions, both local and generalized, including rash).
- Pregnancy outside the uterus (an ectopic pregnancy) and multiple pregnancies have also been reported. These side effects are not considered to be related to the use of corifollitropin alfa, but to Assisted Reproductive Technology (ART) or subsequent pregnancy.
- In rare instances, blood clots (thrombosis) that formed inside a blood vessel, broke off, and travelled inside the bloodstream to block another blood vessel (thromboembolism) have been associated with Corifollitropin alfa therapy as with other gonadotropins.

13. Study Assessments and Procedures

13.1 Study Assessments

13.1.1 Screening

A responsible investigator will identify potential eligible women for this study. They will be screened and documented as eligible submitted to inclusion criteria. The investigator will obtain informed consents for participation in the study using local IRB approved informed consent forms.

13.1.2 Baseline

A normal baseline ultrasound (<6 months before starting) has to be available before inclusion in the trial.
Before starting treatment, the hormonal profile will be checked with a blood analysis, performed on day 2 or 3 of the menstrual cycle.

13.1.3 Treatment Period

The duration of the treatment itself will depend on every patient's response. The frequency of further follow-up visits with endocrine monitoring and ultrasound is individualized and is based on the growth of the follicles.

13.1.4 Follow-Up

Follow-up in case of pregnancy will be planned until 7-8 weeks of amenorrhea. From that moment patients are referred to their treating obstetrician. Information on the pregnancy and delivery will be collected once available.

13.1.5 End of Study

46 patients (23 in each group) need to be included.

13.1.6 Laboratory Evaluation

Blood samples

During the course of the treatment, several blood samples will be taken. All of them are collected in order to evaluate the hormonal profile and the response to ovarian stimulation (LH, FSH, E, P). Furthermore, follow-up with hCG will take place 12 days after the embryo transfer (overview of all additional blood samples: tables and figures, fig.2)

Follicular fluid and cumulus cells

In both groups, measurement (mean diameter) of all aspirated follicles will be performed on the day of the first and second OR (for group B). At each OPU, the follicles will be individually aspirated and searched for the presence of COCs. All oocytes will be further cultured separately, therefore allowing individual tracing of the oocyte and its correspondent embryo. The correlation between the follicular size and the embryological/reproductive outcome will be further evaluated. In addition, from each individual follicle two samples will be collected i) follicular fluid (FF) and ii) cumulus cells (CC) from the corresponding COC. The COCs will be individually collected and denuded before ICSI. The CCs obtained after denudation will be individually collected in cryo-vials and labelled with the same identification code as the FF from where the COC was recovered. The FF and CC samples will be kept at -196 or -80˚C. Both CC and FF are in the standard procedure not used for the patient and are therefore considered waste material. The CC and FF will be analysed in a later stage.

14. Safety Monitoring and Reporting

14.1 Adverse Events

An AE is any untoward medical occurrence in a patient participating in a trial. It includes:

- Any laboratory abnormality, vital sign or finding from physical or gynaecological examination assessed as clinically significant by the investigator (note: findings from assessments and examinations done during screening are not adverse events, but are recorded as medical history).
- Accidental injuries, reasons for any change in medication (drug and/or dose), reasons for any medical, nursing or pharmacy consultation, or reasons for admission to hospital or surgical procedures.

14.2 Suspected Unexpected Serious Adverse Events (SUSAR)

All adverse drug reactions (ADR) to gonadotropins, whether serious or non-serious, are to be captured in the consultations and investigator must record the ADRs in the ADR Log provided in each patient's consultations with information about:

- Adverse Drug Reaction
- Date and time of onset
- Intensity
- Action taken to

- Other action taken
- Date and time of outcome
- Outcome
- Seriousness criteria if applicable
- Short description of ADR (if not serious)

14.3 Procedures for Handling Special Situations

14.3.1 Pregnancy

The first blood test that will be performed on day 2 or 3 of the menstrual cycle will also include the hCG in order to detect possible pregnancies before starting the treatment. Patients with a positive pregnancy test will be excluded from the trial.

14.3.2 Overdose Management

Patients that will use more medications than the one suggested will be monitored in their referral centre with blood test and ultrasound; although, there are no specific side effects due to the overdose of these medications

14.3.3 Annual Safety Report

An annual safety report will be prepared with all the new information and the notifications that the patients will provide within the study period.

15. Data Collection and Management

15.1 Data Collection

Data will be collected in a secure and encrypted eCRF created specifically for the trial using Filemaker Pro® version 13 (Filemaker Inc.) hosted on a dedicated server at the *CRG-UZ Brussel*.

15.2 Database Management and Quality Control

The database has inbuilt validation procedures to ensure the correct introduction of data and avoid cases of missing information where such is not applicable. The doctors, study nurses and research assistants collaborating in the trial will be responsible for the data collection. Data will be stored for 10 years.
No data safety monitoring board will be established.

The results of the study will be publicly disseminated following submission in peer-reviewed scientific journals.

