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Immediate versus postponed single blastocyst transfer in modified natural cycle frozen embryo transfer (mNC-FET): a study protocol for a multicentre randomized controlled trial

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Immediate versus postponed single blastocyst transfer in modified natural cycle frozen embryo transfer (mNC-FET): a study protocol for a multicentre randomized controlled trial

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**Abstract**

*Introduction*

Today, it is widespread practice to postpone frozen embryo transfer (FET) in a modified natural cycle (mNC) for at least one menstrual cycle after oocyte retrieval and failed fresh embryo transfer or freeze-all. The rationale behind this practice is the concern that suboptimal ovarian, endometrial or endocrinological conditions following ovarian stimulation may have a negative impact on endometrial receptivity and implantation. However, two recent systematic reviews and meta-analyses based on retrospective data did not support this practice. As unnecessary delay in time to transfer and pregnancy should be avoided, the aim of this study is to investigate if immediate single blastocyst transfer in mNC-FET is non-inferior to standard postponed single blastocyst transfer in mNC-FET in terms of live birth rate (LBR).

*Methods and analysis*

Multicentre randomized controlled non-blinded trial including 464 normo-ovulatory women aged 18-40 years undergoing single blastocyst mNC-FET after a failed fresh- or freeze-all cycle.

Participants are randomized 1:1 to either FET in the first menstrual cycle following the stimulated cycle (immediate FET) or FET in the second or subsequent cycle following the stimulated cycle.
(postponed FET). The study is designed as a non-inferiority trial and primary analyses will be performed as intention-to-treat and per protocol.

**Ethics and dissemination**

Ethical approval has been granted by the Scientific Ethical Committee of the Capital Region of Denmark (J-nr.: H-19086300). Data will be handled according to Danish law on personal data protection in accordance with the General Data Protection Regulation. Participants will complete written consent forms regarding participation in the study and storage of blood samples in a biobank for future research. The study will be monitored by a GCP-trained study nurse not otherwise involved in the study. The results of this study will be disseminated by publication in international peer-reviewed scientific journals.

**Registration details**

The study is registered at ClinicalTrials.gov NCT04748874.
Article summary

- This is the first randomized controlled trial comparing live birth rates in immediate versus postponed single blastocyst transfer in modified natural cycle (mNC) frozen embryo transfer (FET).
- Systematic reviews and meta-analyses on immediate versus postponed FET indicate that the standard clinical practice of postponing mNC-FET for at least one menstrual cycle after the stimulated cycle is unnecessary.
- The study includes normo-ovulatory women aged 18-40 years undergoing single blastocyst mNC-FET after a failed fresh or a freeze-all cycle, thus securing high generalizability and applicability of study results.
- The study is designed as a non-inferiority trial. Analyses will be performed as intention-to-treat and per-protocol and an inferiority margin of 10% is considered clinically relevant.
Introduction

In recent years, pregnancy rates after frozen embryo transfer (FET) have improved and are now approaching, or even exceeding, those obtained after fresh embryo transfer (1). Thus, FET has become increasingly important in the field of assisted reproductive techniques and can be applied after failed fresh embryo transfer or after elective embryo freezing (freeze-all) on various indications, among them risk of ovarian hyperstimulation syndrome (2). Today, it is standard practice to postpone FET in a modified natural cycle for at least one menstrual cycle after controlled ovarian stimulation and fresh embryo transfer or freeze-all. The rationale behind this practice is the concern that suboptimal ovarian, endometrial and endocrinological conditions after ovarian stimulation may have a negative effect on endometrial receptivity and implantation (3–6) and may increase the risk of small for gestational age babies and preterm delivery (7). However, the elective deferral of FET is an empirical approach founded on assumptions rather than evidence and may unnecessarily delay time to pregnancy and increase costs for embryo freezing.

In women with regular menstrual cycles, FET is often performed in natural cycles (NC) instead of oestrogen- and progesterone supplemented programmed cycles. Advantages of a natural approach include less disruption of hormonal balance and receptivity of the endometrium (8,9) as well as minimal use of drugs, hence fewer side effects and reduced treatment costs. NC are subdivided into true natural cycles (tNC) or human chorionic gonadotropin (hCG) triggered modified natural cycles (mNC). In a tNC, close ultrasonic and endocrine monitoring is required throughout the follicular phase to determine the point of spontaneous ovulation. In mNC-FET, ultrasonic monitoring is generally started in the late follicular phase and ovulation trigger (hCG) is administered when the leading follicle reaches 17-18 mm, the timepoint at which, in the majority of women, the LH surge is induced in the natural cycle (10,11). Reproductive outcomes in tNC- and mNC-FET seem to be comparable (12,13) but mNC-FET is often considered more patient-friendly.

Recently, two systematic reviews and meta-analyses regarding timing of FET have been published, comparing pregnancy outcomes between FET performed in the first menstrual cycle after ovarian stimulation and oocyte retrieval (immediate FET) and FET in the second or subsequent cycle (postponed FET). The reviews are based on retrospective data including a variety of FET protocols, hence, the presence of selection bias is apparent and the quality of
evidence is low. Despite a significant overlap in studies included in the reviews, the results differ slightly, probably due to inclusion of unadjusted (14) versus adjusted (15) results. Huang et al. reported no significant association between timing of FET and pregnancy outcomes; clinical pregnancy rate (CPR) (relative risk (RR) 0.94 (95% CI 0.87–1.03)) and live birth rate (LBR) (RR 0.94 (95% CI 0.85–1.03)) while Bergenheim et al. found a slightly higher CPR (adjusted odds ratio (aOR) 1.22 (95% CI 1.07-1.39)) and LBR (aOR 1.20 (95% CI 1.01-1.44)) in immediate versus postponed FET. Regardless, the standard practice of routinely postponing mNC-FET for at least one menstrual cycle following IVF/ICSI does not seem to be scientifically supported. As unnecessary delay in time to transfer and pregnancy should be avoided, the aim of this study is to investigate, in a multicentre randomized controlled trial, if immediate single blastocyst transfer in mNC-FET is non-inferior to standard postponed single blastocyst transfer in mNC-FET in terms of LBR.

Methods and analysis

Study design

The study is designed as a multicentre randomized controlled non-blinded trial including fertility clinics in Denmark. All clinics are part of an academic hospital setting performing standardized treatments according to the public health care system in Denmark. When determined, a complete list of study sites can be obtained by contacting the steering committee of the study. Patient enrolment is expected to begin in March 2021 and continue until December 2024. We adhered to the SPIRIT recommendations (16) when drafting this protocol.

