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Vaginal Progesterone as Luteal Phase Support in Natural Cycle Frozen-Thawed Embryo Transfer (ProFET): a study protocol for a multi-center open-label randomized controlled trial

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ABSTRACT

Introduction

Vaginal progesterone supplementation is frequently given to patients receiving frozen embryo transfer (FET) in the natural cycle aiming to increase the chance of pregnancy and live birth. To date only a few studies have investigated if progesterone supplementation is beneficial in these cycles and the level of evidence for progesterone supplementation is very low.

Methods and analysis

The ProFET trial is a multicenter, open-label, randomized controlled trial powered for this investigation, including 1800 women with regular menstrual cycles (24-35 days), aged 18-43 years planned for natural cycle frozen embryo transfer (NC-FET) receiving a single blastocyst for transfer. Participants are randomized (1:1:1) to either luteal phase progesterone for 3 weeks, luteal phase progesterone for 7 weeks or no luteal phase progesterone.

Ethics and dissemination

The trial was approved by the Swedish Ethical Review Authority (ID 2020-06774 and 2021-02822) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613). The outcome of this study will be publicly disseminated.

Trial registration number

ClinicalTrials.gov (NCT047258649) and EudraCT (2020-005552-38).

Strengths and limitations of the trial

- The trial has a randomized design, powered to evaluate if luteal support with vaginal progesterone will improve live birth rate in NC-FETs when a single blastocyst is transferred.
- The trial is conducted in women planning FET in natural cycles without exogenous ovulation trigger.
- If overall superiority of progesterone is demonstrated, the sample size will allow evaluation if treatment duration of 7 weeks is superior to 3 weeks.
- The broad inclusion criteria of women with regular menstrual cycles empower high generalizability of the results.

- The study is open-label, neither blinded to participants, nor to treating physicians, which is a limitation, however less likely to introduce bias due to the robust primary outcome; live birth.

INTRODUCTION

In recent years, there has been a dramatic increase in the use of frozen-thawed embryo transfers (FET) cycles in in-vitro fertilization (IVF) all over the world. The FET rate in the United States has doubled since 2015, accounting for 78.8 % of all embryo transfers using non-donor Assisted Reproductive Technology (ART) in 2019(1). Similar changes are taking place in Europe(2) and in Sweden where the FET rate now accounts for 48 % of all IVF-cycles(3). The main reason for this increase is the improved embryo survival and high pregnancy/live birth rates (LBRs) after transfer of vitrified/thawed blastocysts compared to the previously used technique with transfer of thawed slow-frozen cleavage stage embryos(4, 5). Furthermore, high embryo survival rate facilitates the practice of single embryo transfer(6), reducing multiple pregnancy rate and thereby decreasing the risk of adverse perinatal outcomes.

The freeze-all concept, including Gonadotropin Releasing Hormone (GnRH) agonist given to induce oocyte maturation, has substantially changed treatment strategies in ART. Considering efficacy aspects, five large randomized controlled trials (RCTs) have investigated the differences in LBR following fresh embryo transfer (ET) and FET in freeze-all cycles. In 2016, a large RCT including only anovulatory patients, showed a significantly higher LBR and a lower risk of Ovarian Hyperstimulation Syndrome (OHSS) in the freeze–all group compared with fresh embryo transfers(7). However, in patients with regular menstrual cycles, most trials showed no difference in ongoing pregnancy rate or LBR in the freeze–all group compared with fresh embryo transfers (8-10), while one RCT resulted in a higher LBR with frozen embryo transfers(11). The freeze-all concept is also now widely used when pending risk of OHSS, and has almost the risk of eliminated OHSS, a potentially life-threatening condition(12-14).

The most efficient protocol for FET is still not known. A Cochrane review, including 18 RCTs, comparing different cycle regimens for FET, comprising a total of 3815 women did

not support one treatment modality over another when investigating LBR, however, with low certainty of evidence(15).

Safety aspects in ART are of great importance in treatment decision. Recently, interest has risen concerning the role of the corpus luteum (CL) in frozen cycles and studies evaluating the risks of altered vascular adaptation associated with pregnancies following FET according to the presence or absence of CL have been published(16). Corpus luteum is known to produce estrogen and progesterone, but also relaxin, a hormone that regulates the maternal cardiovascular and renal systems and hence mediates the hemodynamic changes occurring during pregnancy. In a prospective cohort study including almost 700 women, programmed cycles (artificial cycles using estrogen and progesterone for endometrial preparation) in FET with no CL present were associated with an almost three-fold increased risk of preeclampsia compared with modified natural cycles (natural cycles triggered by human chorionic gonadotrophin (hCG), for ovulation) with one CL present(16). Furthermore, in a recent Swedish large registry study, including almost 10 000 pregnancies/deliveries after FET, doubled rates of both hypertensive disorders of pregnancy and postpartum hemorrhage were found in programmed cycles compared to natural cycles(17). These studies thus support FET in natural cycles.

The role of progesterone as luteal phase support (LPS) in natural cycles FET (NC-FET) has been briefly studied. A systematic review and meta-analysis from 2020, including one RCT and three retrospective studies, found no evidence of an improved clinical pregnancy rate after progesterone support in NC-FET(18). A more recent systematic and meta-analysis, showed a benefit of progesterone as luteal phase support in NC-FET for LBR(19). However, the two meta-analyses included a mix of RCTs and observational studies and had a wide heterogeneity regarding progesterone treatment regimens. The authors concluded that further large, randomized studies are needed to improve the certainty of evidence.

In view of the limited knowledge concerning a possible advantage of progesterone as luteal phase support in NC-FET , the aim of this large RCT is to investigate if progesterone as luteal phase support increases LBR compared with no progesterone. In addition, assessment of perinatal and obstetric outcomes will be performed. Furthermore, the trial will investigate if the duration of progesterone support matters and assess the association between serum progesterone levels in early luteal phase and IVF outcome(20-22).

OBJECTIVES

Primary objective

To investigate if LPS by vaginal progesterone increases the chance of a live birth after FET in a natural cycle compared with no LPS. If progesterone support is superior to no treatment, we will further investigate if 7 weeks of treatment is more effective than 3 weeks.

Secondary objectives

1. To compare study groups regarding secondary outcomes including biochemical, clinical and ongoing pregnancy, as well as miscarriage.
2. To compare perinatal and obstetrical outcomes.
3. To compare self-reported side effects in women receiving and not receiving LPS with vaginal progesterone.
4. Investigate the association between serum progesterone levels before FET and LBR .

METHODS AND ANALYSIS

Study design

This multicenter, open-label, randomized, controlled Phase IV trial includes the participation of eight fertility clinics in Sweden and one in Iceland. All clinics perform standardized treatment according to the public healthcare system guidelines in Sweden and Iceland. Patient enrollment began in April 2021 and is planned to continue until September 2023.

A total of 1800 women undergoing NC-FET after conventional IVF or intracytoplasmic sperm injection (ICSI) treatment at one of the nine participating clinics will be recruited. As a clinical routine, patients scheduled for NC-FET contact their fertility clinic on the first day of the menstrual bleeding to schedule the treatment. Subsequently, a study nurse or doctor will identify and contact patients who fulfill the inclusion criteria to ask for interest in participating. Study information is sent to the patient by regular mail or through a secured website. Signed written or digitally informed consent is returned to the clinic either by regular mail or by contact through the website.

Eligibility criteria

Inclusion and exclusion criteria are specified in Table 1.

Table 1. ProFET trial. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Natural cycle FET with blastocyst	Oocyte donor cycles
Regular menstrual cycle (24-35 days)	Uterine malformations: cervical anomalies, submucosal uterine fibroid or endometrial polyps requiring surgery.
Age 18-43 years	Hypersensitivity to vaginal progesterone
BMI 18.5-35 (kg/m^2)	Medical contraindication to progesterone treatment
Understand written and spoken Swedish, English or Arabic and have signed a written informed consent.	Serious concomitant disease contraindicating ART and pregnancy
	Preimplantation genetic testing (PGT)
	Previously included in the ProFET study
	Participation in another study with an investigational product within the last 30 days

FET; Frozen embryo transfer, BMI; Body mass index.