15.3 Statistical Considerations and Data Analysis

Analyses were performed in intent-to-treat fashion and per-protocol. Consequently, in the intention-to-treat analysis, all patients will be included in the final analysis as long as after fulfillment of the inclusion criteria they were randomly allocated to one of the treatment groups, whereas the per-protocol analysis will include only those patients who completed the treatment originally allocated. Continuous data will be presented as mean ± SD or median [range] and categorical data will be described by number of cases, including numerator and denominator, and percentages, in line with the CONSORT statement (www.consort-statement.org). Categorical data and continuous data that do not show a normal distribution will be analyzed by Pearson's χ2test/Fisher exact test or Mann-Whitney`s U test as appropriate.
The normality of the distributions will be assessed with Kolmogorov-Smirnov's test and graphical methods. All tests will be two-sided. Differences will be considered as statistically significant if the null hypothesis could be rejected with >95% confidence ($p<0.05$). All tests will be performed with the STATA version 13.

Power/Sample Size:
Our sample size of 46 patients (23 per group) has a power of at least 90% to detect superiority of the double trigger group (mean 5.4 MII) versus control group (mean 3.4 MII), standard deviation (SD=2), using a two-sided, t test, at significance level alpha of 0.05.

16. Ethical Considerations

16.1 Ethical conduct of the study

16.1.1 Declaration of Helsinki

The study protocol will follow the ethical principle established in the declaration of Helsinki.

16.1.2 Ethics Committee

The protocol will be submitted to the local Ethics Committees of Universitair Ziekenhuis Brussel. Strict confidentiality of all personal and research data will be ensured.
No specific reimbursement will be given to patients participating in the study.

16.2 Informed Consent

The informed consents were written in Dutch, French and English.

16.3 Patient and Study Data Protection

Participation in this study is voluntary and women are free to withdraw from the study at any time and for any reason without prejudice.
All information about the treatment received during the study remains confidential.
The subjects name and any data related to her identity will not be disclosed when the results of the study will be published for scientific purposes.
Access to the medical record and information obtained regarding participation in the trial will be limited to the study staff, the Ethics Committee and the health authorities.
The investigator will never reveal the subjects name in the context of a publication or conference but he/she will also encode (the identity will be replaced by an ID code in the study) the data before sending them to the manager of the database of collected data. The investigator and his/her team will therefore be the only ones to be able to establish a link between the data transmitted throughout the study and the medical records. For the study data manager designated by the head investigator, the data transmitted will not allow the subject to be identified. The latter is responsible for collecting the data gathered by all investigators taking part in the study, processing them and protecting them in accordance with the requirements of the Belgian law on the protection of privacy and by the law on patient's rights of 22 August 2002.
The new European General Data Protection Regulation (GDPR), in force since 25 May 2018, imposes additional requirements on how companies or organizations may use personal data. One of these requirements is that the data controller provides the following information.
As also indicated in the informed consent form, personal data are collected in the context of the investigation in which the subject participates. The sponsor is responsible for the correct processing and the information obligation that goes with it.
In addition to the personal data, such as data on age and sex, 'special categories' of personal data are also collected (such as ethnic background, state of health and medical conditions, including medical history, treatments and response to treatments, biological samples, e.g. blood samples, tissue, and the results of their analysis, medical imagery, e.g. scans, x-rays, and the results of their evaluation).
Of course, personal data may only be used for the scientific research purposes described in the form for informed consent.
The data may be accessed by persons located in countries that do not use the same standards of legal data protection as the EU. The conditions of the European and Belgian legislation on the protection of personal data must be respected.
In accordance with the relevant legislation, the data collected as part of the research will be kept for at least 20 years, or 30 years if these data are also part of the medical file.

According to the GDPR, the subject has a number of rights regarding the processing of the data. If any further questions about this, the subject can always contact the investigator.
The data protection officer of the research department is also available for contact details: DPO (data protection officer) via dpo@vub.be The subject also has the right to submit a complaint about how the information is handled. This can be done with the Belgian supervisory authority responsible for enforcing data protection legislation.

16.4 Subject Identification

Every woman will be identified by a study specific subject number.

16.5 Conflict of Interest

There are no conflicts of interest to declare.

17. Finance and Insurance

If the patient will decide to take part in this study, this will not involve any extra costs for her or her insurer. The additional costs of the study (extra medication for prolonged stimulation, ovum pick-up, additional sampling) will be covered by founding from MSD.
Any participation in a clinical study involves a risk, however small it is. Even if there is no fault, the investigator accepts responsibility for damage caused to the participant (or in the event of death, his/her dependents) and directly or indirectly linked to his/her participation in the study. The sponsor has taken out insurance for this responsibility.

18. Reporting and Dissemination

Missing efficacy and safety outcomes will be considered as negative, regardless of the cause which may justify the lack of said information (*e.g.* cycle cancelled due to the survival after warming of no embryo or loss to follow-up). These patients will be included in the analysis following the ITT principle.

19. Conflict of interest statement

This study is financially supported by MSD.
None of the other investigators have conflict of interest to declare.