Eligibility criteria

Inclusion criteria: patients eligible for FET in a modified natural cycle; 18-40 years; regular menstrual cycle (23-35 days); ≥1 vitrified blastocyst with Gardner score ≥ 3BB at vitrification on day 5 or 6 after oocyte retrieval. Exclusion criteria: uterine malformations or presence of hydrosalpinx; submucosal uterine myomas; uterine polyps; severe ovarian hyperstimulation syndrome (OHSS) during the fresh cycle (defined as need for ascites drainage and/or hospital admission due to OHSS); oocyte donation; testicular sperm aspiration; male or female HIV or Hepatitis B/C; preimplantation genetic testing in the fresh cycle; contraindication or allergy to standard fertility medication (i.e., hCG used for ovulation trigger). Patients can withdraw from
participation in the study at any time without accounting for any reason. Further, participation can be interrupted by a treating or non-treating doctor if (i) the patient’s general condition contradicts participation in the study or (ii) protocol violation which the investigator considers having influence on the study outcome. After withdrawal from the study, patients may continue receiving standard treatment at the fertility clinic.

Study population and recruitment
The study population consists of patients undergoing mNC-FET after a fresh IVF/ICSI cycle that did not result in pregnancy, or after an elective freeze-all cycle. All eligible patients interested in receiving information about the study, will be contacted telephonically if they have at least one vitrified blastocyst with Gardner score ≥ 3BB. After receiving oral and written information, patients interested in participating in the study are scheduled for a visit at the fertility clinic on day 2-5 of the first cycle following oocyte retrieval. Here, they will receive further information about the project and have the opportunity to pose questions before signing the informed consent forms. Each participant can be included once, and in the first mNC-FET cycle following the stimulated cycle only. Care providers enrolling and treating patients in the trial will receive all the information and training necessary for uniform handling of patients across trial sites. All trial sites are highly experienced in performing clinical trials.

Randomization
Randomization will be carried out on day 2-5 of the first menstrual cycle following oocyte retrieval by a member of the research team, using an electronic randomization program. Allocation concealment will be ensured, as the service will not reveal the allocation before the randomization procedure, that is, at the end of the baseline visit. Patients are randomized 1:1 by simple randomization to one of the following groups:

1. FET immediate; mNC-FET in the menstrual cycle immediately following oocyte retrieval and fresh embryo transfer or oocyte retrieval and freeze-all.

1. FET postponed; mNC-FET at least one full menstrual cycle after the fresh embryo transfer or freeze-all cycle, i.e., the first FET following the fresh cycle is not started until the second menstrual bleeding or later.
The intervention arm differs from the standard treatment arm regarding timing of the first mNC-FET following the stimulated cycle only.

**Interventions**

FET is performed in hCG triggered mNC. Patients will be monitored by TVUS in the late follicular phase (day 8-12) of the cycle of treatment (immediate or postponed) to assess the dominant follicle and the endometrium. When the dominant follicle reaches 17-18 mm, ovulation trigger (6500 IU hCG sc.) is timed at 10 pm that evening. If the dominant follicle does not meet the size criteria on cycle day 8-12, further scans are performed until criteria are met. In case a preovulatory follicle cannot be confirmed, or the endometrium appears abnormal, the cycle will be cancelled but the participant will remain included in the study.

Single blastocyst warming and ultrasound guided transfer is performed 6 days after administration of hCG trigger. If logistically required, blastocyst transfer may be performed 7 days after hCG trigger. Plasma hCG-level is measured 16 (+/-1) days after hCG trigger. If spontaneous ovulation occurs in between follicular scans, the FET cycle can be continued if the following criteria are met (i) disappearance or typical change in the shape of the leading follicle, (ii) appropriate rise in plasma-progesterone concentration (>4.8 nmol/l)[1]. In case of spontaneous ovulation, blastocyst transfer can be performed 4-5 days later.

**Data collection**

An overview of study visits is depicted in Table 1. Patient- and treatment related data are collected at all time points; (1) baseline (day 2-5 of the cycle immediately following oocyte retrieval, all patients) (2) cycle day 2-5 of the treatment cycle in the postponed group (3) day of hCG trigger (4) early luteal phase (hCG trigger+4) (5) day of blastocyst transfer (hCG trigger+6) (6) mid-luteal phase (hCG trigger+11) (7) day of pregnancy testing (hCG trigger+16). In case of pregnancy and delivery, data will be collected from the patient’s medical records as well as the new-born child’s birth records for registration of obstetric and neonatal outcomes up to 1 year after delivery. Data on quality of life and psychosocial status is digitally obtained at timepoint 1 (immediate group) or 2 (postponed group) and 6 by validated self-reported surveys expressed by Likert based 5-scale items. If the woman has a partner, he or she will be asked to fill out separate questionnaires at the same timepoints. Any protocol deviations or unintended effects of trial conduct will be registered.
All affiliated personnel will be trained in data collection and entering, handling of discrepancies in data and in procedures to be conducted during study visits. Data collection forms can be obtained by contacting the steering committee of the study.

**Blood sample collection**

Blood samples are collected as outlined in Table 1. Consecutive analyses including LH, FSH, progesterone and oestradiol are measured at all time-points. Sample collection at time points 4 and 6 is for patients recruited at Rigshospitalet only. Plasma hCG is measured at baseline and 16(± 1) days after administration of hCG trigger. Blood used for consecutive analyses will be destroyed after analysis as a part of the daily laboratory routine.

**Research biobank and biobank for future research projects**

In addition to the samples for consecutive analyses, blood samples of a total of 12 ml (whole blood, serum and plasma) will be drawn at every sampling occasion and stored in a -20/-80 degrees Celsius freezer at Rigshospitalet. The samples will be identified by anonymous subject ID-numbers to maintain participant confidentiality. Samples may be used as backups for consecutive analyses in the present study in case of missing samples or errors of analysis or saved in a biobank for possible future research projects. Patients are asked to sign a separate informed consent form for storage of blood samples in a biobank for future research. Future projects will require additional approvals from the Danish Scientific Ethics Committee. If samples are not used, they will be destroyed according to the rules of destruction of biological material after end of the study or no later than five years after inclusion of the last patient.

