Summary of clinical trial results

Trial Title: *Randomized controlled trial comparing micronized progesterone (Amelgen®) 400 mg BID versus 400 mg TID for luteal support in artificial vitrified/warmed single blastocyst transfer cycles with low progesterone on day of embryo transfer*

EU reference number: *2020-004112-10*

Acronym / Protocol code: *PROTECTA*

Investigational device / medicinal product: Amelgen

ClinicalTrials.gov identifier: *NCT04806919*

Sponsor: Ghent University Hospital

Contact details sponsor:

Prof. dr. Stoop

Ghent University Hospital – Department of Reproductive Medicine

Corneel Heymanslaan 10

9000 Ghent

Belgium

National Coordinator / Coordinating Investigator: Prof. dr. Stoop

Funder: Gedeon Richter

Author: Kathleen Wijnant

Date of report: 05/12/2025

By signing this final study report, I acknowledge that the information is accurate and complete.

Name and signature Coordinating Investigator: .....

Date and signature Coordinating Investigator: 15/12/25

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1. Introduction

The serum progesterone concentration on the day of embryo transfer appears to be a significant predictor for live birth after frozen embryo transfer (FRET) in an artificial prepared endometrium (Basnayake et al., 2018; Cédrin-Durnerin et al., 2019; Labarta et al., 2017; Yovich et al., 2015). This has been observed in cycles supplemented with different progesterone regimens such as 200 mg TID micronized progesterone (Basnayake et al., 2018) or with 400 mg BID micronized progesterone (Cédrin-Durnerin et al., 2019; Yovich et al., 2015). This could lead to suboptimal progesterone levels and a resulting suboptimal endometrial receptivity for up to one-third treatment cycles (Basnayake et al., 2018).

Several authors have identified thresholds serum progesterone values under which ongoing pregnancy rates significantly decrease, ranging from 9.2 ng/ml (Labarta et al., 2017) and 10 ng/ml (Basnayake et al., 2018) up to 15.7 ng/ml (50 nmol/ml) (Cédrin-Durnerin et al., 2019). Yovich et al. (2015) identified an optimal progesterone range between 70 and 99 nmol/l, which correlates with 22 and 31.1 ng/ml.

Most studies report a wide range in mid-luteal progesterone concentrations and it remains unclear how dosing could be further optimized by individualization based on patient characteristics. A study by Duijkers et al. (2018) found the best secretory transformation in cycles with 400 mg BID regimen in comparison to lower dosed and/or less frequently administered progesterone regimens.

Labarta et al. (2017) found reassuring pregnancy rates after administration of a daily injection of subcutaneous 25 mg progesterone (Inprosub®) to patients with suboptimal progesterone levels. However, this study was not randomized and it may be preferable for patients to achieve adequate serum progesterone levels without the need for additional daily progesterone injections.

To date, the golden standard at our clinic for artificial FRET is the use of 600 mg micronized progesterone (200 mg TID).

The aim of this study is to evaluate the effect of luteal phase dose adjustments (Amelgen® 400 mg TID instead of BID) on the likelihood of ongoing pregnancy, in patients undergoing IVF or ICSI treatment with a suboptimal serum progesterone level (defined as < 10 mcg/l) on the day of blastocyst transfer. The study assesses how the reduced implantation potential in patients with low progesterone levels on day of embryo transfer can be overcome by augmentation of the daily progesterone dosage.

Secondly, this study proposal also offers the opportunity to study 'endometrial compaction' or the progesterone induced, relative change (ideally decrease) in endometrial thickness between the last day of the estrogen phase (estradiol valerate administration) and the day of embryo transfer. Retrospective data by Haas et al. (2019) found a highly significant inverse correlation between the ongoing pregnancy rate and the change of endometrial thickness.

2. Objectives of the study

2.1 Primary objectives

The primary objective is to investigate the effect of an increased dose of vaginal progesterone supplementation (Amelgen® 400 mg BID vs Amelgen® 400 mg TID) on the ongoing pregnancy (fetal heartbeat during TVUS between week 6 and 8 of pregnancy) rate for patients undergoing IVF or ICSI treatment with a suboptimal serum progesterone level (defined as <10 mcg/l) on the day of blastocyst transfer in an artificial prepared endometrium cycle.

2.2 Secondary objectives

Secondary objectives are to assess:

- If the degree of endometrial impaction is associated with reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate (defined as fetal heartbeat during TVUS between

week 6 and 8 of pregnancy), biochemical pregnancy rate, miscarriage rate) in patients undergoing IVF or ICSI treatment (with progesterone levels either below or above 10 mcg/l);

- If progesterone level on the day of blastocyst transfer in an artificial prepared endometrium cycle (< 10 mcg/l versus ≥ 10 mcg/l) is associated with reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate, biochemical pregnancy rate, miscarriage rate) in patients undergoing IVF or ICSI treatment (with progesterone levels either below or above 10 mcg/l) who are receiving standard of care (hence not Amelgen® 400 mg TID);

- If intercourse frequency (registered by patient diary) is associated with progesterone level on the day of blastocyst transfer in an artificial prepared endometrium cycle and with reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate (defined as fetal heartbeat during TVUS between week 6 and 8 of pregnancy), biochemical pregnancy rate, miscarriage rate) in patients undergoing IVF or ICSI treatment (with progesterone levels either below or above 10 mcg/l) who are receiving standard of care (hence not Amelgen® 400 mg TID).

2.3 Exploratory Objectives

- To evaluate patient comfort and side effects on the day of embryo transfer and day of the initial pregnancy test in patients undergoing IVF or ICSI treatment (with progesterone levels either below or above 10 mcg/l)

- To determine if there are patient characteristics (e.g. length, weight, BMI, age, endometrium thickness on day of micronized progesterone initiation…) that can predict low progesterone level at day of embryo transfer (e.g. sexual life and intercourse frequency) in patients undergoing IVF or ICSI treatment (with progesterone levels either below or above 10 mcg/l) who are receiving standard of care (hence not Amelgen® 400 mg TID).

3. Investigational Medicinal Product

3.1 Amelgen® 400 mg

Amelgen® 400 mg pessaries

3.2 Composition and active substance of the IMP

Each pessary contains 400 mg progesterone (the other ingredient is hard fat).

3.3 Manufacturer and Distributor of the IMP

Manufacturer:

Accord-UK Limited

Whiddon Valley

Barnstaple, North Devon

EX32 8NS, United Kingdom

Distributor:

Gedeon Richter Pharma GmbH

Mergenthalerallee 15-21

65760 Eschborn, Germany

Release site:

Accord-UK Limited

Whiddon Valley

Barnstaple, North Devon

EX32 8NS, United Kingdom

Gedeon Richter Plc.

Gyömröi út 19-21

1103 Budapest

Hungary

3.4 Preparation + Dosage + administration of the IMP

Depending on the allocated arm, the pessaries have to be administered vaginally twice a day (according to the SmPC) or three times a day (experimental).

3.5 Permitted dose adjustments and interruption of treatment

If Amelgen® pessaries are used twice a day, the first pessary needs to be administered in the morning (between 6 and 8h) and the second pessary needs to be administered in the evening (between 22 and 24h). If Amelgen® pessaries are used three times a day (in case serum progesterone level < 10 mcg/l on the day of embryo transfer and subject is allocated to the intervention group), a third pessary needs to be administered in the afternoon (between 14 and 16h). If subject has forgotten to administer a pessary and she discovers it the same day, the forgotten pessary should be administered as soon as possible. If subject discovers she has forgotten to administer one or more pessaries in the previous days no action should be taken.

3.6 Duration of treatment

In case of a positive confirmative pregnancy test (D16 (± 2 days) + 2 days), micronized progesterone pessaries (Amelgen® 400 mg BID (control group) or Amelgen® 400 mg TID (intervention group)) are maintained until the day of performance of a TVUS in which an embryo with a heartbeat is documented (between week 6 and 8 of gestation) (= end of study). The use of Amelgen is continued after the TVUS between 6 and 8 weeks of gestation per standard of care. If subjects have left-over IMP after participation in the trial they are allowed to use this after an accountability check by the study team.

In case no (ongoing) pregnancy is achieved, micronized progesterone pessaries can be stopped on the day of the negative pregnancy test or when the diagnosis of a non-viable pregnancy is made (= end of study).

3.7 Packaging and Labeling of the IMP

Packaging/labeling of Amelgen® will be in accordance with the relevant GMP guidelines.

Amelgen 400 mg ovules/ovules/Vaginalzäpfchen
EudraCT N°: 2020-004112-10
TRIAL subject ID nr: _____ Initialen: _____ Datum visite: _____
ENKEL VOOR KLINISCHE STUDIES
Gebruiksaanwijzing: De aanbevolen dosering is 400 mg tweemaal daags, vaginaal in te brengen. Deze dosering zal u gedurende 5 dagen gebruiken. Daarna zal u mede gedeeld worden of u de ovules tweemaal daags of driemaal daags dient in te brengen, afhankelijk van de studie arm waarin u zich bevindt - 15 ovules - Elke ovule bevat 400 mg progesteron – Voor vaginaal gebruik - Buiten het zicht en bereik van kinderen houden - Bewaren beneden 30 °C.
DESTINÉ UNIQUEMENT AUX ESSAIS CLINIQUES
Mode d'emploi: La dose recommandée est de 400 mg deux fois par jour par insertion vaginale - Vous utiliserez ce dosage pendant 5 jours. Ensuite, on vous dira si vous devez insérer les ovules deux ou trois fois par jour, selon le groupe d'étude dans lequel vous vous trouvez - 15 ovules - Chaque ovule contient 400 mg de progestérone - Pour usage vaginal - Tenir hors de la vue et de la portée des enfants - A conserver à une température ne dépassant pas 30 °C.
NUR FÜR KLINISCHE STUDIEN
Gebrauchsanweizung: Die empfohlene Dosis beträgt 400 mg zweimal täglich durch Einführen in die Vagina - Sie werden diese Dosierung 5 Tage lang anwenden. Danach wird Ihnen mitgeteilt, ob Sie die Eizellen zwei- oder dreimal täglich einsetzen müssen, je nachdem, in welchem Studienarm Sie sich befinden - 15 Vaginalzäpfchen - Jedes Vaginalzäpfchen enthält 400 mg Progesteron - Für vaginale Anwendung - Bewahren Sie dieses Arzneimittel für Kinder unzugänglich auf - Nicht über 30 °C lagern.
Vervaldatum/date d'expiration/Verfallsdatum: XX/XXXX
Lotnummer/Numéro de lot/Chargennummer: XXXXX
Prof. Dr. Stoop Tel: 09 332 49 03
SPONSOR: UZ GENT C. Heymanslaan 10 9000 GENT

At UZ Gent, labeling will be performed by study site staff after delivery of the IMP by the pharmacy. Labeling of the IMP that will be used in AZ Delta will be organized in AZ Delta.