Treatment and intervention

At the endogenous surge of LH (luteinizing hormone ; a hormone that naturally rises to trigger ovulation), study participants are randomized 1:1:1 to one of three groups:

A. No vaginal progesterone
B. Vaginal progesterone for 3 weeks
C. Vaginal progesterone for 7 weeks

Patients randomized to luteal phase progesterone are instructed to administrate 100 mg vaginal progesterone (Lutinus□; Ferring Pharmaceuticals, Saint-Prex, Switzerland) three times daily starting 3 days after the LH-surge. Participants are asked to leave a blood sample for analysis of serum progesterone regardless of group-allocation. A blood sample will be

drawn in the morning 3 days after the LH-surge, before any start of progesterone. The result will not be available to the patient. On day 5-6 after LH-surge, a blastocyst is transferred according to standard embryo transfer procedure. Patients randomized to vaginal progesterone will continue administration of progesterone until a pregnancy test. In the case of a positive pregnancy test patients will continue with vaginal progesterone for a total of 3 or 7 weeks respectively. In the case of a negative pregnancy test or miscarriage later on the patient will stop progesterone treatment. See figure 1.

Randomization

Study data are recorded in an electronic case report file (e-CRF) designed by Medicase (Sahlgrenska Science Park, Gothenburg, Sweden) which also includes a randomization program. Randomization is stratified for:

- Previous ET not resulting in positive pregnancy test, number (0-2, ≥3)
- Parity 0/≥1
- Age (<35/≥35 years)
- Treatment site

Blinding procedure

The trial is not blinded, neither to patients nor to treating physicians. Analyses are done by an independent statistician.

Data collection

Patient-related data are collected and variables are registered in the e-CRF program at the following time points:

1) screening before LH-surge
2) randomization at LH-surge
3) FET at LH + 5 or 6 days
4) result of pregnancy-test (urine sample)
5) early pregnancy scan (7 weeks + 5 days to 9 weeks + 0 days) in case of positive pregnancy test
6) through a follow up (by telephone) after gestational week 23 + 0 days
7) from the patient's and the newborn's medical records after delivery

Sample collection

Blood samples will be collected at LH + 3 days, whenever possible. A blood sample of 5 ml is analyzed for serum progesterone level. The blood samples will be sent to and analyzed at the Swedish certified laboratory Unilabs and are then discarded.

Transvaginal ultrasound scans

If the patient conceives, an early transvaginal pregnancy scan will be made at gestational age 7 weeks + 5 days to 9 weeks + 0 days, for estimation of number of gestational sacs, number of fetuses, crown-rump length and viability.

Questionnaires

The participants will be asked to fill out a questionnaire regarding registration of possible study medication side effects. The form is filled out regardless of group allocation and submitted in connection with vaginal ultrasound at gestational age 7 weeks + 5 days to 9 weeks + 0 days – or earlier in the case of a negative pregnancy test or miscarriage. Specified reported symptoms will be recorded as adverse events (AEs) in the e-CRF. Serious adverse events (SAEs) will be followed until two weeks after delivery.

Data management

Data is transferred to an online e-CRF; Medicase. The Medicase database is based on coded subject ID numbers used in the trial. Data are stored on a server located at Sahlgrenska University Hospital, Gothenburg, Sweden, with a daily backup. Only research staff at the Sahlgrenska University Hospital will have access to the final dataset. Ownership of data is determined by co-operation agreements as well as data processing agreements between Sahlgrenska University Hospital and the participating clinics.

Data sharing plan

The data underlying this article will be shared on reasonable request to the main responsible author.

STATISTICS

Outcome measurements

Primary outcome is live birth. Secondary outcomes include biochemical and clinical pregnancy rates, miscarriage rates and obstetric and neonatal outcomes in the study groups. Self-reported side effects will be reported as mild, moderate or severe. Progesterone levels 3 days after LH-surge will be measured in units of nmol/L.

Sample size calculation

In order to find an effect size of a 7% increase in LBR per transfer, measured as a difference in proportions between no progesterone (0.33) and any progesterone group (0.40), 1800 subjects are needed if allocated 1:2. In order to find a difference between progesterone 3 weeks and progesterone 7 weeks, as well as between no progesterone and progesterone for 3 weeks and 7 weeks respectively, 1200 subjects are needed if allocated 1:1. For all comparisons above, except for the primary analysis, a difference between groups of 8% is used. If 1800 women are allocated 1:1:1, 600 to no progesterone, 600 to progesterone 3 weeks and 600 to progesterone 7 week, all four sample size calculations are fulfilled under the condition of a power of 0.80, a significance level 0.05 and a two-sided Fisher's exact test.

Statistical analyses

The main analyses will be on the full analysis set (FAS) without imputation. Sensitivity analyses will be performed on the Intention-to-treat population with imputed data. Complementary analyses will be performed on the per protocol population. For unadjusted comparison between two groups Fisher's exact test will be used for dichotomous variables, Fisher's non-parametric permutation test will be used for continuous variables and Mantel-Haenszel Chi-square test for ordered categorical variables. Mean difference with 95% confidence interval (CI) will be given for all dichotomous and continuous variables. For dichotomous variables relative risk and odds ratio will be given with 95% CI.

For adjusted analyses between two groups, multivariable logistic regression will be used for dichotomous variables. If model assumption is fulfilled the corresponding model with link=log will be given to present adjusted relative risk (RR) with 95% CI. For continuous variables analysis of covariance (ANCOVA) will be used for adjusted analysis between two groups.

The primary efficacy analysis regarding live birth will be conducted with multiple logistic regression adjusting for all stratification variables and other predefined important predictors on the FAS population. The proportions will be given with exact 95% CI. The distribution of continuous variables will be given as mean, standard deviation (SD), median, first and third quartiles (Q1, Q3), minimum and maximum. All significance tests will be two-sided and conducted at the 5% significance level.

Monitoring

All study participants are monitored to meet the inclusion criteria and a check is made that voluntarily informed consent for each study participants is obtained and documented. For all study participants, the main parameters in the study are checked (live birth, clinical pregnancy, miscarriage and ectopic pregnancy). The first two study participants at each center will be monitored with a complete source data verification. Thereafter, a complete source data verification will be performed on every fifth randomly selected study participant.

ETHICS AND DISSEMINATION

The study was approved by the Swedish Ethical Review Authority (ID 2020-06774 and 2021-02822) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613).

The safety of participants in this study is high. As the medication/treatment with vaginal progesterone is well known, SAEs or suspected unexpected serious adverse reactions (SUSARs) are unlikely. If, however, a participant should experience a SAE or a SUSAR the local investigator will contact the principal investigator with no delay and the individual treatment will be stopped immediately.

The results of this trial will be presented at national as well as international scientific congresses and published in international scientific journals. The results of the research will also be disseminated to public through broadcasts, popular science articles, and newspapers.

Patient and public involvement

Development of this study protocol was done without patient involvement. The final study results will be disseminated to participants on request.

DISCUSSION

The rapidly increasing use of FET worldwide and the limited evidence concerning cycle regiments for FET demands further well designed large randomized trials. Progesterone supplementation in NC-FET is widely used despite scarce evidence. Two RCTs with LBR as main outcome have been published(23, 24). In a Swedish study where mainly cleavage stage embryos and single- as well as double embryo transfer were used, a significantly higher LBR was found(23). Further, a small study from Israel, including only 59 patients, using a modified NC-FET protocol, also found a significantly higher LBR after LPS compared with no progesterone(24). The study included a mix of cleavage stage embryos and blastocysts and up to three embryos were transferred.

Available retrospective studies on LPS reveal the use of different embryo stages at embryo transfer; two-nucleus stage(25) cleavage stage embryos(26) and both cleavage embryo and blastocyst transfers(27). All these studies used human Chorionic Gonadotropin (hCG) as ovulation trigger and administration of progesterone supplementation was started at different time points after LH-surge and was administrated either as intramuscular injections or as vaginal suppositories with different doses and duration of treatment.