**Transvaginal ultrasound**

TVUS is performed according to clinical routine in FET cycles. At baseline and cycle day 2-5 of the postponed treatment cycle, TVUS is used to determine endometrial thickness and number of antral follicles. In the late follicular phase, i.e. cycle day 8-12 depending on the length of the patient’s menstrual cycle, endometrial thickness and size of the dominant follicle is estimated. TVUS is repeated until the dominant follicle reaches 17-18 mm, fulfilling the criteria for hCG-trigger. On the day of hCG trigger, thickness, echogenicity and presence of a trilaminar structure of the endometrium, as well as the number, size and echogenicity of follicular ovarian structures, is recorded. In case of conception, an early pregnancy scan will be performed at 7-8 weeks of gestation to assess foetal viability and crown-rump length.
In order to compare ovarian morphology of the first cycle immediately following oocyte retrieval to the standard postponed cycle, a number of parameters, including ovarian volume and size and appearance of follicular structures >10 mm, will be assessed with 2- and 3D TVUS at cycle day 2-5 of the treatment cycle and at the day of hCG-trigger. 2D scans will be performed for all participants. 3D scans will be performed on a subgroup of participants at the same time points.

**Data management**

In accordance with the written consent signed by all study participants, patient files can be directly accessed by the research group and regulatory authorities to follow up on health conditions of critical relevance to the study, as well as to perform intern- and quality control. Data will be transferred to an electronic case report form in REDCap; a secure platform for building and managing online databases. REDCap is based on anonymous subject identification numbers used in the trial and has a full audit trail. For numerical data, intervals are programmed to detect severe typing errors. The platform is secured with password-protected access systems. Data are backed up daily and stored on a server located in a locked facility in Denmark. Printed documents containing identifying information will be stored in a separate, locked file in an area with limited access. A GCP-trained study nurse, not otherwise involved in the project, will review the source documents as needed, to determine whether data reported in REDCap are complete and accurate. The monitoring nurse will audit overall quality of data collection and confirm that the centre has complied with the requirements of the protocol. The data handling plan is approved by the regional centre for data review. Data will be handled according to Danish law on personal data protection in accordance with the General Data Protection Regulation. Data processing agreement forms between the primary (Rigshospitalet) and secondary trial sites will be compiled. The complete dataset will be accessible by investigators at Rigshospitalet and the monitoring nurse only.

**Data sharing plan**

Data from the trial will be shared according to the ICJME guidelines. On request, data can be shared with parties presenting relevant aims for the use of data. Purposes and financial aspects of the other party must be approved by the steering committee of the “FET-immediate” research team. Data will not be shared with groups presenting research projects with the same aims or purposes as the present study's. No data will be shared until three months after publication of
papers reporting the primary and secondary outcomes of the trial. Any new research project must be approved by Danish authorities. The requesting party will cover the costs for data sharing.

**Objectives**

**Primary objective**

The primary objective of the study is to investigate if immediate single blastocyst transfer in mNC-FET is non-inferior to standard postponed single blastocyst transfer in mNC-FET in terms of LBR. Intention-to-treat (ITT) and per protocol (PP) analyses will be performed with a non-inferiority margin of 10%.

**Secondary objectives**

Assessment of the following endpoints in the immediate versus postponed group:

1. LBR per blastocyst transfer
2. Positive hCG rate
3. Ongoing pregnancy rate
4. Miscarriage rate
5. Cancelled cycle rate
6. Reasons for cycle postponement or cancellation
7. Day of ovulation calculated from the first day of menstrual bleeding
8. Endocrinology of the luteal phase by means of hormone levels at pre-defined timepoints
9. Number of ovarian follicular structures >10 mm at baseline and on the day of hCG-trigger
10. Time-to-pregnancy from the start of ovarian stimulation in the fresh cycle to the date of ongoing pregnancy
11. Time-to-live birth from the start of ovarian stimulation in the fresh cycle to the date of delivery
12. Pregnancy related complications including pre-eclampsia, pregnancy related hypertension, medically assisted delivery and postpartum haemorrhage (>1000 ml)
13. Neonatal outcomes including preterm birth, low birth weight, small-for-gestational age, large-for-gestational age and perinatal mortality
14. Quality of life/patient satisfaction
Non-inferiority design and power calculation

The rationale for using a non-inferiority trial design is that FET immediate, as the new treatment, is expected to yield a similar LBR while offering important advantages over the present standard treatment (FET postponed) in terms of shorter time-to-pregnancy, convenience for the patients, and lower costs due to shorter freezing time. We consider a non-inferiority margin of 10% clinically relevant. We expect a LBR of 25% per randomized study participant after postponed single blastocyst transfer in mNC-FET, which is considered the standard treatment. If there is truly no difference between the standard- and intervention treatment (25% in both groups) 464 patients (n=232 in each group) are required to be 80% sure that the upper limit of a one-sided 95% confidence interval (or equivalently a 90% two-sided confidence interval) will exclude a difference in favour of the standard group of more than 10%.

Dropouts and cancelled cycles

Dropouts are defined as randomized women who, at their own initiative, decide to leave the study. Dropouts will be replaced by inclusion of a corresponding number of patients until n= 232 participants is reached in both groups. Cancelled cycles are defined as randomized women who have their cycle cancelled because a dominant follicle cannot be confirmed up to and including cycle day 21, suspicion of endometrial pathology on TVUS or in case the thawed blastocyst does not survive. Numbers and reasons for dropout and cancellation will be tabulated for the two treatment groups and descriptive tables will be compiled for comparison of characteristics of dropouts, cancelled cycles and completers within and between the groups. We anticipate a dropout rate of at most 5% and a cancellation rate of at most 5%. In case of a differential or larger than expected dropout or cancellation rate, potential biases will be discussed along with any discrepancies between the results of the ITT, PP and per-transfer analyses and conclusions will be drawn accordingly.

Statistical analysis and interpretation of data

ITT analyses include dropouts and cancelled cycles, PP analyses include cancelled cycles but not dropouts, and per-transfer analyses exclude both dropouts and cancelled cycles. Differences in LBR will be evaluated by means of risk differences with one-sided 95% confidence intervals (CI) (or equivalently two-sided 90% CI). Non-inferiority will be concluded if the CI excludes a difference of more than 10% in favour of the present standard treatment (postponed FET) in ITT and PP.
analyses. Difference in LBR per-transfer will be assessed as a secondary outcome by risk
difference with 95% CI. Rate of positive hCG, ongoing pregnancy, miscarriage and cancelled
cycles will be assessed by risk differences with 95% CI in ITT, PP and per-transfer analyses as
outlined for LBR. Mean day of ovulation and mean levels of hormones will be compared with T-
test. Hormone levels known to have a skewed distribution will be log-transformed prior to analysis.
Number of ovarian follicular structures >10 mm will be assessed with χ²-test in a PP analysis.
Time-to-pregnancy and live-birth per delivery will be compared in Kaplan-Meier plots and using
log-rank test. Rates of pregnancy-related complications and adverse neonatal outcomes per
delivery will be assessed using Fisher’s exact test. Data on quality of life and psychosocial status
will be obtained in a validated self-reported survey expressed by Likert based 5-scale items and
compared by non-parametric Mann-Whitney U-test. Any missing data will be handled using
pairwise deletion. Statistical analyses will be performed using R.