3.8 Storage conditions of the IMP

The Marketing Authorization holder, Gedeon Richter Plc., has agreed to provide Amelgen® for free without any strings attached. The micronized progesterone pessaries will be supplied to the local hospital pharmacy for the participants' use only. The local hospital pharmacy will have a transit function, which means that the pessaries are stored at the fertility clinic. Upon receipt, the pessaries will be stored at room temperature (below 30°C). Temperature logs will be kept in accordance with local pharmacy practice to ensure proper storage conditions. No temperature deviations are expected as the room temperature is not expected to exceed 30°C. However, Gedeon Richter Plc. will be contacted if a temperature deviation occurs and asked for advice if the medication can still be used. The site is responsible to keep track of the supply of the micronized progesterone pessaries (Amelgen® 400 mg), its handling and distribution to the participants only on an accountability log. Each subject (n = 807), willing to participate in the study, will receive 3 packs of 15 micronized progesterone pessaries (Amelgen® 400 mg) before initiation of progesterone is decided. On D16 (± 2 days) subjects from the intervention group will receive 1 pack of 15 micronized progesterone pessaries (Amelgen® 400 mg) when they sign up for the initial pregnancy test. In case the pregnancy test is positive, subjects from the intervention group will receive 5 packs of 15 micronized progesterone pessaries on the day of the confirmative pregnancy test D16 (± 2 days) + 2 days. Subjects from the control group will receive 3 packs of 15 micronized progesterone pessaries on the day of the confirmative pregnancy test D16 (± 2 days) + 2 days in case the pregnancy test is positive. Drug compliance will be checked through the study diary that will be provided. In addition, the subject needs to bring the empty packaging to the site in order to establish drug accountability. Remaining study medication, which was assigned to a subject but not used in the study, can be kept by the subject for later use (outside of the clinical trial) after drug accountability and compliance have been verified. At the end of the study the remaining study medication, which was not assigned to a subject, will be destroyed per local guidelines (SOP destruction of study medication) and a destruction certificate will be generated and filed in the Investigator Site File (ISF).

3.9 Known side effects of the medication

Common side effects (may affect up to 1 in 10 people):

- Abdominal distension (swelling in the abdomen), abdominal pain, constipation

- Sleepiness
- Tiredness
- Hot flushes
- Breast pain

Uncommon side effects (may affect up to 1 in 100 people):

- Headache, dizziness
- Mood changes
- Change in taste, vomiting, flatulence, diarrhoea, bloat (gastric dilatation), rectal neoplasia
- Night sweats, skin rash or itching
- Joint pain
- Pelvic pain, ovarian enlargement, vaginal bleeding
- Frequent urination, involuntary excretion of urine
- Weight increase
- Bleeding
- Itching at the application site, feeling cold or body temperature change or general discomfort

4. Study Protocol Summary

4.1 Study design

This is a prospective, open label, randomized, bicenter, superiority phase 3 controlled clinical trial in subjects with a suboptimal serum progesterone level (defined as < 10 mcg/l) on the day of blastocyst transfer in an artificial prepared endometrium cycle.

The study also encloses an observational arm, including subjects undergoing IVF or ICSI treatment with serum progesterone level ≥ 10 mcg/l.

In total, it is expected that 807 subjects will be included, of which approximately 30% (n = 242) will have a suboptimal progesterone level (< 10 mcg/l). Subjects with a suboptimal progesterone level (< 10 mcg/l) will be randomized to either the intervention group (Amelgen® 400 mg TID) or the control group (Amelgen® 400 mg BID) (allocation ratio 1:1). The study will stop when there are 242 subjects randomized. Subjects with serum progesterone level ≥ 10 mcg/l, will be treated standard of care and are kept within the study until reaching study endpoints.

The study population is aimed at a broad population undergoing IVF or ICSI treatment and undergoing a single vitrified/warmed single transfer in an artificial prepared endometrium cycle.

4.2 Inclusion criteria

- Informed consent form (ICF) dated and signed
- Age ≥ 18 and < 43 years old at the time of signing ICF
- Body Mass Index (BMI) ≥ 18.5 kg/m² and < 35 kg/m²
- Less than 5 failed previous Assisted Reproductive Technologies (ART) cycles since live birth or in case of no live birth: since start fertility treatment
- Current pregnancy wish
- Patients undergoing a single vitrified/warmed single transfer in an artificial prepared endometrium cycle (IVF or ICSI)

4.3 Exclusion criteria

- Simultaneous participation in another clinical study
- Previous participation in this study
- Known reasons for impaired implantation
- (specifically: presence of an hydrosalpinx; presence of a type I, II or III fibroid; Asherman's syndrome; uterine malformations, intrauterine adhesions, ≥ grade 3 endometriosis according to the ASRM classification, endometrial tuberculosis)
- Repeated miscarriages
- (> 2 miscarriages)
- Untreated and uncontrolled thyroid dysfunction
- Tumors of the ovary, breast, uterus, pituitary or hypothalamus
- Abnormal vaginal bleeding without a known/diagnosed cause
- Ovarian cysts or enlarged ovaries
- Fibroid tumors of the uterus incompatible with pregnancy
- Malformations of the reproductive organs incompatible with pregnancy
- Previous antibiotic hypersensitivity reactions (streptomycin and/or neomycin)
- Risk factors for thromboembolic events, such as a personal or family history, severe obesity or thrombophilia
- Ongoing pregnancy
- Use of carbamazepine, rifampicin or phenytoin
- Those unable to comprehend the investigational nature of the proposed study

4.4 Primary endpoint

The primary end point is the ongoing pregnancy rate. The diagnosis of an ongoing pregnancy is made if a fetal heartbeat is documented during TVUS between week 6 and 8 of pregnancy.

4.5 Secondary endpoints

The secondary end points are:

- the endometrial impaction as measured with TVUS on the day of the embryo transfer (D5) and possible impact on reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate (defined as fetal heartbeat during TVUS between week 6 and 8 of pregnancy), biochemical pregnancy rate, miscarriage rate)
- the comparison of reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate (defined as fetal heartbeat during TVUS between week 6 and 8 of pregnancy), biochemical pregnancy rate, miscarriage rate) of subjects with progesterone levels above and below the 10 mcg/L threshold;
- the intercourse frequency (registered by patient diary) and possible impact on progesterone levels and on reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate (defined as fetal heartbeat during TVUS between week 6 and 8 of pregnancy), biochemical pregnancy rate, miscarriage rate)

4.6 Procedures

Flowchart with a schematic overview of the data collection and study specific interventions (i.e. blue text).

	Study Arm 1 (Control group: Amelgen® 400 mg BID)	Study Arm 2 (Intervention group: Amelgen® 400 mg TID)
Screening	The subject's socio-demographics, relevant medical history, reproductive history and anthropometry will be recorded at the time of study enrollment (= study-specific intervention)	
Endometrium preparation (= standard of care)	Initiation of a daily dose of 6 mg estradiol valerate (Progynova® 2 mg) for at least 10 days, with a maximum of 21 days, starting on day 1 or day 2 of menstruation or withdrawal bleeding or when E_2 < 80 ng/l. Measurement of E_2, Progesterone, FSH and LH takes place when estradiol valerate (Progynova® 2 mg TID) is not started on day 1 or day 2 of menstruation or withdrawal bleeding, before initiation of estradiol valerate (Progynova® 2 mg TID). When E_2 is lower than 80 ng/l estradiol valerate (Progynova® 2 mg TID) can be initiated.	
Evaluation (= standard of care)	TVUS: measurement of endometrial thickness and quality assessment between 10 days and 14 days after start of 6 mg estradiol valerate (Progynova® 2 mg TID) administration * If endometrial thickness is > 7mm and triple line endometrium: start progesterone * If endometrial thickness is < 7mm or no triple line endometrium: new evaluation after at least 3 days * If no endometrial thickness of 7 mm or no triple line endometrium is reached after up to 21 days of estradiol valerate (Progynova® 2 mg) administration: start progesterone Blood sample collection for FSH, E_2, LH and progesterone needs to be done each time a TVUS evaluation is planned.	
Start of progesterone (= D0)	As soon as endometrial thickness is > 7 mm and triple line, unless D21 of Progynova® 2 mg TID treatment is reached: Patient diary will be delivered to the subject (= study-specific intervention) + Initiation of vaginally administered micronized progesterone pessaries (Amelgen® 400 mg) (= day 0). The first administration is situated in the evening of day 0 (between 22-24h) (= standard of care). From day 1, the micronized progesterone pessaries (Amelgen® 400 mg) are administered twice daily (between 6-8h and 22-24h) in a dose of 400 mg until the day of randomization (D6) (= standard of care).	
Day of ET (= D5)	Standard of care: Blood sample collection for progesterone on day of embryo transfer. Embryo transfer is performed in the afternoon of day 5 of micronized progesterone pessaries (Amelgen® 400 mg BID) administration. Study-specific intervention: TVUS: measurement of endometrial thickness just before embryo transfer procedure. Questionnaire (ET) about comfort and side effects for the subject.	
D6	Feedback to subject before 14h: If serum progesterone level on day of transfer (D5) was < 10 mcg/l: randomization (= study-specific intervention). If serum progesterone level on day of transfer (D5) was ≥ 10 mcg/l: no action needs to be taken and micronized progesterone pessaries (Amelgen® 400 mg) need to be continued twice daily according to the standard of care.	
Further management (from D6 until D16 (±2 days))	-Micronized progesterone pessaries (Amelgen® 400 mg BID) (between 6-8h and 22-24h) are maintained until the day of the pregnancy test (from D6 until D16 (± 2 days)). In case of a positive pregnancy test the micronized progesterone pessaries (Amelgen® 400 mg BID) are maintained until the day of the confirmative pregnancy test 2 days later (= standard of care) -D16 (± 2 days): hCG and progesterone assessment (= pregnancy test) (= standard of care)	-Augmentation in micronized progesterone pessaries (Amelgen® 400 mg TID) (between 6-8h, 14-16h and 22-24h) until the day of the pregnancy test (from D6 until D16 (± 2 days)) (= study-specific intervention). In case of a positive pregnancy test the micronized progesterone pessaries (Amelgen® 400 mg TID) are maintained until the day of the confirmative pregnancy test 2 days later (= standard of care)

<table>
<tr><td></td><td>-Questionnaire (initial pregnancy test D16 (± 2 days)) about comfort and side effects for the subject (= study-specific intervention)

-The study ends for subjects with a negative pregnancy test (hCG < 5 U/l).</td><td>-D16 (± 2 days): hCG and progesterone assessment (= pregnancy test) (= standard of care)

-Questionnaire (initial pregnancy test D16 (± 2 days)) about comfort and side effects for the subject (= study-specific intervention)

-The study ends for subjects with a negative pregnancy test (hCG < 5 U/l).</td></tr>
<tr><td></td><td colspan="2">Subjects who are not randomized because of progesterone ≥ 10 mcg/L are followed up for the collection of the outcome data* (identically to the randomized subjects), which is up to and including the first negative pregnancy test (D16 ± 2 days or D16 (± 2 days) + 2 days) (if not pregnant). Or up to and including the pregnancy ultrasound at W6-W8 of gestation (if pregnant), or until a diagnosis of a non-viable pregnancy is made.</td></tr>
<tr><td>Pregnancy follow-up

(from D16 (±2 days) + 2 days until first pregnancy TVUS)</td><td>-In case of a positive pregnancy test on D16 (± 2 days) a confirmative assessment of hCG and progesterone needs to be done 2 days later to exclude biochemical pregnancies (= standard of care).

-If the confirmative pregnancy test is positive (hCG > 5 U/l) micronized progesterone pessaries (Amelgen® 400 mg BID) are maintained until the day of performance of a TVUS in which an embryo with a heartbeat is documented (between week 6 and 8 of gestation) or until a diagnosis of a non-viable pregnancy is made (= standard of care).

-If the confirmative pregnancy test is negative (hCG < 5 U/l) micronized progesterone can be stopped and the study ends.</td><td>-In case of a positive pregnancy test on D16 (± 2 days) a confirmative assessment of hCG and progesterone needs to be done 2 days later to exclude biochemical pregnancies (= standard of care).

-If the confirmative pregnancy test is positive (hCG > 5 U/l) micronized progesterone pessary (Amelgen® 400 mg TID) are maintained until the day of performance of a TVUS in which an embryo with a heartbeat is documented (between week 6 and 8 of gestation) or until a diagnosis of a non-viable pregnancy is made (= standard of care).