This presented ongoing large open-label multicenter randomized clinical trial aims to investigate if vaginal LPS in NC-FET is superior to no LPS. In this set up, not only the differences in LBR and clinical pregnancy rates will be investigated, but also, the obstetrical and perinatal outcomes. This study will contribute to recommendations regarding LPS in NC-FET in the future.

The strength of this trial is the multicenter, randomized design and a large sample size of 1800 women. Broad inclusion criteria representing the patient cohort in every day practice will give a high generalizability to the results. The IVF-protocols consists only of natural cycles with no ovulation trigger. The study is not blinded to participants or investigators, which is a limitation, however less likely to introduce bias due to a robust primary outcome – live birth. By publishing the study protocol the study contributes to research-transparency. If progesterone supplementation in natural FET cycles should be shown to significantly increase the chance of live birth, the benefit for the patients, as well as for the society, would mean a) a shorter time to pregnancy, b) fewer IVF cycles needed per patient, c) reduced costs for patients and society, d) less environmental burden due to less cycles to achieve live birth

and thus less use of hormonal IVF-treatment. On the other hand, if no beneficial effect of this treatment can be shown, it should be abandoned and thereby implicate less financial burden for patients as well as for society, less treatment burden for the patient and less environmental impact associated with the use of LPS.

Contributors

CB and ÅM were the primary initiators of the study, who designed and wrote the first study protocol. AK, AS and CS contributed to the revision and editing of the study protocol. AK, AS, CB, CS, EL, GWe, GWi, KHO, KRW, MB, MK, SL and ÅM will all be involved in the recruitment of patients and data collection. All authors approved the final version of the study protocol. AS and CS applied to the Swedish Ethical Review Authority. CB and ÅM applied to the Swedish Medical Products Agency. AK and CS wrote the first draft of this manuscript which was revised by AS, CB and ÅM. Finally all committed authors approved this protocol.

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Disclaimer

Ferring Pharmaceuticals has not been involved in the design of the study protocol, nor will they be involved in the conduct of the study or any analysis neither the reporting of the results.

Competing interests

AS has support by Ferring Pharmaceuticals. CB has support by Ferring Pharmaceuticals, Merck A/S and Gedeon Richter. ÅM has support by Ferring Pharmaceuticals, Merck Serreno and Gedeon Richter. All these supports are given for lectures on own research.
MB has 4 % stocks in EUGIN Sweden.

Patient consent for publication

Not required.

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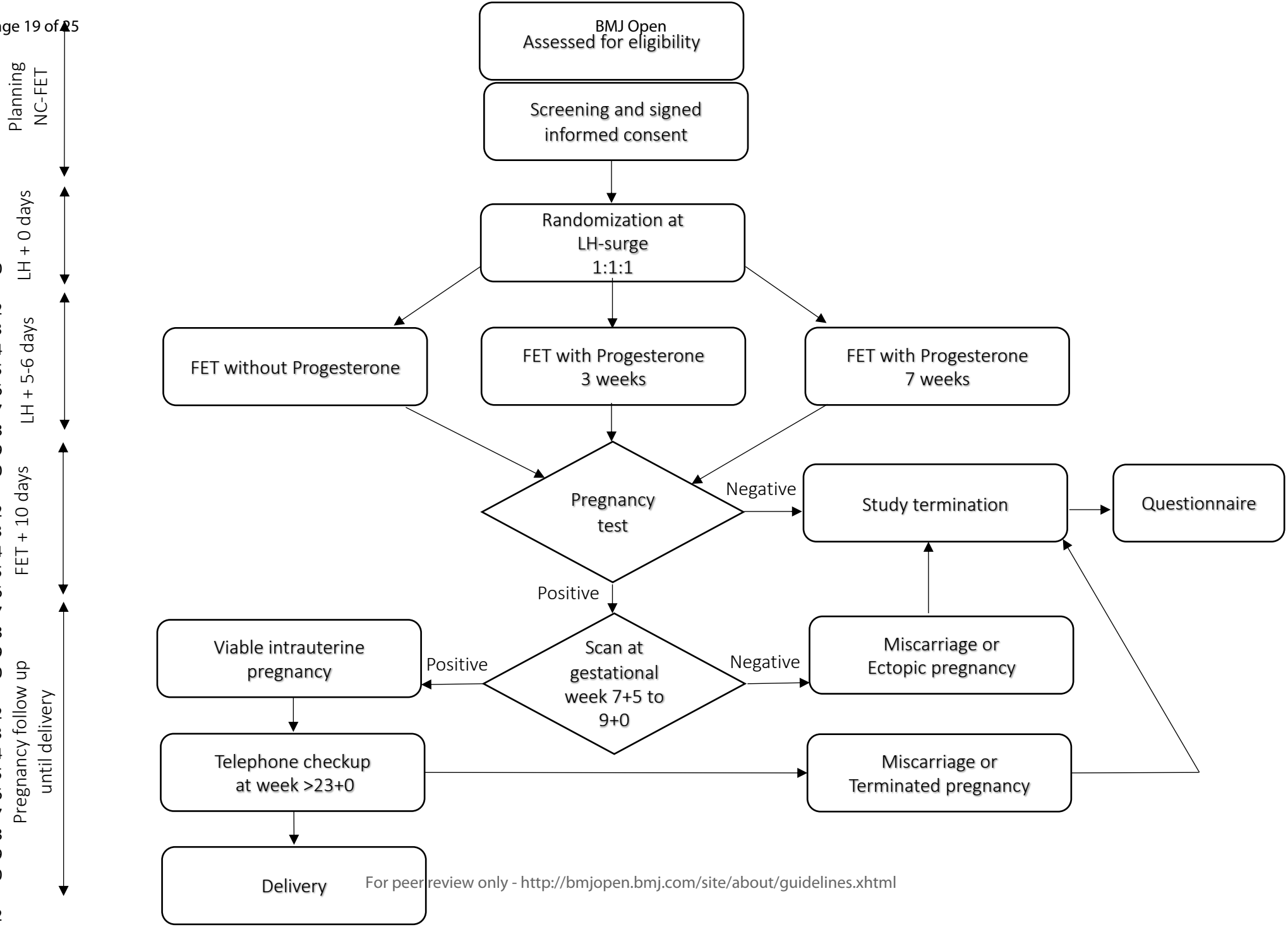
Legends – the ProFET trial

Table 1: ProFET trial, inclusion and exclusion criteria. FET; Frozen embryo transfer, BMI; Body mass index.

Figure 1: ProFET trial, flowchart. LH; Luteinizing hormone, FET; Frozen embryo transfer, NC-FET; Natural cycle frozen embryo transfer.



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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Vaginal Progesterone as Luteal Phase Support in Natural Cycle Frozen-Thawed Embryo Transfer (ProFET): a study protocol for a multi-center open-label randomized controlled trial
Trial registration	2a	Progesterone as Luteal Support in Frozen IVF Natural Cycles (ProFET) ClinicalTrials.gov Identifier: NCT04725864
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Roles and responsibilities	5a	CB and ÅM were the primary initiators of the study, who designed and wrote the first study protocol. AK, AS and CS contributed to the revision and editing of the study protocol. AK, AS, CB, CS, EL, GWe, GWi, KHO, KRW, MB, MK, SL and ÅM will all be involved in the recruitment of patients and data collection. All authors approved the final version of the study protocol. AS and CS applied to the Swedish Ethical Review Authority. CB and ÅM applied to the Swedish Medical Products Agency. AK and CS wrote the first draft of this manuscript which was revised by AS, CB and ÅM.
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	5c	Ferring Pharmaceuticals has not been involved in the design of the study protocol, nor will they be involved in the conduct of the study or any analysis neither the reporting of the results.

	5d	The team at Reproductive Medicine, Sahlgrenska Hospital consisting of principal investigator ÅM, trial sponsor CB and co-workers CS, AS and AK are responsible for the study design, trial registration, ethical approvements, data collection and ongoing contact with all study sites.