Feasibility
With an inclusion period of four years, it is feasible to include the desired number of patients. The
collaboration between several large trial sites in Denmark, will secure an extensive pool of eligible
participants.

Patient and public involvement statement
The study was formulated without patient involvement. However, the research question has
repeatedly been raised by patients failing to get pregnant after fresh embryo transfer and patients
receiving freeze-all. The study results will be disseminated to participants on request by a treating
doctor at the fertility clinic.

Ethics and dissemination
The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark (J-nr.: H-19086300). Any modifications to the protocol which may impact on the administration, design, conduct or safety of the study will require a formal amendment to the committee. Data will be handled according to Danish law on personal data protection in accordance with the General Data Protection Regulation. Details on data management are given elsewhere in this paper. In order to
increase transparency in the research, the study protocol is published and the study is registered at clinicaltrials.gov (NCT04748874).

The safety of the participants of the trial is considered high. The intervention differs from standard treatment solely regarding timing of the first mNC-FET following a fresh IVF/ICSI cycle and we do not anticipate any timing-related risks in performing immediate instead of postponed mNC-FET.

Apart from extra visits; baseline visit for all participants; day 2-5 of treatment cycle for participants randomized to postponed FET; early- and mid-luteal phase for participants enrolled at Rigshospitalet, blood samples (which is not standard clinical practice in FET cycles in most Danish clinics, except for pregnancy testing after transfer) and assessment of ovarian morphology, performed in continuation with the standard care ultrasound scans, there will be no discomfort or harm done to the patients. Treatment with ovulation trigger is according to conventional IVF procedure and the most common side-effects are fatigue, gastro-intestinal discomfort and headache. When drawing blood, patients may experience pain and discomfort and a smaller bruise may appear. There will be no additional financial expenses for study participants, except for transportation costs. Study participants will not receive economical compensation for participating in the study. A potential benefit of participation is that monitoring of endocrinology in the luteal phase may uncover suboptimal conditions for implantation and suggest future changes in the individual treatment strategy.

Results of this study will be disseminated by publication in scientific journals and at clinicaltrials.gov. Results will be presented at national as well as international scientific meetings and published in high-impact peer-reviewed international scientific journals targeting reproductive medicine. Results of common interest will be reported in public press.

A major strength of this study is its multicentre randomized controlled design, focusing on mNC-FET and single blastocyst transfer. To further improve the study method, a double blinded design was considered. However, double blinding would not be possible since the ultrasound appearance immediately after a stimulated cycle differs from that of a natural cycle, a difference that presumably would be recognizable to a fertility doctor. Further, the timing of FET after oocyte pick-up would be apparent to the study participants. Due to these facts, as well as the feasibility in daily
clinical practice, it was decided to keep the study non-blinded. Other strengths of this study are the
high generalisability of results and the transparency in research.

Robust evidence regarding the optimal timing of FET following ovarian stimulation is yet lacking.
As previously discussed, two systematic reviews and meta-analyses regarding timing of FET have
recently been published. Both reviews refute the current standard practice of postponing FET for at
least one menstrual cycle following oocyte retrieval. The reviews are based on retrospective data;
hence, the presence of selection bias is apparent. Particularly, lack of transparency regarding
cancellation rates may increase the risk of selection bias, by means of women with a good
prognosis, in favour of immediate FET. To this date, no randomized controlled trials on the subject
have been published, the reason why this study is highly relevant. If our hypothesis that immediate
mNC-FET is non-inferior to standard postponed mNC-FET is scientifically supported, we can
minimize time to transfer, pregnancy and delivery, saving patients from burdensome waiting time.
With no delay in time to transfer, patients may be encouraged to choose a freeze-all strategy,
thereby reducing the risk of ovarian hyperstimulation syndrome which is one of the most severe
side-effects of IVF, often leading to extended hospital admissions. The results of this study can be
implemented immediately after publication for the sake and time saving of our patients. Further,
storage time of frozen embryos will be reduced, saving costs for fertility clinics. Thus, with this
study we hope to set new national as well as international standards in IVF.

Author’s contributions
KL, AP, JF and SB participated in the conception, design, writing and editing of the study protocol.
SB wrote the first draft and KL, AP, SB, JF, MS, NP, ECL, NH, MF, JWB, SZ, NCF, BN, LFA and
PH and SZ were involved in the critical revision of this paper. All authors have approved the final
version of the manuscript prior to submission.

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Disclaimer
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execution, analyses, interpretation of data, or decision to submit results. Merck KGaA, Darmstadt,
Germany reviewed the manuscript for medical accuracy only before journal submission. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

Competing interest’s statement

AP and KL has received a research grant from Merck supporting the present study. SB has received a research grant from Rigshospitalet’s research fund supporting the present study. As a partner of Reprounion, AP has received a grant from Ferring as well as a research grant from Gedeon Richter. AP has received consulting fees from Novo Nordisk, honoraria from Merck and Ferring, honoraria as well as support for attending meetings from Gedeon Richter. KL has received honoraria from pharmakon and support for attending meetings from Gedeon Richter. PH has received unrestricted grants from Merck, Gedeon Richter, IBSA, Ferring and MSD as well as honoraria for lectures from Merck and Gedeon Richter. MF has received a research grant from Gedeon Richter. NCF has received an unrestricted grant from Gedeon Richter, honoraria for lectures from Merck and support for attending meetings by Ferring, Merck and Gedeon Richter. Since 2018, NCF is head of the steering committee for Danish fertility guidelines.
List of abbreviations

AC – Artificial Cycle
FET – Frozen Embryo Transfer
hCG – human Chorionic Gonadotropin
IVF – In Vitro Fertilization
LH – Luteinizing Hormone
mNC – modified Natural Cycle
NC – Natural Cycle
tNC – true Natural Cycle
TVUS – Transvaginal Ultrasound