-If the confirmative pregnancy test is negative (hCG < 5 U/l) micronized progesterone can be stopped and the study ends.</td></tr>
<tr><td></td><td colspan="2">Subjects who are not randomized because of progesterone ≥ 10 mcg/L are followed up for the collection of the outcome data* (identically to the randomized subjects), which is up to and including the first negative pregnancy test (D16 ± 2 days or D16 (± 2 days) + 2 days) (if not pregnant). Or up to and including the pregnancy ultrasound at W6-W8 of gestation (if pregnant), or until a diagnosis of a non-viable pregnancy is made.</td></tr>
<tr><td>Pregnancy follow-up

(first pregnancy TVUS between week 6 and 8 of gestation)</td><td colspan="2">-Performance of a TVUS between week 6 and 8 of gestation (= standard of care) + Blood sample collection (5ml) hCG and progesterone (= study-specific intervention)

-Diagnosis of an ongoing pregnancy (visualization of an embryo with a heartbeat) or a non-viable pregnancy is made (= standard of care)

-Micronized progesterone pessaries (Amelgen® 400 mg BID (control group) or TID (intervention group)) is mostly continued after the first pregnancy TVUS per standard of care. If progesterone supplementation is continued after study participation, the subject can use the leftover Amelgen® (after drug accountability) or she can collect it with a prescription at the local pharmacy. The study ends for all pregnant subjects after the first pregnancy TVUS (= standard of care) with blood sample collection (study-specific intervention).</td></tr>
<tr><td></td><td colspan="2">Subjects who are not randomized because of progesterone ≥ 10 mcg/L are followed up for the collection of the outcome data* (identically to the randomized subjects), which is up to and including the first negative pregnancy test (D16 ± 2 days or D16 (± 2 days) + 2 days) (if not pregnant). Or up to and including the pregnancy ultrasound at W6-W8 of gestation (if pregnant), or until a diagnosis of a non-viable pregnancy is made.</td></tr>
</table>

4.7 Randomization and blinding

Subjects with a suboptimal serum progesterone level (defined as < 10 mcg/l) on the day of blastocyst transfer in an artificial prepared endometrium cycle are randomised to either Amelgen® 400 mg BID or Amelgen® 400 mg TID according to a 1:1 allocation ratio using permuted block randomization with blocks of varying size (without stratification). A static randomization list will be developed by an independent statistician and uploaded in the randomization module of a centralized computer-generated electronic randomization system, REDCap (Research Electronic Data Capture), to allow for randomisation by a member of the study team.

The study investigators will not have access to the randomization list. All eligible participants with a suboptimal serum progesterone level will be randomly allocated and will receive a treatment/randomization number. The randomization number identifies the subject for all procedures occurring after treatment allocation. Once a randomization number is assigned to a subject, it can

never be re-assigned to another subject. A single participant cannot be assigned more than one randomization number.

This is an open label study, there is no deblinding procedure necessary.

4.8 Monitoring and quality measures

4.8.1 General

Monitoring of the study will be performed in compliance with GCP E6(R2) and the applicable regulatory requirements. The study team will be trained in an initiation visit by the monitor. A detailed description of the monitoring tasks can be found in the latest version of the (study-specific) 'Monitoring plan'.

4.8.2 Monitoring team

Monitoring services will be provided by HIRUZ CTU. All relevant contact details (e.g. primary contact person) can be found in the 'Monitoring plan'.

4.8.3 Scope

Monitoring services will consist of the following (non-exhaustive list):

- Review of informed consents and the followed process
- Check on recruitment status
- Checking for protocol deviations/violations
- Checking GCP compatibility
- Check on safety reporting compliance
- IMP handling and storage
- Review of study data

4.8.4 Protocol deviation policy

Sponsor and all investigators agree to take any reasonable actions to correct protocol deviations/violations noted during monitoring/inspection, in consultation with the monitoring team. All deviations must be documented on a protocol deviation log by the study team that is kept available at any time for monitoring/inspection purposes. Under emergency circumstances, deviations from the protocol to protect the rights, safety or well-being of human subjects may proceed without prior approval of the sponsor and the EC.

4.8.5 Serious breach to GCP and/or the protocol

Critical issues that significantly affect patient safety, data integrity and/or study conduct should be clearly documented and will be communicated with the Coordinating Investigator, HIRUZ CTU and possibly both the applicable Ethics Committee(s) and Competent authority.

5. Study analysis

5.1 Sample size calculations

A total sample size of 242 patients with a suboptimal serum progesterone level (< 10 mcg/l) on the day of blastocyst transfer (121 in each arm) is needed to have at least 80% power to detect a clinically relevant difference in absolute risk for "ongoing pregnancy confirmed by TVUS between week 6 and 8 after gestation" of 15% between micronized progesterone (Amelgen®) 400 mg TID versus 400 mg BID, using a Chi-square test for proportion difference at the two-sided 5% significance level, when the proportion of ongoing pregnancies confirmed by TVUS between week 6 and 8 after gestation is 15%

with 400 mg BID (and hence 30% with 400 mg TID) and when the design is balanced (1:1 allocation ratio).This sample size calculation was performed using the software SAS Power and sample size.

5.2 Type of statistical methods described in the protocol

A binary logistic regression model will be fitted that regresses "ongoing pregnancy confirmed by TVUS between week 6 and 8 after gestation" on allocated arm, centre, age and embryo quality.

We will estimate the marginal (unconditional) relative risk as treatment effect (rather than a conditional odds ratio). A relative risk is more intuitive to interpret than an odds ratio, and it is less affected by variability in the ongoing pregnancy rate compared to a risk difference. The covariate adjusted estimation approach for estimation of a marginal treatment effect, as recommended in the U.S. Food and Drug Administration Guidance on covariate adjustment (FDA 2023), will be applied. A 95% confidence interval around the marginal relative risk will be computed using the non-parametric BCa bootstrap method (Efron and Tibshirani 1994).

5.3 Statistical team analysis team

The statistical analysis of our data will be performed by the Biostatistics Unit of the Faculty of Medicine and Health Sciences at Ghent University, Corneel Heymanslaan 10, 5K3, 9000 Ghent, Belgium. Phone: 09 332 19 61- E-mail: statcel@UGent.be

5.4 Interim analysis described within the protocol

Three interim analyses for futility for the primary objective will be performed: when 25%, 33%, and 50% of the primary endpoints have been collected. The study will stop, when the conditional power under the original design at an interim analysis is less than 50%.

These interim analyses do not effect our type I error rate, but may come with a small cost in statistical power.

5.5 Statistical analysis

A binary logistic regression model was fitted that regresses the outcomes HCG positive result, ongoing pregnancy confirmed by TVUS between Week 6 and 8 after gestation (Number of gestational sacs with fetal heart rate) and pregnancy loss on progesterone groups. In adjusted models extra covariates were included. Models were fitted including only age, embryo quality and weight next to progesterone at day of transfer. Building more complex models had no added value, prone to overfitting. In these models, next to the P4 serum level, also the group (= whether rescue strategy is applied or not: TID or BID) and an interaction term between group and P4 serum level were included, because it might be expected that the relationship between P4 serum level and the reproductive outcome is different according to whether a rescue strategy is applied or not (TID or BID).

For unadjusted and adjusted models including age, embryo quality and weight, binary logistic regression was used.

For clinical interpretation, the marginal (unconditional) relative risk (risk ratio) was estimated as treatment effect (rather than a conditional odds ratio). A risk ratio is more intuitive to interpret than an odds ratio, and it is less affected by variability in the pregnancy rate compared to a risk difference. The covariate adjusted estimation approach for estimation of a marginal treatment effect, as recommended in the U.S. Food and Drug Administration Guidance on covariate adjustment (FDA 2023), was applied. A 95% confidence interval around the marginal relative risk is computed using the non-parametric BCa bootstrap method (Efron and Tibshirani 1994).

Predicted probabilities were calculated based on all patients to obtain higher power. Marginal predictions (probabilities) per group were also reported. The R-package 'marginaleffects' is used to calculate the average counterfactual predictions and average counterfactual risk ratio's with confidence intervals.

To determine whether there is an optimal 'cut-off' value of progesterone concentration at day of ET related to a higher probability of ongoing pregnancy where it could be assumed a rescue strategy is beneficial, 2 different analyses were performed: a ROC analysis and a Generalized Additive Model (GAM).

6. Regulatory Authorities

OVERVIEW APPROVED DOCUMENTS	
Initial submission: • Protocol v1.0, dd. 2020-11-12 • ICF v2.0, dd. 2020-12-14 • SmPC Amelgen 400 mg, dd 2020-03 • IMP label, v1.0 dd. 2020-11-12 • Questionnaire Initial Pregnancy Test, v1.0 dd 2020-11-12 • Patient diary, v1.0 dd 2020-11-12 • Subject card, v1.0 dd 2020-11-12	**Approval date EC:** 2020-12-17 **Approval date FAMHP:** 2020-12-10
Amendment 1 (substantial): • Protocol v2.0, dd. 2022-02-28 • ICF v3.0, dd. 2022-02-28 • Patient diary AZ Delta, v1.0 dd 2020-11-12 • Announcement on website and in Facebook group, v1.0 dd 2022-02-28 • Addition of AZ Delta Roeselare, • Extension of study duration until 2024-01-01	**Approval date EC:** 2022-06-01 **Approval date FAMHP:** 2022-05-06
Temporary Halt • Until approval of Substantial Amendment n°2	**EC notification:** 2024-04-05 **FAMHP notification:** 2024-04-05
Amendment 2 (substantial): • Protocol, v3.0 dd. 2024-01-19 • ICF v4.0 dd. 2024-01-19 • Request for approval to use data of non-randomized patients • Extension of study duration until 2025-12-01	**Approval date EC:** 2024-06-18 **Approval date FAMHP:** 2024-03-04
Amendment 3 (substantial): • Restart of the trial	**Approval date EC:** 2024-07-10 **Approval date FAMHP:** 2024-07-18

7. Results

7.1 Subject enrollment and demographics

Subject enrollment

In total 807 approved subject were planned for recruitment, of which approximately 30 % (n=242) were forecasted to have a suboptimal serum progesterone level (< 10 mcg/l). Resulting in an estimated amount of 565 subjects that won't fulfill the requirements for randomization.

This prospective study was conducted from April 2021, with the first subject first visit on April 2th, 2021 to January 2025, with the last subject last visit on January 3th, 2025. The end of trial date was documented on January 13th, 2025 and the close-out visit was held March 11th, 2025. The study was initiated in two fertility centers; Ghent University Hospital in Belgium, this center had both the Sponsor and Site Investigator Role and AZ delta in Roeselare, who had only a Site Investigator Role. From the 362 signed informed consents in the Ghent University Hospital fertility center and the 12 signed informed consents in AZ delta in Roeselare, data of 268 women undergoing a single blastocyst transfer in one of both centers were included in the analysis. This study included a randomized controlled trial (RCT) introducing a rescue strategy with micronized vaginal progesterone (MVP) 400mg for those cycles with a serum progesterone on day of transfer < 10ng/ml involving 85 of the 268 patients. Patients were randomized to either control or intervention group (1:1 allocation). The expected accrual rate of 2 to 7 subjects per month with a frozen embryo transfer within the study was not reached. This can mainly be explained by the changes in fertility treatment strategies over the years, involving a decay in artificial vitrified/warmed single blastocyst transfers and an increase in natural transfer cycles. A temporarily arrest of the study also led in part to a lower recruitment rate. During the arrest, additional secondary (and exploratory) objectives / endpoints were added to the protocol, enabling the collection of outcome data of subjects who were not randomized because of progesterone values ≥ 10 mcg/L.

Subject withdrawal

Subject withdrawals were considered subjects who consented and participated in some study procedures, but who withdrew or were withdrawn from the study.

Subjects were free to withdraw consent from participation in the study at any time upon request. The withdrawal was clearly documented in the electronic medical records as well as the eCRF, after which no further data was collected from the subject.

Reasons for prematurely discontinue from the study interventions:

- On the subjects own request;
- When the investigator felt it was not in the best interest of the subject to continue, i.e. the occurrence of clinical adverse event (AE), laboratory abnormality, or other medical condition or situation;
- After notification of significant study intervention non-compliance
- After inclusion, if the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation, i.e. fresh embryo transfer instead of vitrified/warmed embryo transfer
- When the subject was considered lost to follow-up (i.e. failing to return for 2 scheduled visits and unable to be contacted by the study site staff).