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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Vaginal progesterone as luteal phase support in natural cycle frozen-thawed embryo transfer (ProFET): protocol for a multicenter, open-label, randomized controlled trial

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Vaginal progesterone as luteal phase support in natural cycle frozen-thawed embryo transfer (ProFET): protocol for a multicenter, open-label, randomized controlled trial

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ABSTRACT

Introduction

Vaginal progesterone supplementation is frequently given to patients receiving frozen embryo transfer (FET) in the natural cycle aiming to increase the chance of pregnancy and live birth. To date only a few studies have investigated if progesterone supplementation is beneficial in these cycles and the level of evidence for progesterone supplementation is very low.

Methods and analysis

The ProFET trial is a multicenter, open-label, randomized controlled trial powered for this investigation, including 1800 women with regular menstrual cycles (24-35 days), aged 18-43 years planned for natural cycle frozen embryo transfer (NC-FET) receiving a single blastocyst for transfer. Participants are randomized (1:1:1) to either luteal phase progesterone for 3 weeks, luteal phase progesterone for 7 weeks or no luteal phase progesterone. The

participating study centers consist of twelve IVF-clinics in Sweden and one in Iceland. The primary outcome is to investigate if luteal phase support (LPS) by vaginal progesterone increases the chance of a live birth per randomized patient in a natural FET cycle compared with no LPS.

Ethics and dissemination

The trial was approved by the Swedish Ethical Review Authority (ID 2020-06774, 2021-02822 and 2022-01502-02) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613). All participants are required to provide written informed consent. The outcome of this study will be disseminated to the public through broadcasts, newspapers and presentations at scientific congresses as well as publications in international scientific journals.

Trial registration number

ClinicalTrials.gov (NCT04725864) and EudraCT (2020-005552-38).

Strengths and limitations of this study

- The trial has a randomized design, powered to evaluate if luteal support with vaginal progesterone will improve live birth rate in natural cycle frozen embryo transfers (NC-FETs) when a single blastocyst is transferred.
- The trial is conducted in women planning FET in natural cycles without exogenous ovulation trigger.
- If overall superiority of progesterone is demonstrated, the sample size will allow evaluation of whether treatment duration of 7 weeks is superior to 3 weeks.
- The broad inclusion criteria of women with regular menstrual cycles will ensure high generalizability of the results.
- The study is open label, blinded neither to participants nor to treating physicians, which is a limitation; however, this limitation is countered by the use of a robust primary outcome (live birth).

INTRODUCTION

In recent years, there has been a dramatic increase in the use of frozen-thawed embryo transfers (FET) cycles in in-vitro fertilization (IVF) all over the world. The FET rate in the United States has doubled since 2015, accounting for 78.8 % of all embryo transfers using non-donor Assisted Reproductive Technology (ART) in 2019(1). Similar changes are taking place in Europe(2) and in Sweden where the FET rate now accounts for 48 % of all IVF-cycles(3). The main reason for this increase is the improved embryo survival and high pregnancy/LBR after transfer of vitrified/thawed blastocysts compared to the previously used technique with transfer of thawed slow-frozen cleavage stage embryos(4, 5). Furthermore, high embryo survival rate facilitates the practice of single embryo transfer(6), reducing multiple pregnancy rate and thereby decreasing the risk of adverse perinatal outcomes.

Recently, the freeze-all concept has gained high popularity all over the world. Several large trials, comparing freeze-all vs fresh transfer, have shown similar live birth rates in ovulatory patients(7-10) while freeze-all has been shown to be beneficial in anovulatory patients(11). The freeze-all concept is also widely used when pending risk of ovarian hyperstimulation syndrome (OHSS), and has almost eliminated the risk of OHSS, a potentially life-threatening condition(12-14).

The most efficient protocol for FET is still not known. A Cochrane review, including 18 RCTs, comparing different cycle regimens for FET, comprising a total of 3815 women did not support one treatment modality over another when investigating LBR, however, with low certainty of evidence(15).

Safety aspects in ART are of great importance in treatment decision. Recently, interest has risen concerning the role of the corpus luteum (CL) in frozen cycles and studies evaluating the risks of altered vascular adaptation associated with pregnancies following FET according to the presence or absence of CL have been published(16). The CL, developing after ovulation, is known to produce estrogen and progesterone, but also relaxin, a hormone that regulates the maternal cardiovascular and renal systems and hence mediates the hemodynamic changes occurring during pregnancy. In a prospective cohort study including almost 700 women, programmed cycles (artificial cycles using estrogen and progesterone for endometrial preparation) in FET with no CL present were associated with an almost three-fold increased risk of preeclampsia compared with modified natural cycles (natural cycles triggered by

human chorionic gonadotrophin (hCG), for ovulation) with one CL present(16). Furthermore, in a recent Swedish large registry study, including almost 10 000 pregnancies/deliveries after FET, doubled rates of both hypertensive disorders of pregnancy and postpartum hemorrhage were found in programmed cycles compared to natural cycles(17). These studies thus support FET in natural cycles.

Luteal phase support (LPS) in fresh IVF cycles has been proven mandatory(18). Less is known regarding the role of LPS with progesterone in natural FET cycles. A natural ovulatory cycle would suggest that no supplementation needs to be given. However, the luteinizing hormone peak – used as a urine sample to detect ovulation - does not guarantee a subsequent ovulation. Furthermore, several studies have shown that corpus luteum deficiency with midluteal serum progesterone levels <10ng/ml could be a reason to support implantation and early pregnancy with LPS, even in a cycle where ovulation has occurred(19, 20). A study from 2018(21) showed that low but also high levels of progesterone were associated with a reduction in clinical pregnancy rate and LBR compared to normal levels. This has also been confirmed in a more recent study(22). Not only the doses, but also the duration of luteal phase support is widely discussed and differ between studies.

A systematic review and meta-analysis from 2020, including one RCT and three retrospective studies, found no evidence of an improved clinical pregnancy rate after progesterone support in NC-FET(23). A more recent systematic and meta-analysis, showed a benefit of progesterone as luteal phase support in NC-FET for LBR(24). However, the two meta-analyses included a mix of RCTs and observational studies and had a wide heterogeneity regarding progesterone treatment regimens. The authors concluded that further large, randomized studies are needed to improve the certainty of evidence.

In view of the limited knowledge concerning a possible advantage of progesterone as luteal phase support in NC-FET, the aim of this large RCT is to investigate if progesterone as luteal phase support increases LBR compared with no progesterone. In addition, assessment of perinatal and obstetric outcomes will be performed. Furthermore, the trial will investigate if the duration of progesterone support matters and assess the association between serum progesterone levels in early luteal phase and IVF outcome(20, 25, 26).

OBJECTIVES

Primary objective

To investigate if LPS by vaginal progesterone increases the chance of a live birth after FET in a natural cycle compared with no LPS. If progesterone support is superior to no treatment, we will further investigate if 7 weeks of treatment is more effective than 3 weeks.

Secondary objectives

1. To compare study groups regarding secondary outcomes including biochemical, clinical and ongoing pregnancy, as well as miscarriage.
2. To compare perinatal and obstetrical outcomes.
3. To compare self-reported side effects in women receiving and not receiving LPS with vaginal progesterone.
4. Investigate the association between serum progesterone levels before FET and LBR .

METHODS AND ANALYSIS

Study design

This multicenter, open-label, randomized, controlled Phase IV trial includes the participation of twelve fertility clinics in Sweden and one in Iceland. All clinics perform standardized treatment according to the public healthcare system guidelines in Sweden and Iceland. Patient enrollment began in May 2021 and is planned to continue until June 2024.