Definitions

Live birth - delivery of a live born child ≥22+0 weeks of gestation
Positive hCG - serum β-hCG >5 IU/L
Ongoing pregnancy - visualization of an intrauterine gestational sac containing a foetus with heartbeat, by TVUS at 7-8 weeks of gestation

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Table 1
Overview of study visits

| Baseline<sup>a</sup> | CD 2-5 immediate treatment cycle | CD 2-5 postponed treatment cycle | Late follicular phase CD 8-12 | hCG trigger trigger+4 | Early luteal phase trigger+6 | Blastocyst transfer trigger +6 | Mid luteal phase trigger+11 | Pregnancy testing trigger +16 | GA 7-8 | Follow-up 1 year |
|----------------------|---------------------------------|---------------------------------|-----------------------------|------------------------|-----------------------------|-------------------------------|-------------------------------|--------------------------------|--------|-----------------|
| Information and counselling | x | | | | | | | | | |
| Signing of informed consent | x | | | | | | | | x<sup>e</sup> | | |
| Treatment related data collection | x | x<sup>c</sup> | x | x | x<sup>d</sup> | x | x<sup>d</sup> | x | x | x | |
| Randomisation | x | | | | | | | | | | |
| Transvaginal ultrasound scan | x | x<sup>c</sup> | x | x | | | | | x | |
| Ovarian morphology UL scan | x | x<sup>c</sup> | x | | | | | | | |
| Blood sample | x | x<sup>c</sup> | x | x<sup>d</sup> | x | x<sup>d</sup> | x | x | |
| Quality of life questionnaire | x<sup>b</sup> | x<sup>c</sup> | x | x<sup>d</sup> | x | x<sup>d</sup> | x | x | |

<sup>a</sup>All participants included in the study are invited to a baseline visit which is also day 2-5 of the treatment cycle for immediate FET.

<sup>b</sup>Participants randomized to immediate FET only.

<sup>c</sup>Participants randomized to postponed FET only.

<sup>d</sup>Only at Rigshospitalet

<sup>e</sup>in case of pregnancy custodians sign informed consent regarding access to the future child’s records
Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

**Instructions to authors**

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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| Reporting Item | 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

Trial registration:  

**#2b** All items from the World Health Organization Trial data set

Registration Data Set

Separate file

Protocol version  

**#3** Date and version identifier

All pages

Funding  

**#4** Sources and types of financial, material, and other support

Roles and responsibilities:  

**#5a** Names, affiliations, and roles of protocol contributors

Roles and responsibilities:  

**#5b** Name and contact information for the trial sponsor

Roles and responsibilities:  

**#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

Roles and responsibilities:  

**#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)

**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

#6b Explanation for choice of comparators

**Objectives**

#7 Specific objectives or hypotheses

**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, non-inferiority, exploratory)

**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
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)

Interventions: **#11a** Interventions for each group with sufficient detail to allow description replication, including how and when they will be administered

Interventions: **#11b** Criteria for discontinuing or modifying allocated modifications interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)

Interventions: **#11c** Strategies to improve adherence to intervention adherance protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)

Interventions: **#11d** Relevant concomitant care and interventions that are permitted or prohibited during the trial concomitant care

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
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)

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

Recruitment  #15 Strategies for achieving adequate participant enrolment to reach target sample size

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

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

**Allocation:** #16c Who will generate the allocation sequence, who will implement enrolment, and who will assign participants to interventions

**Blinding (masking):** #17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how

Blinding (masking): #17b If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial

**Methods: Data collection, management, and analysis**

Data collection plan #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
Data collection plan: **#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

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

Statistics: outcomes **#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

Statistics: additional analyses **#20b** Methods for any additional analyses (eg, subgroup and adjusted analyses)

Statistics: analysis population and missing data **#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)

**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.

| Data monitoring: | 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 | n/a |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Interim analysis| #21b                                                                                                                                                                                            |     |
| Harms           | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 6   |
| #22             |                                                                                                                                                                                                  |     |
| Auditing        | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 7   |
| #23             |                                                                                                                                                                                                  |     |
| Ethics and      | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval                                                                                                                                                                     |
| Dissemination   | #24                                                                                                                                                                                            | 10  |
| Research ethics |                                                                                                                                                                                                  |     |
| Approval        |                                                                                                                                                                                                  |     |
| Protocol        | 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) | 10  |
| Amendments      | #25                                                                                                                                                                                            |     |
| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) |
|------------------|------|----------------------------------------------------------------------------------------------------------------------------------|
| Consent or assent: #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable |
| 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 |
| Declaration of interests | #28 | Financial and other competing interests for principal investigators for the overall trial and each study site |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators |
| 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 |
| Dissemination policy: trial results | #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 |
Dissemination policy: **#31b** Authorship eligibility guidelines and any intended use of professional writers

Dissemination policy: **#31c** Plans, if any, for granting public access to the full reproducible protocol, participant-level dataset, and statistical code

**Appendices**

Informed consent **#32** Model consent form and other related documentation given to participants and authorised surrogates Separate file

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

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Immediate versus postponed single blastocyst transfer in modified natural cycle frozen embryo transfer (mNC-FET): a study protocol for a multicentre randomized controlled trial

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Immediate versus postponed single blastocyst transfer in modified natural cycle frozen embryo transfer (mNC-FET): a study protocol for a multicentre randomized controlled trial

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Abstract

Introduction

Today, it is widespread practice to postpone frozen embryo transfer (FET) in a modified natural cycle (mNC) for at least one menstrual cycle after oocyte retrieval and failed fresh embryo transfer or freeze-all. The rationale behind this practice is the concern that suboptimal ovarian, endometrial or endocrinological conditions following ovarian stimulation may have a negative impact on endometrial receptivity and implantation. However, two recent systematic reviews and meta-analyses based on retrospective data did not support this practice. As unnecessary delay in time to transfer and pregnancy should be avoided, the aim of this study is to investigate if immediate single blastocyst transfer in mNC-FET is non-inferior to standard postponed single blastocyst transfer in mNC-FET in terms of live birth rate (LBR).

Methods and analysis

Multicentre randomized controlled non-blinded trial including 464 normo-ovulatory women aged 18-40 years undergoing single blastocyst mNC-FET after a failed fresh- or freeze-all cycle.