Demographic data

Results of 268 women could be included in the analysis.

Mean maternal age was 32.8 years old, and the mean BMI was 24.3kg/m2. The most common causes for infertility were male factor or unexplained infertility. Mean AMH was 3.98ng/ml and mean antral follicle count was 21.22. Primary infertility was slightly more common (53%) than secondary infertility (47%). 37% of patients were undergoing a first IVF-attempt. Mean total dose of estradiol valerate (Progynova® 2mg TID) before initiation of MVP was 82.23mg. Mean endometrial thickness at day of initiation of MVP was 8.41mm (Table 1.).

Table 1. Baseline characteristics of patients and cycles

	All participants N = 268	RCT group		P4 >10 ng/ml N = 183	p-value[2]
		P4 <10 ng/ml BID N = 42[1]	P4 < 10 ng/ml TID N = 43[1]		
Maternal age (years)	32.79 (4.25)	32.29 (4.05)	30.81 (3.41)	33.38 (4.34)	<0.001***
Maternal length (cm)	167.76 (6.90)	168.48 (7.14)	167.67 (6.94)	167.61 (6.87)	>0.9
Maternal weight (kg)	68.42(12.70)	70.61 (14.21)	72.00 (14.65)	67.08 (11.65)	0.090
Maternal BMI (kg/m2)	24.28 (4.10)	24.85 (4.66)	25.52 (4.52)	23.85 (3.80)	0.080
Parity					0.13
0	185 (69%)	29 (69%)	24 (56%)	132 (72%)	
1	70 (26%)	13 (31.0%)	18 (42%)	39 (21%)	
2	11 (4.1%)	0 (0%)	1 (2.3%)	10 (5.5%)	
3	1 (0.4%)	0 (0%)	0 (0%)	1 (0.5%)	
5	1 (0.4%)	0 (.0%)	0 (0%)	1 (0.5%)	
Relation type					0.2
Heterosexual	240 (90%)	34 (81%)	39 (91%)	167 (91%)	
Lesbian	10 (3.7%)	4 (9.5%)	1 (2.3%)	5 (2.7%)	
Single	18 (6.7%)	4 (9.5%)	3 (7.0%)	11 (6.0%)	
Type of infertility					0.3
primary	141 (53%)	23 (55%)	18 (42%)	100 (55%)	
secondary	127 (47%)	19 (45%)	25 (58%)	83 (45%)	
Cause of infertility					
Ovulation dysfunction	42 (16%)	7 (17%)	6 (14%)	29 (16%)	>0.9
Male factor	168 (63%)	21 (50%)	28 (64%)	119 (65%)	>0.9
Tubary factor	18 (6.8%)	2 (4.8%)	2 (4.7%)	14 (7.7%)	0.7
Endometriose	16 (6.0%)	2 (4.8%)	3 (7.0%)	11 (6.0%)	0.5
Unexplained	50 (19%)	5 (12%)	7 (16%)	38 (21%)	0.4
Other	109 (40%)	20 (48%)	19 (44%)	69 (38%)	0.4
Number of IVF cycles					0.4
0	99 (37%)	16 (38%)	11 (26%)	72 (39%)	
1	114 (43%)	19 (45%)	20 (47%)	75 (41%)	
2	35 (13%)	5 (12%)	6 (14%)	24 (13%)	
3	18 (6.7%)	1 (2.4%)	6 (14%)	11 (6.0%)	
4	2 (0.7%)	1 (2.4%)	0 (0%)	1 (0.5%)	
Total dose of estradiol valerate (mg)	82.82 (18.43)	77.41 (19.57)	89.3 (22.87)	82.51 (16.58)	0.012*
Endometrial thickness at last ultrasound	8.40 (1.49)	8.45 (1.53)	7.93 (1.21)	8.50 (1.53)	0.043
E2 at last ultrasound (ng/L)	223.60 (117.61)	199.24 (82.79)	224.98 (89.74)	228.28 (129.34)	0.3
LH at last ultrasound	10.38 (7.79)	9.14 (6.68)	9.51 (7.03)	10.78 (8.18)	0.3

[1]Mean (SD); n (%), [2]Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test.

7.2 Study specific results

Study population

Consecutive women who attended the center for IVF/ICSI to undergo a single vitrified/warmed blastocyst transfer in an artificially prepared transfer cycle were screened and recruited if they fulfilled the selection criteria. The inclusion criteria were the following: women aged ≥18 years <43 years at the time of inclusion, with current pregnancy wish, those with Body Mass Index ≥18.5kg/m2 and <35kg/m2, with less than five failed previous Assisted Reproductive Technologies (ART) cycles since live birth (or in case of no live birth since start of fertility treatment). Women were excluded if they participated simultaneously in another clinical study or if they participated earlier in this study, if they had known reasons for impaired implantation (presence of hydrosalpinx, presence of a type I, II or III fibroid, Asherman's syndrome, uterine malformations, intrauterine adhesions, ≥grade 3 endometriosis according to ASRM classification, endometrial tuberculosis), repeated (>2) miscarriages, untreated and uncontrolled thyroid dysfunction, tumors of the ovary, breast, uterus, pituitary or hypothalamus, abnormal vaginal bleeding without a known/diagnosed cause, ovarian cysts or enlarged ovaries, fibroid tumors of the uterus incompatible with pregnancy, malformations of the reproductive organs incompatible with pregnancy, previous antibiotic hypersensitivity reactions streptomycin and/or neomycin), risk factors for thromboembolic events (such as a personal or family history, severe obesity or thrombophilia), use of carbamazepine, rifampicin or phenytoin. Those who were unable to comprehend the investigational nature of the proposed study were also excluded.

All women were recruited during consultation for infertility advice, diagnosis and/ or treatment at Ghent University Hospital or AZ Delta (Roeselare). Patients were counseled extensively, and informed written consents were obtained before participation. All participants voluntarily joined this study, and there was no financial compensation for them during the study period.

Endpoints

Ongoing pregnancy rate was the primary endpoint. Diagnosis of an ongoing pregnancy was made if a fetal heartbeat was documented during transvaginal ultrasound (TVUS) between Week 6 and 8 of pregnancy. Secondary endpoints were the comparison of reproductive outcome (pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate, biochemical pregnancy rate, miscarriage rate) of subjects with progesterone levels above and below the 10mcg/l threshold and the endometrial impaction as measured with TVUS on day of embryo transfer (D5) and possible impact on reproductive outcomes.

Study protocol

At enrollment, socio-demographic data, medical and reproductive history, and anthropometry were recorded. On the first or second day of menstruation or withdrawal bleeding, 6mg estradiol valerate (Progynova® 2mg) was initiated for at least 10 days, up to a maximum of 21 days. If estradiol could not be started on days 1 or 2, estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone (P4) were measured. Estradiol valerate was initiated if E2 was <80 ng/l and P4 <1.5 ng/ml. TVUS was performed 10-14 days after starting estradiol to assess endometrial quality and thickness. If endometrial thickness was ≥7mm with a triple-line appearance, MVP was started. If not, a re-evaluation was scheduled after at least 3 days. If no endometrial thickness of 7mm or triple-line endometrium was reached after up to 21 days, MVP was started. The first MVP dose was administered in the evening (22-24h), followed by 400mg twice daily until randomization (D6).

Embryo transfer occurred on day 5 of MVP, under transabdominal ultrasound guidance by a trained clinician according to the center's protocol. Serum P4 levels were measured on D5 using a validated electrochemiluminescence immunoassay (Cobas e801 Roche, ECLIA. If P4 <10 mcg/l, patients were randomized into either the intervention group (group 1A) or control group (group 1B). In the intervention group, MVP was increased to 400mg TID until the pregnancy test, while the control group

maintained the standard dose (BID). If P4 ≥10 mcg/l (group 2), MVP was continued twice daily per standard care.

A pregnancy test was performed 11 days after embryo transfer (D16 (± 2 days), with a confirmatory test 2 days later if the first test was positive. If the confirmatory pregnancy test was positive (hCG >5 U/l), MVP was continued until a TVUS between weeks 6-8 or until a non-viable pregnancy was diagnosed. If the test was negative (hCG <5 U/l), MVP was discontinued, and the study ended. All pregnant participants completed the study after the first pregnancy TVUS, planned between Week 6 and 8 of gestation.(Figure 1.)

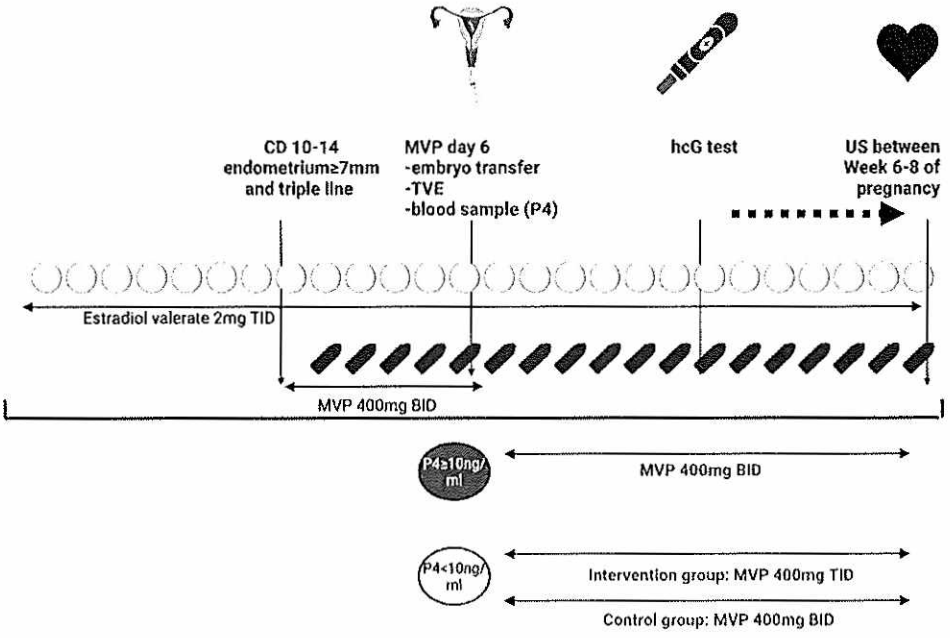

Fig 1. Study protocol

At the time of study enrollment, the subject's socio-demographics, relevant medical history, reproductive history and anthropometry were recorded. On the first or second day of menstruation or withdrawal bleeding a daily dose of 6mg estradiol valerate (Progynova® 2mg) was initiated for at least 10 days, with a maximum of 21 days. If estradiol valerate could not be started on day 1 or day 2 of menstruation of withdrawal bleeding, measurement of estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone (P4) took place and estradiol valerate could then be initiated if E2 was lower than 80ngl/l. TVUS was performed between 10 and 14 days after start of 6mg estradiol valerate administration to assess endometrial quality and thickness. If endometrial thickness was ≥7mm and aspect was triple line, micronized vaginal progesterone (MVP) was initiated. If endometrial thickness was <7mm or aspect was not triple line, a new evaluation was planned after at least 3 days. If after up to 21 days of estradiol valerate administration no endometrial thickness of 7mm or no triple line endometrium was reached, MVP was started either way. The first administration of MVP was situated in the evening (D0, between 22-24h). From day 1, MVP (Amelgen® 400 mg) was administered twice daily (between 6- 8h and 22-24h) in a dose of 400 mg until the day of randomization (D6). Embryo transfer was performed in the afternoon of day 5 of MVP administration. Just before embryo transfer procedure, endometrial thickness was measured by TVUS. Transfers were performed under transabdominal ultrasound guidance by a trained clinician according to the center's protocol. Serum P4 levels were measured using a validated electrochemiluminescence immunoassay (Cobas e801 Roche, ECLIA). If serum P4 level on day of transfer (D5) was < 10 mcg/l, patients were randomized the next day into the intervention (further referred to as group '1A') or control group (further referred to as group '1B'). Patients in the intervention group augmented the dose MVP (Amelgen® 400 mg TID) (between 6-8h, 14-16h and 22- 24h) until the day of the pregnancy test.