A total of 1800 women undergoing NC-FET after conventional IVF or intracytoplasmic sperm injection (ICSI) treatment at one of the nine participating clinics will be recruited. As a clinical routine, patients scheduled for NC-FET contact their fertility clinic on the first day of the menstrual bleeding to schedule the treatment. Subsequently, a study nurse or doctor will identify and contact patients who fulfill the inclusion criteria to ask for interest in participating. Study information is sent to the patient by regular mail or through a secured website. Signed written or digitally informed consent is returned to the clinic either by regular mail or by contact through the website. (supplemental file 1)

Eligibility criteria

Inclusion and exclusion criteria are specified in Table 1.

Table 1. ProFET trial inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Natural cycle FET with blastocyst	Oocyte donor cycles
Regular menstrual cycle (24-35 days)	Uterine malformations: cervical anomalies, submucosal uterine fibroid or endometrial polyps requiring surgery.
Age 18-43 years	Hypersensitivity to vaginal progesterone
BMI 18.5-35 (kg/m^2)	Medical contraindication to progesterone treatment
Understand written and spoken Swedish, English or Arabic and have signed a written informed consent.	Serious concomitant disease contraindicating ART and pregnancy
	Preimplantation genetic testing (PGT)
	Previously included in the ProFET study
	Participation in another study with an investigational product within the last 30 days

FET; Frozen embryo transfer, BMI; Body mass index.

Treatment and intervention

At the endogenous surge of LH (luteinizing hormone ; a hormone that naturally rises to trigger ovulation), study participants are randomized 1:1:1 to one of three groups:

A. No vaginal progesterone
B. Vaginal progesterone for 3 weeks
C. Vaginal progesterone for 7 weeks

Patients randomized to luteal phase progesterone are instructed to administrate 100 mg vaginal progesterone (Lutinus□; Ferring Pharmaceuticals, Saint-Prex, Switzerland) three times daily starting 3 days after the LH-surge. Participants are asked to leave a blood sample for analysis of serum progesterone regardless of group-allocation. A blood sample will be drawn in the morning 3 days after the LH-surge, before any start of progesterone. The result will not be available to the patient, neither to the treating clinician until the end of the study.

On day 5-6 after LH-surge, a blastocyst is transferred according to standard embryo transfer procedure. Patients randomized to vaginal progesterone will continue administration of progesterone until a pregnancy test. In the case of a positive pregnancy test patients will continue with vaginal progesterone for a total of 3 or 7 weeks respectively. In the case of a negative pregnancy test or miscarriage later on the patient will stop progesterone treatment. See figure 1.

Randomization

Study data are recorded in an electronic case report file (e-CRF) designed by Medicase (Sahlgrenska Science Park, Gothenburg, Sweden) which also includes a randomization program. Randomization is stratified for:

- Previous ET not resulting in positive pregnancy test, number (0-2, ≥3)
- Parity 0/≥1
- Age (<35/≥35 years)
- Treatment site

Blinding procedure

The trial is not blinded, neither to patients nor to treating physicians. Analyses are done by a statistician, blinded to group allocation.

Data collection

Patient-related data are collected and variables are registered in the e-CRF program at the following time points:

1) screening before LH-surge
2) randomization at LH-surge
3) FET at LH + 5 or 6 days
4) result of pregnancy-test (urine sample)
5) early pregnancy scan (7 weeks + 5 days to 9 weeks + 0 days) in case of positive pregnancy test
6) through a follow up (by telephone) after gestational week 23 + 0 days
7) from the patient's and the newborn's medical records after delivery

Sample collection

Blood samples will be collected at LH + 3 days, whenever possible. A blood sample of 5 ml is analyzed for serum progesterone level. The blood samples will be sent to and analyzed at the Swedish certified laboratory Unilabs and are then discarded.

Transvaginal ultrasound scans

If the patient conceives, an early transvaginal pregnancy scan will be made at gestational age 7 weeks + 5 days to 9 weeks + 0 days, for estimation of number of gestational sacs, number of fetuses, crown-rump length and viability.

Questionnaires

The participants will be asked to fill out a questionnaire regarding registration of possible study medication side effects. The form is filled out regardless of group allocation and submitted in connection with vaginal ultrasound at gestational age 7 weeks + 5 days to 9 weeks + 0 days – or earlier in the case of a negative pregnancy test or miscarriage. Specified reported symptoms will be recorded as adverse events (AEs) in the e-CRF. Serious adverse events (SAEs) will be followed until two weeks after delivery.

Data management

Data is transferred to an online e-CRF; Medicase. The Medicase database is based on coded subject ID numbers used in the trial. Data are stored on a server located at Sahlgrenska University Hospital, Gothenburg, Sweden, with a daily backup. Only research staff at the Sahlgrenska University Hospital will have access to the final dataset. Ownership of data is determined by co-operation agreements as well as data processing agreements between Sahlgrenska University Hospital and the participating clinics.

STATISTICS

Outcome measurements

Primary outcome is live birth. Secondary outcomes include biochemical and clinical pregnancy rates, miscarriage rates and obstetric and neonatal outcomes in the study groups. For a complete list of secondary outcomes, see supplemental file 2. Self-reported side effects will be reported as mild, moderate or severe. Progesterone levels 3 days after LH-surge will be measured in units of nmol/L.

Sample size calculation

In order to find an effect size of a 7% increase in LBR per randomized patient, measured as a difference in proportions between no progesterone (0.33) and any progesterone group (0.40), 1800 subjects are needed if allocated 1:2. In order to find a difference between no progesterone (0.33) and progesterone for 3 weeks (0.41) and 7 weeks (0.41) respectively, 1200 subjects are needed if allocated 1:1. Also, for the comparison between the progesterone groups, 1200 subjects are needed if allocated 1:1, to detect a difference of 8%, (0.38 for 3 weeks of progesterone vs 0.46 for 7 weeks. For all comparisons above, except for the primary analysis, a difference between groups of 8% is used. If 1800 women are allocated 1:1:1, 600 to no progesterone, 600 to progesterone 3 weeks and 600 to progesterone 7 week, all four sample size calculations are fulfilled under the condition of a power of 0.80, a significance level 0.05 and a two-sided Fisher's exact test.

We thus have two primary superiority analyses in this study. The first is the comparison of LBR between no progesterone and the combined group of any progesterone with Fisher's exact test on significance level 0.05. If this test is significant the probability mass of 5% will be transferred to the second comparison of live birth between progesterone for 3 weeks compared with progesterone for 7 weeks. If the first test is significant, we have been able to show that any progesterone gives significantly higher LBR than in women without progesterone. If in the second comparison 7 weeks shows significantly higher LBR than 3 weeks, we have also confirmed superiority regarding 7 weeks over 3 weeks. If the first analysis is non-significant, we have not been able to show any confirmative results in this study. The comparisons between no progesterone and 3 weeks progesterone and between no progesterone and 7 weeks progesterone is performed to calculate mean difference with 95% CI between these groups.

Statistical analyses

The main analyses will be on the full analysis set (FAS) without imputation. Complementary analyses will be performed on the per protocol population. The primary efficacy analysis regarding live birth will be conducted with multivariable logistic regression adjusting for all stratification variables on the FAS population. The first sensitivity primary analysis will be the same analysis also adjusted for the following other predefined important predictors:

- body-mass index
- smoking status
- duration of subfertility
- previous miscarriage (yes/no)
- blastocyst (day 5/day 6 at cryopreservation)
- number of embryos transferred

The second sensitivity primary analysis will be the same analysis as the primary efficacy analysis but performed on the intention-to-treat population with multiple imputation based on 100 datasets. Both primary outcome and stratified variables will be imputed. For adjusted analyses between two groups, multivariable logistic regression will be used for dichotomous variables. If model assumption is fulfilled the corresponding model with link=log will be given to present adjusted relative risk (RR) with 95% CI. For continuous variables analysis of covariance (ANCOVA) will be used for adjusted analysis between two groups.

Explorative unadjusted mean difference between the two groups with 95% confidence interval (CI) will be given for dichotomous variables and continuous variables together with effect sizes. For continuous variables these 95% CI will be based on T-test or Fisher's non-parametric permutation test. For dichotomous variables relative risk and odds ratio will be given with 95% CI. Proportions will be given with exact 95% CI.