Participants are randomized 1:1 to either FET in the first menstrual cycle following the stimulated cycle (immediate FET) or FET in the second or subsequent cycle following the stimulated cycle.
(postponed FET). The study is designed as a non-inferiority trial and primary analyses will be performed as intention-to-treat and per protocol.

Ethics and dissemination

Ethical approval has been granted by the Scientific Ethical Committee of the Capital Region of Denmark (J-nr.: H-19086300). Data will be handled according to Danish law on personal data protection in accordance with the General Data Protection Regulation. Participants will complete written consent forms regarding participation in the study and storage of blood samples in a biobank for future research. The study will be monitored by a GCP-trained study nurse not otherwise involved in the study. The results of this study will be disseminated by publication in international peer-reviewed scientific journals.

Registration details

The study is registered at ClinicalTrials.gov NCT04748874.
Strengths and limitations of this study

- This is the first randomized controlled trial comparing live birth rates after immediate versus postponed single blastocyst transfer in modified natural cycle (mNC) frozen embryo transfer (FET)
- Including normo-ovulatory women aged 18-40 years undergoing single blastocyst mNC-FET after a failed fresh or freeze-all cycle, thus securing high generalizability and applicability of study results
- Non-inferiority design with intention-to-treat and per protocol analyses performed with a non-inferiority margin of 10%
- Publication of study protocol and trial registration secures transparency in research
- Non-blinded to patients, researchers and clinicians due to nature of study
Introduction

In recent years, pregnancy rates after frozen embryo transfer (FET) have improved and are now approaching, or even exceeding, those obtained after fresh embryo transfer (1). Thus, FET has become increasingly important in the field of assisted reproductive techniques and can be applied after failed fresh embryo transfer or after elective embryo freezing (freeze-all) on various indications, among them risk of ovarian hyperstimulation syndrome (2). Today, it is standard practice to postpone FET in a modified natural cycle for at least one menstrual cycle after controlled ovarian stimulation and fresh embryo transfer or freeze-all. The rationale behind this practice is the concern that suboptimal ovarian, endometrial and endocrinological conditions after ovarian stimulation may have a negative effect on endometrial receptivity and implantation (3–6) and may increase the risk of small for gestational age babies and preterm delivery (7). However, the elective deferral of FET is an empirical approach founded on assumptions rather than evidence and may unnecessarily delay time to pregnancy and increase costs for embryo freezing.

In women with regular menstrual cycles, FET is often performed in natural cycles (NC) instead of oestrogen- and progesterone supplemented programmed cycles. Advantages of a natural approach include less disruption of hormonal balance and receptivity of the endometrium (8,9) as well as minimal use of drugs, hence fewer side effects and reduced treatment costs. NC are subdivided into true natural cycles (tNC) or human choriogonadotropin (hCG) triggered modified natural cycles (mNC). In a tNC, close ultrasonic and endocrine monitoring is required throughout the follicular phase to determine the point of spontaneous ovulation. In mNC-FET, ultrasonic monitoring is generally started in the late follicular phase and ovulation trigger (hCG) is administered when the leading follicle reaches 17-18 mm, the timepoint at which, in the majority of women, the LH surge is induced in the natural cycle (10,11). Reproductive outcomes in tNC- and mNC-FET seem to be comparable (12,13) but mNC-FET is often considered more patient-friendly.

Recently, two systematic reviews and meta-analyses regarding timing of FET have been published, comparing pregnancy outcomes between FET performed in the first menstrual cycle after ovarian stimulation and oocyte retrieval (immediate FET) and FET in the second or subsequent cycle (postponed FET). The reviews are based on retrospective data including a variety of FET protocols, hence, the presence of selection bias is apparent and the quality of
evidence is low. Despite a significant overlap in studies included in the reviews, the results differ slightly, probably due to inclusion of unadjusted (14) versus adjusted (15) results. Huang et al. reported no significant association between timing of FET and pregnancy outcomes; clinical pregnancy rate (CPR) (relative risk (RR) 0.94 (95% CI 0.87–1.03)) and live birth rate (LBR) (RR 0.94 (95% CI 0.85–1.03)) while Bergenheim et al. found a slightly higher CPR (adjusted odds ratio (aOR) 1.22 (95% CI 1.07-1.39)) and LBR (aOR 1.20 (95% CI 1.01-1.44)) in immediate versus postponed FET. Regardless, the standard practice of routinely postponing mNC-FET for at least one menstrual cycle following IVF/ICSI does not seem to be scientifically supported. As unnecessary delay in time to transfer and pregnancy should be avoided, the aim of this study is to investigate, in a multicentre randomized controlled trial, if immediate single blastocyst transfer in mNC-FET is non-inferior to standard postponed single blastocyst transfer in mNC-FET in terms of LBR.

Methods and analysis

Study design
The study is designed as a multicentre randomized controlled non-blinded trial including fertility clinics in Denmark. All clinics are part of an academic hospital setting performing standardized treatments according to the public health care system in Denmark. When determined, a complete list of study sites can be obtained by contacting the steering committee of the study. Patient enrolment is expected to begin in March 2021 and continue until December 2024. We adhered to the SPIRIT recommendations (16) when drafting this protocol.

Eligibility criteria
Inclusion criteria: patients eligible for FET in a modified natural cycle; 18-40 years; regular menstrual cycle (23-35 days); ≥1 vitrified blastocyst with Gardner score ≥ 3BB at vitrification on day 5 or 6 after oocyte retrieval. Exclusion criteria: uterine malformations or presence of hydrosalpinx; submucosal uterine myomas; uterine polyps; severe ovarian hyperstimulation syndrome (OHSS) during the fresh cycle (defined as need for ascites drainage and/or hospital admission due to OHSS); oocyte donation; testicular sperm aspiration; male or female HIV or Hepatitis B/C; preimplantation genetic testing in the fresh cycle; contraindication or allergy to standard fertility medication (i.e., hCG used for ovulation trigger). Patients can withdraw from
participation in the study at any time without accounting for any reason. Further, participation can be interrupted by a treating or non-treating doctor if (i) the patient’s general condition contradicts participation in the study or (ii) protocol violation which the investigator considers having influence on the study outcome. After withdrawal from the study, patients may continue receiving standard treatment at the fertility clinic.