Patients in the control group maintained MVP (Amelgen® 400 mg BID) (between 6-8h and 22-24h) until the day of the pregnancy test. If serum P4 level on day of transfer (D5) was ≥ 10 mcg/l (further referred to as group 2), MVP (Amelgen® 400 mg) could be continued twice daily according to the standard of care.

A first pregnancy test was performed 11 days after embryo transfer (D16 (± 2 days)). In case of a positive pregnancy test on D16 a confirmative assessment of hCG and P4 was done 2 days later. If the confirmative pregnancy test was positive (hCG > 5 U/l) MVP (Amelgen® 400mg TID in the intervention group; Amelgen® 400 mg BID in the control group and for patients with P4>10mcg/l on day of transfer) was maintained until the day of performance of a TVUS between week 6 and 8 of gestation or until a diagnosis of a non-viable pregnancy was made. If the confirmative pregnancy test was negative (hCG < 5 U/l), MVP was stopped and the study ended. TVUS was planned between Week 6 and 8 of gestation. MVP was continued after the first pregnancy TVUS per standard of care. Patients could use the leftover Amelgen® (after drug accountability) or could collect it with a prescription at the local pharmacy. The study ended for all pregnant subjects after the first pregnancy TVUS (= standard of care).

Statistical analysis

A binary logistic regression model was fitted that regresses the outcomes HCG positive result, ongoing pregnancy confirmed by TVUS between Week 6 and 8 after gestation (Number of gestational sacs with fetal heart rate) and pregnancy loss on progesterone groups. In adjusted models extra covariates were included. Models were fitted including only age, embryo quality and weight next to progesterone at day of transfer. Building more complex models had no added value, prone to overfitting. In these models, next to the P4 serum level, also the group (= whether rescue strategy is applied or not: TID or BID) and an interaction term between group and P4 serum level were included, because it might be expected that the relationship between P4 serum level and the reproductive outcome is different according to whether a rescue strategy is applied or not (TID or BID). For unadjusted and adjusted models including age, embryo quality and weight, binary logistic regression was used. For clinical interpretation, the marginal (unconditional) relative risk (risk ratio) was estimated as treatment effect (rather than a conditional odds ratio). A risk ratio is more intuitive to interpret than an odds ratio, and it is less affected by variability in the pregnancy rate compared to a risk difference. The covariate adjusted estimation approach for estimation of a marginal treatment effect, as recommended in the U.S. Food and Drug Administration Guidance on covariate adjustment (FDA 2023), was applied. A 95% confidence interval around the marginal relative risk is computed using the non-parametric BCa bootstrap method (Efron and Tibshirani 1994). Predicted probabilities were calculated based on all patients to obtain higher power. Marginal predictions (probabilities) per group were also reported. The R-package 'marginaleffects' is used to calculate the average counterfactual predictions and average counterfactual risk ratio's with confidence intervals. To determine whether there is an optimal 'cut-off' value of progesterone concentration at day of ET related to a higher probability of ongoing pregnancy where it could be assumed a rescue strategy is beneficial, 2 different analyses were performed: a ROC analysis and a Generalized Additive Model (GAM).

Results: Impact of serum progesterone on reproductive outcome

Population: all patients

No significant association could be found between ongoing pregnancy and serum progesterone (P4) levels (aOR=1.00, 95% CI 0.94-1.05, p=0.899). Similarly, there was no significant association between positive HCG and P4 serum level (aOR=1.03, 95%CI 0.98-1.08, p=0.290), neither between pregnancy loss and P4 (aOR=1.03, 95%CI 0.96-1.11, p=0.389).

Maternal age was the only variable significantly associated with all reproductive outcomes.

Predicted probabilities for ongoing pregnancy were 33.7% (95% CI 0.270-0.404) for group 2 (P4 ≥10 mcg/l), 27.3% (95% CI 0.150-0.425) for patients in group 1B (P4<10mcg/l, 400mg BID) and 25.1%

(95% CI 0.136-0.391) for group 1A (P4<10mcg/l, 400mg TID). For positive HCG, the predicted probabilities were 55.1% (95% CI 0.479-0.627) for group 2, 44.3% (95% CI 0.309-0.597) for group 1B and 42.5 % (95% CI 0.275-0.566) for group 1A. For pregnancy loss, the predicted probabilities were 38.5% (95% CI 0.300-0.493) for group 2, 39.5% (95% CI 0.177-0.662) for group 1B and 41.6% (95% CI 0.213-0.684) for group 1A.

Patients with P4≥ and <10ng/ml using MPV 400mg BID

No significant difference in ongoing pregnancy rates (OPR) was observed between patients with P4 ≥10ng/ml and those with P4 <10ng/ml using MPV 400 mg BID (aOR 1.46, 95%CI 0.69-3.26; p=0.339).

No association was found between HCG and P4-group (aOR 1.65, 95%CI 0.82-3.35, p=0.162). Pregnancy loss showed no significant differences between patients with P4≥ and <10ng/ml using MPV 400mg BID (aOR 0.85, 95%CI 0.29-2.61, p=0.763).

The only variable significantly associated with all 3 outcomes, is maternal age.

ROC curve analysis was performed for reproductive outcomes. The AUC was estimated at 0.482 (Fig 1.), 0.519 and 0.548 for ongoing pregnancy, HCG positive and pregnancy loss respectively. A clear cut-off point of progesterone concentration to predict reproductive outcomes with both good sensitivity and good specificity could not be identified.

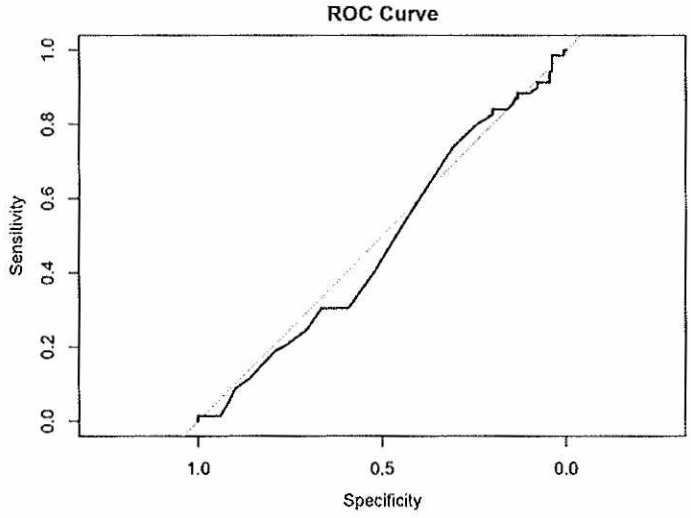

Fig 1. ROC curve analysis for reproductive outcomes

Furthermore, spline and glm were plotted on the same graph to visualize progesterone concentration versus ongoing pregnancy to explore a possible cut-off in P4 concentration.(Fig.2) The GAM smoother shows a straight line without knots or bends, indicating there is no clear cut-off in progesterone concentration at day of ET for which the probability of pregnancy suddenly changes.

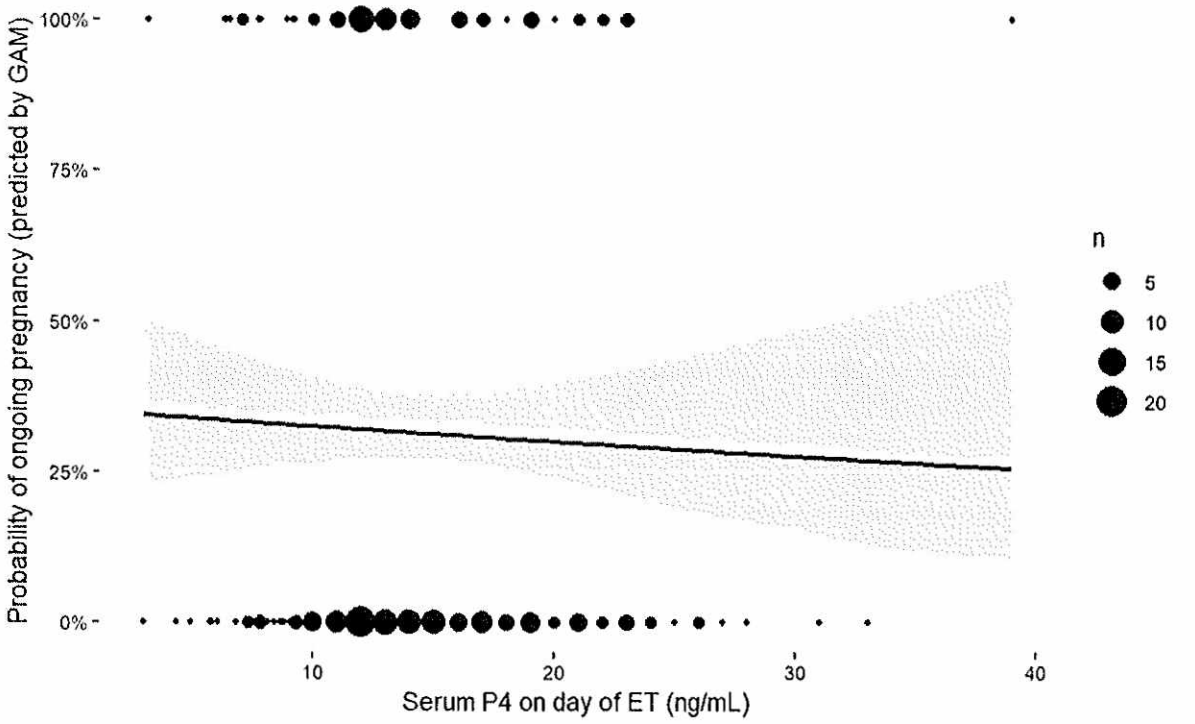

Fig 2. Generalized Additive Model (GAM) used to model the probability of ongoing pregnancy as a smooth, potentially non-linear function of progesterone concentration at day of ET for the patients without rescue strategy (BID patients). The observed outcome per patient (ongoing pregnant or not) is shown as black dots and the fitted probability curve predicted by GAM is shown as a red curve.

RCT group

Increasing the dose of MVP to TID in the intervention group did not improve OPR (aOR 0.91, 95% CI 0.34-2.42; p=0.850). HCG positive and pregnancy loss rates were also similar and did not differ between control and intervention group (aOR 0.88 95% CI 0.35-2.19; p=0.783 and aOR 0.99, 95% CI 0.24-4.03). The adjusted predicted probability of an ongoing pregnancy among patients with progesterone levels <10 ng/mL is 29.9% (95% CI: 0.162–0.468) for those receiving MVP 400 mg twice daily and 28.0% (95% CI: 0.154–0.425) for those receiving MVP 400 mg three times daily. The average treatment effect, expressed as the marginal risk ratio, is 0.94 (95% CI: 0.417–2.025). Adjusted predicted probability for positive HCG is 47.3% for patients with P4<10 using MVP 400mg BID (95% CI 0.317-0.626) and 44.3% for patients with P4<10 using MVP 400mg TID (95% CI 0.298-0.597). Marginal risk ratio is 0.94 (95% CI 0.58-1.64). For pregnancy loss, predicted probability is 37.0% for patients in the BID group (CI 0.150-0.589) and 36.7% for patients in the TID group (95% CI 0.149-0.585). Marginal risk ratio is 0.99 (0.144-1.844).