For comparison between two groups Mantel – Haenszel Chi square test will be used for ordered categorical variables and Fisher's exact test for dichotomous variables.
The distribution of continuous variables will be given as mean, standard deviation (SD), median, first and third quartiles (Q1, Q3), minimum and maximum. All significance tests will be two-sided and conducted at the 5% significance level.

Monitoring

All study participants are monitored to meet the inclusion criteria and a check is made that voluntarily informed consent for each study participants is obtained and documented. For all study participants, the main parameters in the study are monitored (live birth, clinical pregnancy, miscarriage and ectopic pregnancy). The first two study participants at each center will be monitored with a complete source data verification. Thereafter, a complete source data verification will be performed on every fifth randomly selected study participant.

Patient and public involvement

Development of this study protocol was done without patient or public involvement. The final study results will be disseminated to participants on request.

ETHICS AND DISSEMINATION

The study was approved by the Swedish Ethical Review Authority (ID 2020-06774 and 2021-02822) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613). All participants are required to sign a written informed consent form before study entry (supplemental file 1). The safety of participants in this study is high. As the medication/treatment with vaginal progesterone is well known, SAEs or suspected unexpected serious adverse reactions (SUSARs) are unlikely. If, however, a participant should experience a SAE or a SUSAR the local investigator will contact the principal investigator with no delay and the individual treatment will be stopped immediately.

The results of this trial will be presented at national as well as international scientific congresses and published in international scientific journals. The results of the research will also be disseminated to public through broadcasts, popular science articles, and newspapers.

DISCUSSION

The rapidly increasing use of FET worldwide and the limited evidence concerning cycle regiments for FET demands further well designed large randomized trials. Progesterone supplementation in NC-FET is widely used despite scarce evidence. Two RCTs with LBR as main outcome have been published(27, 28). In a Swedish study where mainly cleavage stage embryos and single- as well as double embryo transfer were used, a significantly higher LBR was found(27). Further, a small study from Israel, including only 59 patients, using a modified NC-FET protocol, also found a significantly higher LBR after LPS compared with no progesterone(28). The study included a mix of cleavage stage embryos and blastocysts and up to three embryos were transferred.

Available retrospective studies on LPS reveal the use of different embryo stages at embryo transfer; two-nucleus stage(29) cleavage stage embryos(30) and both cleavage embryo and blastocyst transfers(31). All these studies used human Chorionic Gonadotropin (hCG) as

ovulation trigger and administration of progesterone supplementation was started at different time points after LH-surge and was administrated either as intramuscular injections or as vaginal suppositories with different doses and duration of treatment.

This presented ongoing large open-label multicenter randomized clinical trial aims to investigate if vaginal LPS in NC-FET is superior to no LPS. In this set up, not only the differences in LBR and clinical pregnancy rates will be investigated, but also, the obstetrical and perinatal outcomes. This study will contribute to recommendations regarding LPS in NC-FET in the future.

The strength of this trial is the multicenter, randomized design and a large sample size of 1800 women. Broad inclusion criteria representing the patient cohort in everyday practice will ensure a high generalizability. The IVF protocols consist only of natural cycles with no ovulation trigger. The study is not blinded to participants or investigators, which is a limitation, however, the use of a robust primary outcome (live birth) makes this less likely to introduce bias.

If progesterone supplementation in natural FET cycles should be shown to significantly increase the chance of live birth, the benefit for the patients, as well as for the society, would mean a) a shorter time to pregnancy, b) fewer IVF cycles needed per patient, c) reduced costs for patients and society, d) less environmental burden due to less cycles to achieve live birth and thus less use of hormonal IVF-treatment. On the other hand, if no beneficial effect of this treatment can be shown, it should be abandoned and thereby implicate less financial burden for patients as well as for society, less treatment burden for the patient and less environmental impact associated with the use of LPS.

Contributors

CB and ÅM were the primary initiators of the study, who designed and wrote the first version of the study protocol. AK, AS and CS contributed to the revision and editing of the study protocol. AK, AS, CB, CS, EL, GWe, GWi, KHO, KRW, MB, MK, SL and ÅM will all be involved in the recruitment of patients and data collection. All authors approved the final version of the study protocol. AS and CS applied to the Swedish Ethical Review Authority. CB and ÅM applied to the Swedish Medical Products Agency. AK and CS wrote the first

draft of this manuscript which was revised by AS, CB and ÅM. All authors approved this protocol.

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The project will be funded by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG-965526 and ALFGBG-720291), by an unrestricted grant from Ferring Pharmaceuticals (SU 2020-05958), by Sahlgrenska University Hospital´s Research Foundation, by Gothenburg Medical Society and by the Hjalmar Svensson foundation. We will also apply for further funding.

Disclaimer

Ferring Pharmaceuticals has not been involved in the design of the study protocol, nor will they be involved in the conduct of the study or any analysis or reporting of the results.

Competing interests

CB and ÅM declare support from Ferring Pharmaceuticals, Merck Sereno and Gedeon Richter. MB has 4 % stocks in EUGIN Sweden. None of the other authors hade conflicts of interest.

Patient consent for publication

Not required.

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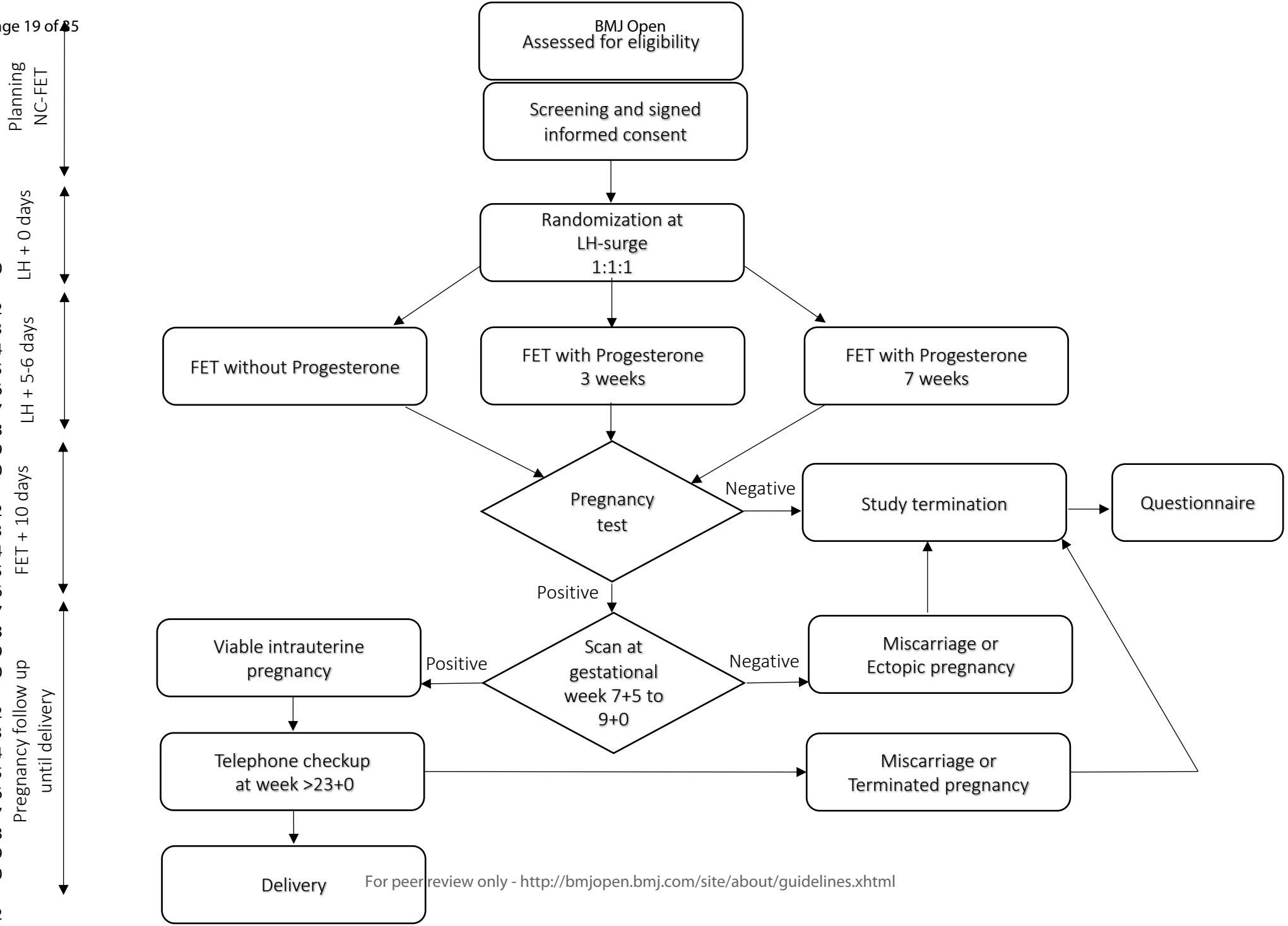
FIGURE LEGENDS

Figure 1: ProFET trial flowchart

LH; Luteinizing hormone, FET; Frozen embryo transfer, NC-FET; Natural cycle frozen embryo transfer.