**Study population and recruitment**

The study population consists of patients undergoing mNC-FET after a fresh IVF/ICSI cycle that did not result in pregnancy, or after an elective freeze-all cycle. All eligible patients interested in receiving information about the study, will be contacted telephonically if they have at least one vitrified blastocyst with Gardner score ≥ 3BB. After receiving oral and written information, patients interested in participating in the study are scheduled for a visit at the fertility clinic on day 2-5 of the first cycle following oocyte retrieval. Here, they will receive further information about the project and have the opportunity to pose questions before signing the informed consent forms. Each participant can be included once, and in the first mNC-FET cycle following the stimulated cycle only. Care providers enrolling and treating patients in the trial will receive all the information and training necessary for uniform handling of patients across trial sites. All trial sites are highly experienced in performing clinical trials.

**Randomization**

Randomization will be carried out on day 2-5 of the first menstrual cycle following oocyte retrieval by a member of the research team, using an electronic randomization program. Allocation concealment will be ensured, as the service will not reveal the allocation before the randomization procedure, that is, at the end of the baseline visit. Patients are randomized 1:1 by simple randomization to one of the following groups:

1. **FET immediate;** mNC-FET in the menstrual cycle immediately following oocyte retrieval and fresh embryo transfer or oocyte retrieval and freeze-all.

1. **FET postponed;** mNC-FET at least one full menstrual cycle after the fresh embryo transfer or freeze-all cycle, i.e., the first FET following the fresh cycle is not started until the second menstrual bleeding or later.
The intervention arm differs from the standard treatment arm regarding timing of the first mNC-FET following the stimulated cycle only. The first menstrual cycle refers to the initial vaginal bleeding after egg retrieval and fresh transfer or freeze-all.

**Interventions**

FET is performed in hCG triggered mNC. Patients will be monitored by TVUS in the late follicular phase (day 8-12) of the cycle of treatment (immediate or postponed) to assess the dominant follicle and the endometrium. When the dominant follicle reaches 17-18 mm, ovulation trigger (6500 IU hCG sc.) is timed at 10 pm that evening. If the dominant follicle does not meet the size criteria on cycle day 8-12, further scans are performed until criteria are met. In case a preovulatory follicle cannot be confirmed, or the endometrium appears abnormal, the cycle will be cancelled but the participant will remain included in the study.

Single blastocyst warming and ultrasound guided transfer is performed 6 days after administration of hCG trigger. If logistically required, blastocyst transfer may be performed 7 days after hCG trigger. Plasma hCG-level is measured 16 (+/-1) days after hCG trigger. If spontaneous ovulation occurs in between follicular scans (one, or maximum two, days apart) the FET cycle can be continued if one or both of the following criteria are met: (i) disappearance or typical change in the shape of the leading follicle, (ii) appropriate rise in plasma-progesterone concentration (>4.8 nmol/l) (17). In case of spontaneous ovulation, blastocyst transfer can be performed 4-5 days after the TVUS, where the spontaneous ovulation was detected.

**Data collection**

An overview of study visits is depicted in Table 1. Patient- and treatment related data are collected at all time points; (1) baseline (day 2-5 of the cycle immediately following oocyte retrieval, all patients) (2) cycle day 2-5 of the treatment cycle in the postponed group (3) day of hCG trigger (4) early luteal phase (hCG trigger+4) (5) day of blastocyst transfer (hCG trigger+6) (6) mid-luteal phase (hCG trigger+11) (7) day of pregnancy testing (hCG trigger+16). In case of pregnancy and delivery, data will be collected from the patient’s medical records as well as the new-born child’s birth records for registration of obstetric and neonatal outcomes up to 1 year after delivery. Data on quality of life and psychosocial status is digitally obtained at timepoint 1 (immediate group) or 2 (postponed group) and 6 by validated self-reported surveys expressed by Likert based 5-scale.
items. If the woman has a partner, he or she will be asked to fill out separate questionnaires at the
same timepoints. Any protocol deviations or unintended effects of trial conduct will be registered.
All affiliated personnel will be trained in data collection and entering, handling of discrepancies in
data and in procedures to be conducted during study visits. Data collection forms can be obtained
by contacting the steering committee of the study.
## Table 1

### Overview of study visits

|                      | Baseline<sup>a</sup> CD 2-5 immediate treatment cycle | CD 2-5 postponed treatment cycle | Late follicular phase CD 8-12 | hCG trigger | Early luteal phase trigger+4 | Blastocyst transfer trigger +6 | Mid luteal phase trigger+11 | Pregnancy testing trigger +16 | GA 7±8 | Follow-up 1 year |
|----------------------|--------------------------------------------------------|----------------------------------|-------------------------------|-------------|-----------------------------|-------------------------------|-------------------------------|--------------------------------|--------|------------------|
| **Information and counselling** |                                         | x                                 |                               |             |                             |                               |                               |                                 |        |                  |
| **Signing of informed consent** |                                         | x                                 |                               |             |                             |                               |                               |                                 |        |                  |
| **Treatment related data collection** |                                         | x                                 | x                             | x<sup>d</sup> | x                            | x                             | x                             | x<sup>d</sup>                      |        |                  |
| **Randomisation** |                                         | x                                 | x<sup>d</sup> | x          | x                            | x                             | x                             | x<sup>d</sup>                      |        |                  |
| **Transvaginal ultrasound scan** |                                         | x                                 | x<sup>d</sup> | x<sup>d</sup> | x                            | x                             | x                             | x<sup>d</sup>                      |        |                  |
| **Ovarian morphology UL scan** |                                         | x                                 | x<sup>d</sup> | x          | x                            | x                             | x                             | x<sup>d</sup>                      |        |                  |
| **Blood sample** |                                         | x                                 | x<sup>d</sup> | x          | x                            | x                             | x                             | x<sup>d</sup>                      |        |                  |
| **Quality of life questionnaire** |                                         | x                                 | x<sup>d</sup> | x<sup>d</sup> | x                            | x                             | x                             | x<sup>d</sup>                      |        |                  |

<sup>a</sup>All participants  
<sup>b</sup>Participants randomized to immediate FET only.  
<sup>c</sup>Participants randomized to postponed FET only.  
<sup>d</sup>Only at Rigshospitalet  
<sup>e</sup>In case of pregnancy custodians sign informed consent regarding access to the future child's records
Blood sample collection
Blood samples are collected as outlined in Table 1. Consecutive analyses including LH, FSH, progesterone and oestradiol are measured at all time-points. Sample collection at time points 4 and 6 is for patients recruited at Rigshospitalet only. Plasma hCG is measured at baseline and 16(± 1) days after administration of hCG trigger. Blood used for consecutive analyses will be destroyed after analysis as a part of the daily laboratory routine.