Impact of dose increase of MVP on serum P4 levels

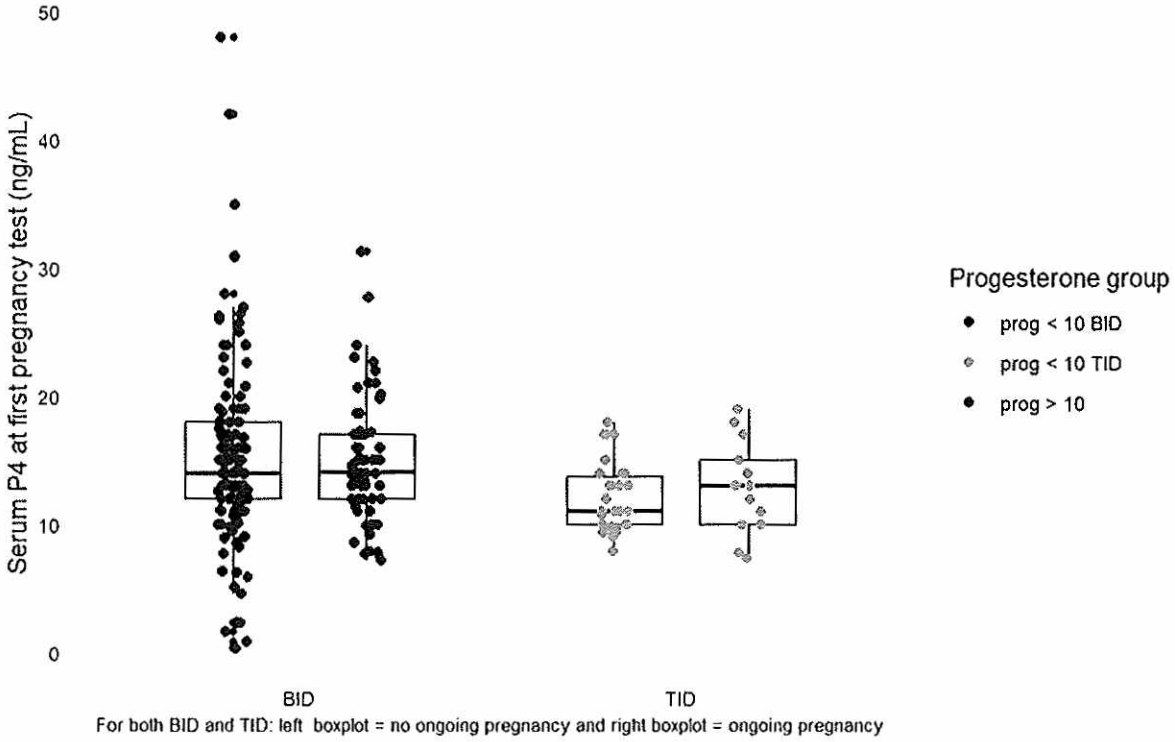

Figure 3 illustrates progesterone levels on the day of the first pregnancy test across the different groups (1A, 1B, and 2), stratified by ongoing pregnancy status.

There appears to be little difference in progesterone levels between the various groups (1A, 1B, and 2). In the BID group, a greater number of outliers (both low and high progesterone values) are observed in patients with no ongoing pregnancy.

Endometrial compaction

No correlation could be found between endometrial compaction and reproductive outcomes.

8. Safety

No SAEs have been reported during the conduct of the trial.

9. Protocol deviations

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
001	Visit Independent	Protocol deviation	1	Minor (1)	1	11-05-2021	Trial assessments (4)		Subject did not complete the QST 'Initial pregnancy test'	Protocol deviation		Complete (2)
003	Visit Independent	Protocol deviation	1	Minor (1)	1	19-12-2024	Trial assessments (4)		Subject didn't complete the survey 'Initial pregnancy test'	Protocol deviation		Complete (2)
006	Visit Independent	Protocol deviation	1	Major (2)	1	06-08-2021	Trial assessments (4)		Patient started study medication too early.	Cycle was cancelled and participation in trail was stopped.		Complete (2)
016	Visit Independent	Protocol deviation	1	Minor (1)	1	27-09-2022	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotransfer'.	Protocol deviation		Complete (2)
017	Visit Independent	Protocol deviation	1	Minor (1)	1	21-09-2021	Trial assessments (4)		Doctor decided to stop the study early in consultation with the patient and increase the medication Progynova to 8mg a day instead of 6 mg a day (protocol) due to insufficient endometrial buildup.	Protocol deviation		Complete (2)
019	Visit Independent	Protocol deviation	1	Minor (1)	1	04-11-2021	Trial assessments (4)		Doctor decided to stop the study early in consultation with the patient and increase the medication Progynova to 8mg a day instead of 6 mg a day (protocol) due to	Protocol deviation		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
									insufficient endometrial buildup.			
020	Visit Independent	Protocol deviation	1	Major (2)	1	09-09-2024	Informed Consent Form (1)		Original ICF not received yet. There isn't a possibility to obtain the original ICF. Patient is lost in follow up and lives in Kenia.	Note to file (section ICF) + Major Protocol Deviation.		Complete (2)
023	Visit Independent	Protocol deviation	1	Major (2)	1	12-12-2024	Informed Consent Form (1)		Incorrect ICF present. Patient signed ICF v2.0 on date 14/10/2021. Copy only was provided to study team. Pre-stamped envelope was sent to patient asking to send ICF v2.0 (original). Patient indicated that ICF v2.0 was lost. Patient sent original ICF v2.0 by mail, but incorrectly entered her date of birth. To date, no correct original ICF v2.0 received. Protocol deviation was made up as well.	Note to file and protocol deviation.		Complete (2)
021	Visit Independent	Protocol deviation	2	Minor (1)	2	02-11-2021	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotransfer'	Protocol deviation		Complete (2)
026	Visit Independent	Protocol deviation	1	Major (2)	1	11-02-2022	Other (5)	Subject received unlabeled study medication	Subject received unlabeled study medication	Study team was reminded to always provide study patients with labeled study medication.		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
031	Visit independent	Protocol deviation	1	Minor (1)	1	02-06-2022	Trial assessments (4)		Survey questionary was not send to patient after transfer	Protocol deviation was made		Complete (2)
034	Visit independent	Protocol deviation	1	Major (2)	1	28-04-2022	Trial assessments (4)		Subject used Utrogestan instead of Ameigen. Because she had left the boxes of Ameigen in the sun and therefore she replaced it by new medication (Utrogestan).	Despite the fact that the subject changed luteal support to non-IMP, she was followed up in the study because she was randomized		Complete (2)
034	Visit independent	Protocol deviation	2	Minor (1)	2	29-09-2023	Trial assessments (4)		Some pages of the patient diary are missing (TVUS on 2022-05-18 while intake of Ameigen was only recorded until 2022-04-29), and the study team is not able to obtain the missing pages anymore*	Protocol deviation Source idea		Complete (2)
034	Visit independent	Protocol deviation	3	Major (2)	3	08-04-2022	Eligibility (3)		It was only noticed after the subject has completed the trial, that she was not eligible after all and was therefore not allowed to be included. The subject seemed to be a screen failure as the dose of estrogen has been elevated during the endometrium preparation phase.	Documented as Major PD. Statistic ans will be informed.		Complete (2)
034	Visit independent	Protocol deviation	4	Minor (1)	4	08-04-2022	Trial assessments (4)		Physician decided to deviate from the protocol and elevate the estrogen dose	Minor PD made. No timely actions could be undertaken as this deviation		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
									during the preparation phase, for which the subject became ineligible to be included in the trial.	was only identified after the subject completed the trial.		
040	Visit independent	Protocol deviation	1	Major (2)	1	11-04-2022	Trial assessments (4)		misuse of medication. Hs started 9/4/22 with 8mg Progynova orally abd Ameigen 400mg vaginally instead of 2mg Progynova vaginally.	Cancel cycle 11/4/22.		Complete (2)
045	Visit independent	Protocol deviation	1	Minor (1)	1	29-04-2022	Trial assessments (4)		Physician decided to deviate from the protocol and elevate the estrogen dose during the preparation phase, for which the subject became ineligible to be included in the trial.	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject completed the trial.		Complete (2)
048	Visit independent	Protocol deviation	1	Minor (1)	1	12-07-2022	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
049	Visit independent	Protocol deviation	1	Minor (1)	1	21-04-2022	Trial assessments (4)		Physician decided to deviate from the protocol and elevate the estrogen dose during the preparation phase, for which the subject became ineligible to be included in the trial.	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject completed the trial.		Complete (2)
049	Visit independent	Protocol deviation	2	Minor (1)	2	18-05-2022	Trial assessments (4)		The subject became ineligible during the preparation phase to be included in the trial. The questionnaire was incorrectly sent to the	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject		Complete (2)

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									patient and was as well as completed by the patient.	completed the trial.		
051	Visit Independent	Protocol deviation	1	Minor (1)	1	29-05-2022	Trial assessments (4)		Decter decided to cancel cycle because of endometrium 4.5 mm on day 22 of cycle. Ameigen was not started. Transfer was not performed.	protocol deviation		Complete (2)
053	Visit Independent	Protocol deviation	1	Minor (1)	1	20-05-2022	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
057	Visit Independent	Protocol deviation	1	Minor (1)	1	25-07-2022	Trial assessments (4)		Patient performed urine test on day 18 after transfer instead of blood collection on day 16 after transfer.	Protocol deviation		Complete (2)
057	Visit Independent	Protocol deviation	2	Minor (1)	2	02-08-2022	Trial assessments (4)		patient did a blood test day 26 after transfer instead of day 16 after transfer. A second blood test was also not performed 2 days after the first (D28).	Protocol deviation		Complete (2)
062	Visit Independent	Protocol deviation	1								no deviation	Complete (2)
062	Visit Independent	Protocol deviation	2	Minor (1)	2	11-07-2022	Trial assessments (4)		Subject performed the first pregnancy blood test day 27 after transfer instead of day 16 after transfer. Furthermore there wasn't performed a second blood test 2 days after the first blood test.	Protocol deviation.		Complete (2)
064	Visit Independent	Protocol deviation	1	Minor (1)	1	02-11-2022	Trial assessments (4)		Pregnancy ultrasound 13w	Protocol deviation		Complete (2)

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									instead of 8weeks			
068	Visit Independent	Protocol deviation	1	Minor (1)	1	25-07-2023	Trial assessments (4)		no blood test performed	prot deviation		Complete (2)
069	Visit Independent	Protocol deviation	1	Minor (1)	1	24-08-2022	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
072	Visit Independent	Protocol deviation	1	Minor (1)	1	29-05-2022	Trial assessments (4)		The daily dose of estrogens were increased to 8mg per day orally instead of 6 mg orally as the protocol specifies.	Protocol deviation		Complete (2)
076	Visit Independent	Protocol deviation	1	Minor (1)	1	02-08-2022	Trial assessments (4)		Subject didn't complete the survey 'Survey after first pregnancy test'.	Protocol deviation.		Complete (2)
090	Visit Independent	Protocol deviation	1	Minor (1)	1	31-08-2022	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
101	Visit Independent	Protocol deviation	1	Minor (1)	1	24-10-2022	Trial assessments (4)		repeat of HCG after 3 days instead of 2 because of sunday day 2 after first HCG	protocol deviation		Complete (2)
116	Visit Independent	Protocol deviation	1	Major (2)	1	17-10-2022	Informed Consent Form (1)		Copy ICF present. Original ICF can no longer be obtained as patient became spontaneously pregnant during screening period.	Major PD made. We tried to contact patient by phone several times.		Complete (2)
122	Visit Independent	Protocol deviation	1	Minor (1)	1	20-01-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
122	Visit Independent	Protocol deviation	2	Minor (1)	2	27-01-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
124	Visit independent	Protocol deviation	1	Minor (1)	1	09-12-2022	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
126	Visit independent	Protocol deviation	1	Minor (1)	1	20-03-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
126	Visit independent	Protocol deviation	2	Minor (1)	2	31-03-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
133	Visit independent	Protocol deviation	1	Minor (1)	1	22-12-2022	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
137	Visit independent	Protocol deviation	1	Minor (1)	1	01-01-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
142	Visit independent	Protocol deviation	1	Minor (1)	1	02-02-2023	Trial assessments (4)		Subject didn't complete the survey 'Survey after first pregnancy test.'	Protocol deviation.		Complete (2)
146	Visit independent	Protocol deviation	1	Minor (1)	1	19-01-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
148	Visit independent	Protocol deviation	1	Minor (1)	1	09-03-2023	Trial assessments (4)		First pregnancy ultrasound happened before 6 weeks.	protocol deviation		Complete (2)
148	Visit independent	Protocol deviation	2	Major (2)	2	09-03-2023	Trial assessments (4)		amelgon was increased from 2 tablets to 3 a day	Protocol deviation		Complete (2)
148	Visit independent	Protocol deviation	3	Minor (1)	3	07-02-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
152	Visit independent	Protocol deviation	1	Minor (1)	1	02-02-2023	Trial assessments (4)		Subject didn't complete the survey 'Survey after first pregnancy test.'	Protocol deviation.		Complete (2)