Study information ProFET

Treatment with Progesterone after IVF with Frozen Embryo Transfer in a natural cycle with ovulation

The ProFET trial has its name from Progesterone, and FET, short for Frozen Embryo Transfer.

Information to participants in the trial

We hereby ask for your participation in the ProFET study. In this document we provide information about the project and what participation may entail.

What kind of research project is this? Why are we asking you to participate?

In IVF treatment (In Vitro Fertilisation) it has become increasingly common to transfer a frozen/thawed embryo. Frozen embryo transfer accounts for 46 % of all IVF treatments in Sweden (www.qivf.se, Annual report 2020). The increased use of frozen embryo transfer is due to improved results after the introduction of new freezing procedures and embryo culture methods.

The corpus luteum, occurring in the ovary after ovulation, produces progesterone to support an early pregnancy. In IVF-cycles, where no ovulation has occurred, extra progesterone is needed and provided as medication after embryo transfer. In the same way, treatment with progesterone is given to all patients undergoing IVF stimulation with transfer of a fresh embryo, as the own hormone production during these treatments is suppressed.

Today, it is not known whether progesterone treatment after a frozen embryo transfer in a natural ovulatory cycle, improves the chance of live birth. Nevertheless, this treatment is sometimes given, despite its lack of known benefits to the patient.

The ProFET trial aims to find out if the addition of progesterone after a frozen embryo transfer in a natural cycle increases the chances of live birth. Each participant will be randomly assigned to one out of three groups. Group A will undergo frozen embryo transfer without the addition of progesterone. This group corresponds to normal clinical routine. Group B will be treated with progesterone, taken as a vaginal tablet, three times daily under three weeks. Group C will be treated with progesterone, taken as a vaginal tablet, three times daily during seven weeks. Some women will be asked for an additional blood sample, to measure their blood progesterone levels, before frozen embryo transfer.

You are asked to participate because you are currently undergoing IVF treatment with a planned frozen embryo transfer in a natural cycle. You are between 18 to 43 years of age, have a BMI (body mass index) between 18.5 – 35 kilogram/m^2, a regular menstrual cycle, and understand Swedish, English or Arabic.

The research principal for the project is the Reproductive Medicine unit at the Sahlgrenska University Hospital, Region Västra Götaland.

Research design

The section below describes the participation in the trial.

1. You will be in contact with a doctor or nurse, in order to plan your frozen embryo transfer. If you meet the criteria for participation and wish to participate, you are asked to sign a consent form at the clinic or a digital consent form via 1177. You will also receive a questionnaire where you will keep notes on any symptoms after embryo transfer. You will receive this form even if you belong to the group that does not take any medicine.

2. Once you have a positive ovulation test, we ask you to contact your clinic according to ordinary routines and schedule an appointment for a frozen embryo transfer. One of the study doctors or nurses will randomly assign you to one of the three groups. The participants in Group A undergo a frozen embryo transfer without any additional treatment. Group B is prescribed vaginal tablets containing progesterone three times daily for three weeks. Group C is prescribed vaginal tablets containing progesterone three times daily for seven weeks. It is not possible, as a participant in the clinical trial, to ask to be placed in a particular group. The group allocation is computerised. If you are allocated to one of the groups treated with progesterone, you commence your treatment three times daily, starting three days after the positive ovulation test. You can pick up the medicine at any pharmacy, e-prescriptions are sent by the responsible doctor.

   When possible, we will draw a blood sample three days after the positive ovulation test, but before starting the progesterone treatment. A blood sample is taken even if you belong to the group that does not receive progesterone. The blood sample is drawn from a vein in the arm. Approximately 5 ml, roughly the amount of a teaspoon is needed. The test is called S-Progesterone and measures the level of progesterone (corpus luteum hormone) in the blood.

3. Frozen embryo transfer.

4. A urinary pregnancy test is taken at home according to routine instructions given after a frozen embryo transfer. Contact via phone with the doctor/nurse responsible for the trial, regarding outcomes/results:

   Not pregnant: Your participation in the trial ends. Questionnaire is handed in. If you belong to one of the groups treated with progesterone (Groups B or C), your treatment with the drug ends.

   Pregnant: If you belong to Groups B or C (treated with progesterone) you continue with your treatment for three or seven weeks, respectively, based on what group you were assigned to. If you belong to the group that will take progesterone tablets for seven weeks, a new e-prescription will be sent for prolonged treatment.
   A transvaginal ultrasound is scheduled for gestational week eight or nine to confirm pregnancy. Questionnaire is handed in. Routine ultrasounds during pregnancy will be offered, as standard for all pregnant women. After gestational week 22 you will receive a phone call by the study nurse, who will ask about how your pregnancy proceeds.

Participants in the trial are required to fill out a questionnaire regarding unexpected symptoms, which can be attributed to the administered drug, or to other causes. Participation may require self-administration of a vaginal tablet three times per day for a period of three or seven weeks.

Expenses for progesterone tablets, will be reimbursed financially by the ProFET trial at the Reproductive Medicine unit at the Sahlgrenska University Hospital.

We will also collect data from national registries (Pregnancy Register, Swedish Neonatal Quality Register, Statistics Sweden) and from medical records on antenatal care and delivery, as well as your child's records, regarding your child's condition.

Possible outcomes and risks

The treatment does not involve any risk unless you have a hypersensitivity to the drug or have any of the diseases contraindicating the use of the study drug. Patient with any of these conditions will be allowed to participate in the trial.

Participation in the trial does not entail discomfort or pain. Some patients experience the vaginal suppository as smudgy. There are no known long-term side effects related to treatment with vaginal progesterone suppositories, that could lead to injury or risk.

Should you experience discomfort or have any questions, you are welcome to contact the study nurse during office hours. In case of acute gynaecological problems outside office hours, you should contact a gynaecological emergency department. If you become ill in some way, are hospitalised or on sick leave, you must report this to the study nurse or the doctor

responsible for the trial, as all illness during an ongoing trial must be reported in accordance with current rules.

Information about your stored data

The project will collect data and keep relevant records about you. The IVF treatment will be recorded in the clinic's regular medical record system and is protected by confidentiality. If a blood sample is drawn, the test result will be entered in your medical chart and in a specific research database. Collected research data will be stored without name or social security number, but instead under a study code number, protected in a designated research data base. All personal data is confidential, and no unauthorized person will be able to access it.

When the collected data is analysed, no individual can be identified. The same also applies when the trial and its result are reviewed by an independent safety committee, and when results from the trial is published in scientific journals.

Data will be archived for fifteen years, in accordance with research regulations. Data analysis is solely for research purposes, and the legal basis is public interest/research in accordance with EU:s data protection regulation for the treatment.