20. Tables and Figures

Fig. 1 study design

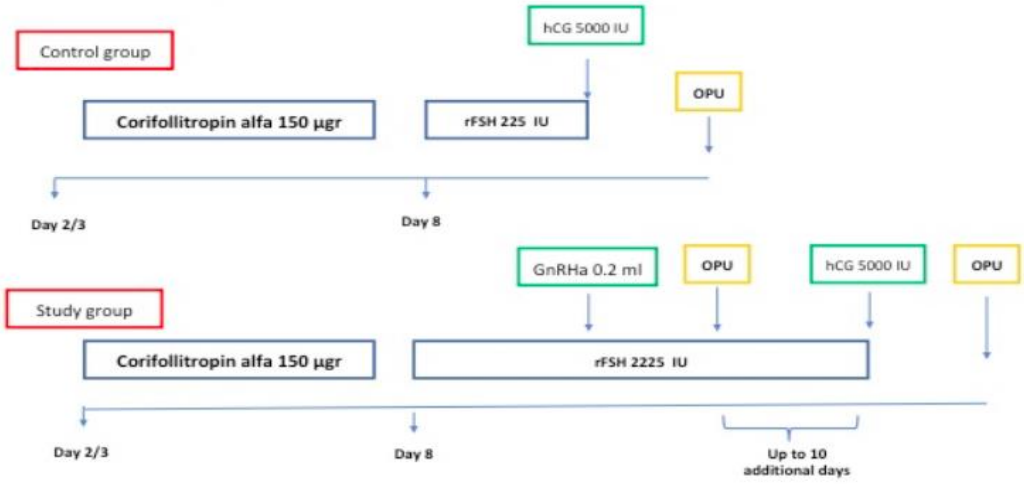

Table 1: Summarizing table of all investigations and procedures

Group A (control group)

	Day 2 of the cycle	Day 8 of the stimulation	Every 1 or 2 days	Final maturation trigger	Ovum pick-up*	Day 3 or 5 after egg retrieval	12 days after embryo transfer
Informed consent	x						
Inclusion/ exclusion criteria	x			x			
Infertility/ menstrual/ reproductive history	x						
Blood sample for endocrine monitoring (LH, FSH, estradiol, progesteron)	x	x	x		x		
Ultrasound with follicle measurement		x	x				
Embryo transfer						x	
Blood sample for pregnancy hormone (hCG)							x

(*) with collection of follicular fluid and cumulus cells

Group B (study group)

	Day 2 of the cycle	Day 8 of the stimulation	Every 1 or 2 days	Final maturation trigger	1st Ovum pick-up* with freezing of all embryos	4 days after first pick-up and 3-4 times after that	2nd Ovum pick-up* with freezing of all embryos	In the next cycle
Informed consent	x							
Inclusion/ exclusion criteria	x			x				
Infertility/ menstrual/ reproductive history	x							
Blood sample for endocrine monitoring (LH, FSH, estradiol, progesteron)	x	x	x		x	x	x	
Ultrasound with follicle measurement		x	x			x		
Embryo transfer								x

(*) with collection of follicular fluid and cumulus cells

21. References

Baerwald, A.R., Adams, G.P., and Pierson, R.A. (2003). A new model for ovarian follicular development during the human menstrual cycle. Fertil Steril 80(1), 116-122.

Devroey, P., Polyzos, N.P., and Blockeel, C. (2011). An OHSS-Free Clinic by segmentation of IVF treatment. Hum Reprod 26(10), 2593-2597. doi: 10.1093/humrep/der251.

Drakopoulos, P., Blockeel, C., Stoop, D., Camus, M., de Vos, M., Tournaye, H., et al. (2016). Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? Hum Reprod 31(2), 370-376. doi: 10.1093/humrep/dev316.

Humaidan, P., Polyzos, N.P., Alsbjerg, B., Erb, K., Mikkelsen, A.L., Elbaek, H.O., et al. (2013). GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: two prospective randomized controlled multi-centre studies in IVF patients. Hum Reprod 28(9), 2511-2521. doi: 10.1093/humrep/det249.

Nivet, A.L., Leveille, M.C., Leader, A., and Sirard, M.A. (2016). Transcriptional characteristics of different sized follicles in relation to embryo transferability: potential role of hepatocyte growth factor signalling. Mol Hum Reprod 22(7), 475-484. doi: 10.1093/molehr/gaw029.

Papanikolaou, E.G., Pozzobon, C., Kolibianakis, E.M., Camus, M., Tournaye, H., Fatemi, H.M., et al. (2006). Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles. Fertil Steril 85(1), 112-120. doi: 10.1016/j.fertnstert.2005.07.1292.

Wirleitner, B., Okhowat, J., Vistejnova, L., Kralickova, M., Karlikova, M., Vanderzwalmen, P., et al. (2018). Relationship between follicular volume and oocyte competence, blastocyst development and live-birth rate: optimal follicle size for oocyte retrieval. *Ultrasound Obstet Gynecol* 51(1), 118-125. doi: 10.1002/uog.18955.