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Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
155	Visit independent	Protocol deviation	1	Minor (1)	1	31-01-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
158	Visit independent	Protocol deviation	1	Minor (1)	1	10-02-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
161	Visit independent	Protocol deviation	1	Minor (1)	1	03-03-2023	Trial assessments (4)		The embryotransfer took place earlier then described per protocol, because of the higher progesterone levels that were seen on 03MAR2023. Based on the blood results of 28/2/2023 and 3/3/2023 the doctors decided that the LH-peak took place on 1/3/23 and that the thaw and transfer can be sheduled on tuesday 7/3/2023.	Protocol deviation		Complete (2)
161	Visit independent	Protocol deviation	2	Minor (1)	2	07-03-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
163	Visit independent	Protocol deviation	1	Minor (1)	1	19-05-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
165	Visit independent	Protocol deviation	1	Minor (1)	1	08-02-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
170	Visit independent	Protocol deviation	1	Minor (1)	1	23-03-2023	Informed Consent Form (1)		ICF later signed by physician because of forgetting. Note by physician was added and signed on ICF.	Protocol deviation		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
171	Visit independent	Protocol deviation	1	Minor (1)	1	30-06-2023	Informed Consent Form (1)		ICF signed at later date by docter	Protocol deviation		Complete (2)
172	Visit independent	Protocol deviation	1	Major (2)	1	03-04-2023	Informed Consent Form (1)		Original ICF received on 23/03/2023. Doctor signed at a later date 03/04/2023. Note post it: original ICF received on 23/03/2023. Signed at later date due to forgetfulness. Patient received info on 05/01/2023	Protocol deviation		Complete (2)
182	Visit independent	Protocol deviation	1	Minor (1)	1	13-05-2023	Trial assessments (4)		Study-specific blood sampling was not performed on day pregnancy ultrasound.	Protocol deviation		Complete (2)
193	Visit independent	Protocol deviation	1	Minor (1)	1	27-04-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
200	Visit independent	Protocol deviation	1	Minor (1)	1	29-06-2023	Trial assessments (4)		Study-specific blood sampling HCG and progesterone on day of first pregnancy ultrasound was not performed. However, HCG and progesterone blood sampling was performed at the phys'cian's request within the routine and at the patient's expense.	Protocol deviation.		Complete (2)

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Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
202	Visit independent	Protocol deviation	1	Minor (1)	1	19-06-2023	Trial assessments (4)		Determination of hCG for confirmative pregnancy test was performed 3 days after first pregnancy test instead of (D16+) 2 days	Protocol deviation		Complete (2)
205	Visit independent	Protocol deviation	1	Minor (1)	1	09-08-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
214	Visit independent	Protocol deviation	1	Minor (1)	1	18-07-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
216	Visit independent	Protocol deviation	1	Minor (1)	1	09-10-2023	Trial assessments (4)		Study-specific blood sampling HCG and progesterone on day of first pregnancy ultrasound was not performed. However, HCG and progesterone blood sampling was performed at the physician's request within the routine and at the patient's expense.	Protocol deviation.		Complete (2)
218	Visit independent	Protocol deviation	1	Major (2)	1	31-07-2023	Trial assessments (4)		Dr. Spileers signed at a later time than the patient due to forgetfulness. Dr. Spileers is no longer employed in the fertility department so she is not in a position to sign and date off the note on the ICF that the patient received information regarding the study on date 08/06/2023.	Major PD made.		Complete (2)

Record ID (record_id)	Event Name (redcap_event_name)	Repeat Instrument (redcap_repeat_instrument)	Repeat Instance (redcap_repeat_instance)	Classification of protocol deviation*: (protocol_deviation_class)	Number of protocol deviation: (protocol_deviation_number)	Date of protocol deviation: (protocol_deviation_date)	Protocol deviation concerning: (prot_deviation_type)	Define other: (protocol_deviation_other)	Description of deviation: (prot_deviation_description)	Action taken: (protocol_deviation_action)	Comments: (prot_dev_comments)	Complete? (protocol_deviation_complete)
219	Visit independent	Protocol deviation	1	Minor (1)	1	22-09-2023	Trial assessments (4)		Study-specific blood sampling HCG and progesterone on day of first pregnancy ultrasound was not performed. However, HCG and progesterone blood sampling was performed at the physician's request within the routine and at the patient's expense.	Protocol deviation.		Complete (2)
220	Visit independent	Protocol deviation	1	Minor (1)	1	09-08-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
224	Visit independent	Protocol deviation	1	Minor (1)	1	16-08-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
234	Visit independent	Protocol deviation	1	Minor (1)	1	17-10-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
234	Visit independent	Protocol deviation	2	Minor (1)	2	27-10-2023	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
245	Visit independent	Protocol deviation	1	Minor (1)	1	17-10-2023	Eligibility (3)		patient diary not completed between 2023-10-17 and 2023-10-24	Protocol deviation		Complete (2)
245	Visit independent	Protocol deviation	2	Minor (1)	2	30-10-2023	Trial assessments (4)		patient diary not completed between as from 2023-10-30 until 2023-11-15	Protocol deviation.		Complete (2)
247	Visit independent	Protocol deviation	1								None	Complete (2)
247	Visit independent	Protocol deviation	2	Minor (1)	1	23-10-2023	Trial assessments (4)		Subject didn't complete the survey 'after	Protocol deviation		Complete (2)

Record ID (record_id)	Event Name (redcap_event_name)	Repeat Instrument (redcap_repeat_instrument)	Repeat Instance (redcap_repeat_instance)	Classification of protocol deviation*: (protocol_deviation_class)	Number of protocol deviation: (protocol_deviation_number)	Date of protocol deviation: (protocol_deviation_date)	Protocol deviation concerning: (prot_deviation_type)	Define other: (protocol_deviation_other)	Description of deviation: (prot_deviation_description)	Action taken: (protocol_deviation_action)	Comments: (prot_dev_comments)	Complete? (protocol_deviation_complete)
									frozen embryotranfer'.			
252	Visit independent	Protocol deviation	1	Minor (1)	1	06-12-2023	Other (5)	miscarriage	Pte. came for checkup earlier because she had blood loss and abdominal pain	echo not confrom term - hCG insufficiently advanced, stop medication - herco echo given		Complete (2)
253	Visit independent	Protocol deviation	1	Minor (1)	1	08-12-2023	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.			Complete (2)
252	Visit independent	Protocol deviation	1	Major (2)	1	20-12-2023	Trial assessments (4)		Dr. Spiteers signed later than the patient due to forgetfulness. Dr. Spiteers is no longer employed in the fertility department so she is not in a position to write a note on the ICF that the patient received information regarding the study on date 20/09/2023 with accompanying initial and date of writing the note.	Major PD made.		Complete (2)
258	Visit independent	Protocol deviation	1	Major (2)	1	26-12-2023	Trial assessments (4)		Dr. Spiteers signed later than da patient because of original ICF received at later date. Dr. Spiteers is no longer employed in the fertility department so she is not in a position to write a note on the ICF that the patient received information regarding the study on date 08/11/2023 with accompanying	Major PD made.		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
									Initial and date of writing the note.			
269	Visit independent	Protocol deviation	1	Minor (1)	1	11-17-2023	Trial assessments (4)		Doctor forgot to do a transvaginal ultrasound to determine endometrial thickness.	Protocol Deviation.		Complete (2)
272	Visit independent	Protocol deviation	1	Major (2)	1	05-12-2023	Informed Consent Form (1)		Dr. Spiteers signed later than the patient due to forgetfulness. Dr. Spiteers is no longer employed in the fertility department so she is not in a position to write a note with the ICF that the patient received information regarding the study on date 08/11/2023 with accompanying initials and date of writing of the note.	Major PD made.		Complete (2)
274	Visit independent	Protocol deviation	1	Major (2)	1	08-04-2022	Informed Consent Form (1)		Patient was included in the study (study number assigned). Original ICF is missing. Only part of patient is present, however electronically signed and no boxes checked. No safety risk as patient not yet started within the study. EOT prepared in consultation with patient. Patient will start in a cryo cycle after the end of the study. All data removed from e-CRF.	Protocol deviation		Complete (2)
275	Visit independent	Protocol deviation	1	Minor (1)	1	22-01-2024	Trial assessments (4)		Patient diary has been completed from 2024-01-	Protocol deviation.		Complete (2)

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									22 on, while the subject started her IMP intake on 2024-01-18.			
276	Visit independent	Protocol deviation	1	Minor (1)	1	18-03-2024	Trial assessments (4)		Subject informed us by email on 18/03/2024 that the blood result HCG was negative, however the specific blood results weren't attached in the email	Results were requested from patient on 19/03/2024 and 27/03/204. However, no response.		Complete (2)
284	Visit independent	Protocol deviation	1	Major (2)	1	05-02-2024	Trial assessments (4)		Dr. Stevens signed at a later time than the patient due to forgetfulness. Dr. Stevens is no longer employed in the fertility department so she is not in a position to write a note on the ICF that the patient received information regarding the study on date 11/12/2023 with accompanying initials and date.	Major PD made.		Complete (2)
298	Visit independent	Protocol deviation	1	Minor (1)	1	14-02-2024	Trial assessments (4)		The survey 'after frozen embryotranfer' was incompletely completed by the subject.	Protocol deviation.		Complete (2)
288	Visit independent	Protocol deviation	2	Minor (1)	2	27-02-2024	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
296	Visit independent	Protocol deviation	1	Minor (1)	1	03-03-2024	Trial assessments (4)		urinary pregnancy test was performed instead of blood sampling	protocol deviation		Complete (2)
286	Visit independent	Protocol deviation	2	Minor (1)	2	21-02-2024	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
297	Visit independent	Protocol deviation	1	Minor (1)	1	30-05-2024	Trial assessments (4)		The patient lost 1 box of the Amelgen. As a result, empty package not received.	Protocol deviation		Complete (2)
293	Visit independent	Protocol deviation	1	Minor (1)	1	12-03-2024	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
302	Visit independent	Protocol deviation	1	Major (2)	1	15-04-2024	Trial assessments (4)		No blood sampling pregesterone performed on day transfer as described in protocol.	Protocol deviation and drop out.		Complete (2)
303	Visit independent	Protocol deviation	1	Minor (1)	1	09-04-2024	Trial assessments (4)		The physician decided to deviate from the protocol and cancel the cycle after cycle day 21 when the endometrium was <7 mm, making the subject ineligible to participate in the study.	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject completed the trial.		Complete (2)
304	Visit independent	Protocol deviation	2	Major (2)	2	06-05-2024	Informed Consent Form (1)		Dr. Stevens signed later than the patient due to forgetfulness. Dr. Stevens is no longer employed in the fertility department so she is not in a position to write a note on the ICF that the patient received the information regarding the study on date 08/03/2024 with accompanying initials and date.	Major PD made.		Complete (2)
310	Visit independent	Protocol deviation	1	Major (2)	1	25-07-2024	Trial assessments (4)		No study specific blood collection HCG and Progesteron was performed on the day of the pregnancy	Protocol Deviation	None.	Complete (2)