The collected data is the responsibility of the board of the Sahlgrenska University Hospital. In accordance with EU:s data protection regulation you are entitled – without cost – to view your own trial records. You are also entitled to have any potentially false data corrected. You are also entitled to request your records being erased or limited in access. If you want to review your records, please contact the Principal Investigator, dr Åsa Magnusson, Reproduktionsmedicin, Sahlgrenska Universitetssjukhuset, e-mail: asa.magnusson@vgregion.se Telephone: 031-342 10 00. Data protection officer is reachable at: Sahlgrenska Universitetssjukhuset, Dataskyddsombudet, 413 45 Göteborg. Telephone 031-343 27 15. sahlgrenska.universitetssjukhuset.dso@vgregion.se. Any complaint with how your personal data is handled, may be submitted to the Integrity Protection Authority which is supervisory authority.

How do I get information about the results from the trial?

The research will be published in international scientific journals. The research team encourages all participating IVF clinics to present the results on their respective websites.

Insurance and compensation

IVF treatment is covered by the Swedish patient injury insurance. Participation in the trial does not involve any additional costs, and therefore no compensation is offered for participation.

Participation is voluntary

Your participation is entirely voluntary, and you may at any time withdraw your consent without giving an explanation. A withdrawal will not impact on your future care or treatment.

Should you wish to stop your participation, please contact the responsible parties for the trial. (See information below.)

Responsible for the trial

The main investigators are:
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- Dr. Caroline Stadelmann, e-mail caroline.stadelmann@vgregion.se telephone 031-343 67 59

both at Reproductive Medicine, Sahlgrenska University Hospital, Gothenburg.

The following sections are edited for each clinic. Locally responsible for the trial are at:

Livio Fertility Center, Gothenburg
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Livio Gärdet
-Dr Camilla Stenfelt, e-mail camilla.stenfelt@livio.se telefon 08-58612000

Consent to participate in the study

I have received oral and written information about the trial and have had the opportunity to ask questions. I may keep the written information.

I agree to participate in the study "ProFET". At the same time, I agree that information about me is processed in the manner described in the research study information and that data from described records and registers may be obtained.

Study participant

Social security number: …….

Place and date	Signature
	Name clarification

Doctor who receives consent:

Place and date	Signature
	Name clarification

Supplemental file 2

The ProFET trial – a complete list of secondary outcomes

1. Number of participants with biochemical pregnancy [Time Frame: 2-3 weeks after embryo transfer.]

   A pregnancy diagnosed only by the detection of beta hCG in serum or urine.

2. Number of participants with clinical pregnancy [Time Frame: 4-8 weeks after embryo transfer.]

   A pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs.

3. Number of participants with ongoing pregnancy [Time Frame: 5-7 weeks after embryo transfer.]

   An intrauterine pregnancy with one or more fetuses with heartbeats measured in gestational week 7+5 to 9+0 with vaginal ultrasound.

4. Number of participants with miscarriage [Time Frame: Up to 20 weeks after embryo transfer.]

   The spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age. Also, the outcome will be reported according to Core Outcome Measure for Infertility Trials (Duffy et al., 2020) in a separate appendix.

5. Number of participants with ectopic pregnancy [Time Frame: Up to 20 weeks after embryo transfer.]

   A pregnancy outside the uterine cavity, diagnosed by ultrasound, surgical visualization, or histopathology.

6. Number of participants with termination of pregnancy [Time Frame: Up to 20 weeks after embryo transfer.]

Defined as the termination of a clinical pregnancy, by deliberate interference that takes place before 22 completed weeks of gestational age. Also, the outcome will be reported according to Core Outcome Measure for Infertility Trials (Duffy et al., 2020) in a separate appendix.

7. Birth weight [Time Frame: Up to 41 weeks after embryo transfer.]

   Defined as weight in grams at birth.

8. Gestational age at delivery [Time Frame: Up to 41 weeks after embryo transfer.]

   The gestational age at FET is calculated by adding the number of culture days to ovulation (ovulation=day 14). Gestational age at delivery is then calculated by adding the number of days since FET.

9. Preterm birth [Time Frame: Up to 35 weeks after embryo transfer.]

   Defined as a child born alive before 37 completed weeks of pregnancy.

10. Very preterm birth [Time Frame: Up to 30 weeks after embryo transfer.]

    Defined as a child born alive before 32 completed weeks of pregnancy.

11. Low birth weight [Time Frame: Up to 41 weeks after embryo transfer.]

    Birth weight less than 2500 g.

12. Very low birth weight [Time Frame: Up to 41 weeks after embryo transfer.]

    Birth weight less than 1500 g.

13. Stillbirth [Time Frame: Up to 41 weeks after embryo transfer.]

    The death of a fetus prior to the complete expulsion or extraction from its mother, after and including 22 completed weeks of gestational age. Also, the outcome will

be reported according to Core Outcome Measure for Infertility Trials (Duffy et al., 2020) in a separate appendix.

14. Perinatal death [Time Frame: Up to 41 weeks after embryo transfer and 7 days after birth.]

    Fetal or neonatal death occurring during late pregnancy (at 22 completed weeks of gestational age and later), during childbirth, or up to seven days after birth. Also, the outcome will be reported according to Core Outcome Measure for Infertility Trials (Duffy et al., 2020) in a separate appendix.

15. Number of children with birth defects [Time Frame: Up to 41 weeks after embryo transfer.]

    Congenital birth defects were defined according the International Statistical Classification of Diseases and Related Health Problems (ICD-10). And further defined according to the EUROCAT classification system.

16. Number of children admitted to Neonatal Intensive Care Unit (NICU)
    [Time Frame: Up to 41 weeks after embryo transfer and 7 days after birth.]

    Defined as children that were admitted to NICU after birth.

17. Number of participants with hypertensive disorders of pregnancy [Time Frame: Up to 41 weeks after embryo transfer including the postpartum period before discharge of mother.]

    Hypertensive disorders of pregnancy defined as high blood pressure disorders including preeclampsia, gestational hypertension and chronic hypertension.

18. Number of participants with placenta previa [Time Frame: Up to 41 weeks after embryo transfer.]

    Defined as a placenta covering the internal os of the cervix, at time of delivery.

19. Number of participants with placenta abruption [Time Frame: Up to 41 weeks after embryo transfer.]

Defined as the premature separation of a normally located placenta from the uterine wall that occurs before delivery of the fetus.

20. Number of participants with postpartum hemorrhage [Time Frame: Up to 41 weeks after embryo transfer.]

    Defined as a cumulative blood loss of greater than 1,000 mL or blood loss accompanied by signs or symptoms of hypovolemia within 24 hours after the birth process.

21. Number of participants with Cesarean section [Time Frame: Up to 41 weeks after embryo transfer.]

    Defined as a surgical procedure used to deliver a baby through incisions in the abdomen and uterus.

22. Number of participants with thromboembolic events [Time Frame: Up to 41 weeks after embryo transfer including the postpartum period before discharge of mother.]

    Defined as formation in a blood vessel of a clot (thrombus) that breaks loose and is carried by the blood stream to plug another vessel.

23. Maternal mortality [Time Frame: Up to 41 weeks after embryo transfer including the postpartum period before discharge of mother.]

    Defined as female deaths from any cause related to or aggravated by pregnancy or its management (excluding accidental or incidental causes) during pregnancy and childbirth.

24. Number of participants with treatment related side effects [Time Frame: Up to 8 weeks after embryo transfer.]

    Side effects reported according to study specific questionnaire. Questions are answered with yes or no. If yes, symptoms are described, but not by using a scale.

25. Number of participants with adverse events [Time Frame: Up to 8 weeks after embryo transfer.]

Any untoward medical occurrence in symptom or disease temporally associated with the use of the medicinal (investigational) product, whether or not related to the medicinal product.

26. Cost effectiveness [Time Frame: After study completion, an average of 1 year.]

Comparison between groups regarding the total costs for the intervention divided by treatment efficacy (live birth).

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	14-15
	5b	Name and contact information for the trial sponsor	15
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14-15

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	1-6
	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6-10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7; table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7; table 1
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11-12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11-12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12-13
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7,13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	3,7
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	8-9

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.