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Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
									ultrasound (w6-8 pregnancy) dice to; forgotten by midwife.			
311	Visit independent	Protocol deviation	1	Minor (1)	1	03-07-2024	Other (5)	cancel cycle	Patient was diagnosed with endometritis requiring initiation of antibiotic treatment before proceeding for embryo transfer.	Treatment for transfer cancelled and further follow-up when antibiotic treatment completed.		Complete (2)
311	Visit independent	Protocol deviation	2	Minor (1)	2	26-08-2024	Trial assessments (4)		Second hcg, prog analyse out of window. Performed 3 days after first blood test instead of 2 days.	Protocol deviation		Complete (2)
313	Visit independent	Protocol deviation	1	Minor (1)	1	26-08-2024	Trial assessments (4)		Second hcg, prog analyse out of window. Performed 3 days after first blood test instead of 2 days.	Protocol deviation.		Complete (2)
313	Visit independent	Protocol deviation	2	Minor (1)	2	13-09-2024	Trial assessments (4)		Pregnancy ultrasound not performed in UZ Gent. Hcg blood analyse was not performed because of this.	Protocol deviation		Complete (2)
315	Visit independent	Protocol deviation	1	Major (2)	1	17-09-2024	Informed Consent Form (1)		ICF was signed by physician at a later date. The study had already ended for subject at this time.	Protocol deviation Physician signed the ICF with a note why she signed at a later date and when (date) the ICF was explained to the subject.		Complete (2)
315	Visit independent	Protocol deviation	2	Minor (1)	2	20-08-2024	Trial assessments (4)		Second questionnaire regarding comfort and side effects Amelgen was not completed by patient.	Protocol deviation		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
315	Visit independent	Protocol deviation	3	Minor (1)	3	10-12-2024	Trial assessments (4)		Patient lost diary. Original is not present.	Protocol deviation		Complete (2)
318	Visit independent	Protocol deviation	1	Minor (1)	1	06-09-2024	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
324	Visit independent	Protocol deviation	1	Minor (1)	1	24-09-2024	Trial assessments (4)		Patient not seen on day of transfer. No vaginal ultrasound performed. No check of diary notes. Study midwife clearly noted that patient was participating in the study. Not noticed by doctor and midwife who performed the transfer.	Protocol deviation.		Complete (2)
326	Visit independent	Protocol deviation	1	Minor (1)	1	01-10-2024	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
327	Visit independent	Protocol deviation	1	Minor (1)	1	30-09-2024	Trial assessments (4)		Ameligen was started on D19 of cycle with an endometrium of 6mm	prot.dev.		Complete (2)
332	Visit independent	Protocol deviation	1	Minor (1)	1	22-11-2024	Trial assessments (4)		Physician decided to deviate from the protocol and elevate the estrogen dose during the preparation phase, for which the subject became ineligible to be included in the trial.	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject completed the trial.		Complete (2)
337	Visit independent	Protocol deviation	1	Minor (1)	1	25-10-2024	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
339	Visit independent	Protocol deviation	1	Minor (1)	1	21-10-2024	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
339	Visit independent	Protocol deviation	2	Minor (1)	2	31-10-2024	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
342	Visit independent	Protocol deviation	1	Minor (1)	1	22-11-2024	Trial assessments (4)		The physitian decided to deviate from the protocol and cancel the cycle after cycle day 21 when the endometrium was <7 mm, making the subject ineligible to participate in the study.	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject completed the trial.		Complete (2)
347	Visit independent	Protocol deviation	1	Minor (1)	1	31-10-2024	Trial assessments (4)		The doctor decided to deviate from the protocol and cancel the cycle after cycle day 15 because of thin endometrium. Which rendered the subject ineligible to participate in the study.	Minor PD made. No timely actions could be undertaken as this deviation was only identified after the subject completed the trial.		Complete (2)
354	Visit independent	Protocol deviation	1	Minor (1)	1	03-12-2024	Trial assessments (4)		Subject didn't complete the survey 'after frozen embryotranfer'.	Protocol deviation.		Complete (2)
354	Visit independent	Protocol deviation	2	Minor (1)	2	13-12-2024	Trial assessments (4)		Subject didn't complete the survey 'initial pregnancy test'.	Protocol deviation.		Complete (2)
360	Visit independent	Protocol deviation	1	Minor (1)	1	17-01-2025	Eligibility (3)		Patient was not assigned a study number and was also not included in the REDCAP database. After signing the ICF on 2022-05-13, this patient was found to be a	Reported to CRA Marjon. See note to file for action taken.		Complete (2)

Record ID record_id	Event Name redcap_event_name	Repeat Instrument redcap_repeat_instrument	Repeat Instance redcap_repeat_instance	Classification of protocol deviation*: protocol_deviation_class	Number of protocol deviation: protocol_deviation_number	Date of protocol deviation: protocol_deviation_date	Protocol deviation concerning: prot_deviation_type	Define other: protocol_deviation_other	Description of deviation: prot_deviation_description	Action taken: protocol_deviation_action	Comments: prot_dev_comments	Complete? protocol_deviation_complete
									screen failure and she was therefore not included (discovered on 2025-01-17).			
361	Visit independent	Protocol deviation	1	Minor (1)	1	20-01-2025	Eligibility (3)		Patient was not assigned a study number and was also not included in the REDCAP database. After signing the ICF on 2022-04-11, this patient was found to be a screen failure and she was therefore not included (discovered on 2025-01-20).	Reported to CRA Marjon. See Note to file for action taken (ISF section 2. Subject enrolment)		Complete (2)
362	Visit independent	Protocol deviation	1	Minor (1)	1	21-01-2025	Eligibility (3)		Patient was not assigned a study number and was also not included in the REDCAP database. After signing the ICF on 2022-03-17, this patient was found to be a screen failure and she was therefore not included (discovered on 2025-01-21).	Reported to CRA Marjon. See Note to file for action taken (ISF section 2. Subject enrolment)		Complete (2)

31/1/25

10. Completion of the study

This prospective study was completed January 13th, 2025 and the close-out visit was held March 11th, 2025. The last ICF signed was on December 6th, 2024 while the last subject last visit was recorded on January 3th, 2025.

The study was initiated in two fertility centers; Ghent University Hospital in Belgium, this center had both the Sponsor and Site Investigator Role and AZ delta in Roeselare, who had only a Site Investigator Role. From the 362 signed informed consents in the Ghent University Hospital fertility center and the 12 signed informed consents in AZ delta in Roeselare, data of 268 women undergoing a single blastocyst transfer in one of both centers were included in the analysis. This study included a randomized controlled trial (RCT) introducing a rescue strategy with micronized vaginal progesterone (MVP) 400mg for those cycles with a serum progesterone on day of transfer < 10ng/ml involving 85 of the 268 patients. Patients were randomized to either control or intervention group (1:1 allocation).

The expected number of subjects, i.e. 807 subjects of which approximately 30% (n = 242) having a suboptimal progesterone level (< 10 mcg/l) was not reached. This can mainly be explained by the changes in fertility treatment strategies over the years, involving a decay in artificial vitrified/warmed single blastocyst transfers and an increase in natural transfer cycles. A temporarily arrest of the study also led in part to a lower recruitment rate. During the arrest, additional secondary (and exploratory) objectives/endpoints were added to the protocol, enabling the collection of outcome data of subjects who were not randomized because of progesterone values ≥ 10 mcg/L.

11. Discussion and overall conclusions

This prospective study evaluated the impact of serum progesterone concentrations on the day of ET and the effect of luteal phase progesterone dose augmentation on OPR in artificially prepared FET cycles. Contrary to several previous reports suggesting a negative impact of serum progesterone levels below 10 ng/ml on pregnancy outcomes (Alsbjerg et al., 2018; Labarta et al., 2017; Melo et al., 2021), our results did not demonstrate a statistically significant association between serum progesterone levels at ET, whether below or above the 10 ng/ml threshold, and ongoing pregnancy rates. Furthermore, increasing the dose of micronized vaginal progesterone (MVP) from 800 mg/day (BID) to 1,200 mg/day (TID) in patients with low serum progesterone did not confer a benefit in reproductive outcomes.

The lack of a clear relationship between serum progesterone levels and pregnancy outcomes in our cohort may reflect multiple factors. First, the well-controlled design, with prospective randomization and strict inclusion criteria, reduces confounding and potential biases inherent to retrospective studies that previously reported cut-off thresholds ranging from 7.8 to 10 ng/ml (Labarta et al., 2017; Melo et al., 2021).

Second, the absence of improved outcomes following progesterone dose augmentation suggests that simply increasing vaginal progesterone dosage may not adequately compensate for low serum progesterone levels in some patients. This challenges the rationale for routine dose escalation as a rescue strategy in artificial FET cycles and underscores the complexity of luteal phase support. The potential mechanisms behind low serum progesterone despite adequate dosing, such as variable vaginal absorption, metabolic clearance, or endometrial progesterone receptor sensitivity, warrant further investigation.

Additionally, maternal age emerged as the only significant predictor of reproductive outcomes in adjusted analyses, consistent with established literature emphasizing its critical role in embryo implantation and pregnancy maintenance. Embryo quality and patient weight, though included as covariates, did not show significant associations, potentially due to limited sample size or homogeneity in embryo grading and patient BMI.

Interestingly, no correlation was observed between endometrial compaction, defined as the relative change in endometrial thickness between the end of the estrogen phase and day of ET, and pregnancy outcomes. This contrasts with earlier retrospective findings by Haas et al. (2019), who reported an inverse correlation between endometrial compaction and ongoing pregnancy rates. The discrepancy might be attributed to differences in study design, patient populations, or timing of ultrasound assessments.

Our study has several strengths, including the prospective randomized design addressing a clinically relevant question, standardized progesterone administration protocols, and rigorous hormonal and ultrasonographic monitoring. However, limitations should be acknowledged. One limitation of this study is that the final sample size was smaller than initially calculated due to slower-than-expected recruitment over the study period. Although the inclusion of 270 patients provided valuable insights, the reduced sample size may have limited the statistical power to detect smaller differences between groups, particularly in the randomized controlled trial subgroup. Future studies with larger cohorts are warranted to confirm these findings and further evaluate the impact of progesterone supplementation strategies on reproductive outcomes. Additionally, serum progesterone was measured at a single time point on the day of ET; serial measurements might better characterize progesterone dynamics and endometrial receptivity. Moreover, the study was limited to micronized vaginal progesterone; alternative routes such as intramuscular or subcutaneous administration were not evaluated and could yield different results.

In conclusion, our findings suggest that serum progesterone concentrations on the day of ET, within the ranges studied, are not a decisive predictor of ongoing pregnancy in artificially prepared FET cycles supported with vaginal micronized progesterone. Moreover, increasing MVP dosing in patients with low serum progesterone did not improve pregnancy outcomes, questioning the routine use of such rescue strategies. Given the absence of significant differences between progesterone subgroups and the neutral effect of dose escalation in our randomized controlled cohort, we believe this trial provides important preliminary evidence suggesting that it remains ethically justifiable to conduct a larger randomized controlled trial. Such a study would be essential to conclusively determine the clinical

efficacy and necessity of rescue strategies for patients with suboptimal serum progesterone concentrations on the day of embryo transfer. Future research should focus on identifying patient-specific factors influencing progesterone absorption and endometrial receptivity, as well as exploring alternative supplementation routes or protocols. Randomized controlled trials with larger cohorts and stratification by progesterone delivery methods will be crucial to optimize luteal phase support and improve ART outcomes.

12. References

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