Research biobank and biobank for future research projects
In addition to the samples for consecutive analyses, blood samples of a total of 12 ml (whole blood, serum and plasma) will be drawn at every sampling occasion and stored in a -20/-80 degrees Celsius freezer at Rigshospitalet. The samples will be identified by anonymous subject ID-numbers to maintain participant confidentiality. Samples may be used as backups for consecutive analyses in the present study in case of missing samples or errors of analysis or saved in a biobank for possible future research projects. Patients are asked to sign a separate informed consent form for storage of blood samples in a biobank for future research. Future projects will require additional approvals from the Danish Scientific Ethics Committee. If samples are not used, they will be destroyed according to the rules of destruction of biological material after end of the study or no later than five years after inclusion of the last patient.

Transvaginal ultrasound
TVUS is performed according to clinical routine in FET cycles. At baseline and cycle day 2-5 of the postponed treatment cycle, TVUS is used to determine endometrial thickness and number of antral follicles. In the late follicular phase, i.e. cycle day 8-12 depending on the length of the patient’s menstrual cycle, endometrial thickness and size of the dominant follicle is estimated. TVUS is repeated until the dominant follicle reaches 17-18 mm, fulfilling the criteria for hCG-trigger. On the day of hCG trigger, thickness, echogenicity and presence of a trilaminar structure of the endometrium, as well as the number, size and echogenicity of follicular ovarian structures, is recorded. In case of conception, an early pregnancy scan will be performed at 7-8 weeks of gestation to assess foetal viability and crown-rump length.

In order to compare ovarian morphology of the first cycle immediately following oocyte retrieval to the standard postponed cycle, a number of parameters, including ovarian volume and size and appearance of follicular structures >10 mm, will be assessed with 2- and 3D TVUS at cycle day 2-5.
of the treatment cycle and at the day of hCG-trigger. 2D scans will be performed for all participants. 3D scans will be performed on a subgroup of participants at the same time points.

Data management
In accordance with the written consent signed by all study participants, patient files can be directly accessed by the research group and regulatory authorities to follow up on health conditions of critical relevance to the study, as well as to perform intern- and quality control. Data will be transferred to an electronic case report form in REDCap; a secure platform for building and managing online databases. REDCap is based on anonymous subject identification numbers used in the trial and has a full audit trail. For numerical data, intervals are programmed to detect severe typing errors. The platform is secured with password-protected access systems. Data are backed up daily and stored on a server located in a locked facility in Denmark. Printed documents containing identifying information will be stored in a separate, locked file in an area with limited access. A GCP-trained study nurse, not otherwise involved in the project, will review the source documents as needed, to determine whether data reported in REDCap are complete and accurate. The monitoring nurse will audit overall quality of data collection and confirm that the centre has complied with the requirements of the protocol. The data handling plan is approved by the regional centre for data review. Data will be handled according to Danish law on personal data protection in accordance with the General Data Protection Regulation. Data processing agreement forms between the primary (Rigshospitalet) and secondary trial sites will be compiled. The complete dataset will be accessible by investigators at Rigshospitalet and the monitoring nurse only.

Data sharing plan
Data from the trial will be shared according to the ICJME guidelines. On request, data can be shared with parties presenting relevant aims for the use of data. Purposes and financial aspects of the other party must be approved by the steering committee of the “FET-immediate” research team. Data will not be shared with groups presenting research projects with the same aims or purposes as the present study’s. No data will be shared until three months after publication of papers reporting the primary and secondary outcomes of the trial. Any new research project must be approved by Danish authorities. The requesting party will cover the costs for data sharing.
Objectives

Primary objective

The primary objective of the study is to investigate if immediate single blastocyst transfer in mNC-FET is non-inferior to standard postponed single blastocyst transfer in mNC-FET in terms of LBR. Intention-to-treat (ITT) and per protocol (PP) analyses will be performed with a non-inferiority margin of 10%.

Secondary objectives

Assessment of the following endpoints in the immediate versus postponed group:

1) LBR per blastocyst transfer
2) Positive hCG rate
3) Ongoing pregnancy rate
4) Miscarriage rate (biochemical and clinical pregnancy loss)
5) Cancelled cycle rate
6) Reasons for cycle postponement or cancellation
7) Day of ovulation calculated from the first day of menstrual bleeding
8) Endocrinology of the luteal phase by means of hormone levels at pre-defined timepoints
9) Number of ovarian follicular structures >10 mm at baseline and on the day of hCG-trigger
10) Time-to-pregnancy from the start of ovarian stimulation in the fresh cycle to the date of ongoing pregnancy
11) Time-to-live birth from the start of ovarian stimulation in the fresh cycle to the date of delivery
12) Pregnancy related complications including pre-eclampsia, pregnancy related hypertension, medically assisted delivery and postpartum haemorrhage (>1000 ml)
13) Neonatal outcomes including preterm birth, low birth weight, small-for-gestational age, large-for-gestational age and perinatal mortality
14) Quality of life/patient satisfaction

Non-inferiority design and power calculation

The rationale for using a non-inferiority trail design is that FET immediate, as the new treatment, is expected to yield a similar LBR while offering important advantages over the present standard treatment (FET postponed) in terms of shorter time-to-pregnancy, convenience for the patients,
and lower costs due to shorter freezing time. We consider a non-inferiority margin of 10% clinically relevant. The power calculation was performed using a computerized algorithm (18). We expect a LBR of 25% per randomized study participant after postponed single blastocyst transfer in mNC-FET, which is considered the standard treatment. If there is truly no difference between the standard- and intervention treatment (25% in both groups) 464 patients (n=232 in each group) are required to be 80% sure that the upper limit of a one-sided 95% confidence interval (or equivalently a 90% two-sided confidence interval) will exclude a difference in favour of the standard group of more than 10%.

**Dropouts and cancelled cycles**

Dropouts are defined as randomized women who, at their own initiative, decide to leave the study. Dropouts will be replaced by inclusion of a corresponding number of patients until n= 232 participants is reached in both groups. Cancelled cycles are defined as randomized women who have their cycle cancelled because a dominant follicle cannot be confirmed up to and including cycle day 21, suspicion of endometrial pathology on TVUS or in case the thawed blastocyst does not survive. Numbers and reasons for dropout and cancellation will be tabulated for the two treatment groups and descriptive tables will be compiled for comparison of characteristics of dropouts, cancelled cycles and completers within and between the groups. We anticipate a dropout rate of at most 5% and a cancellation rate of at most 5%. In case of a differential or larger than expected dropout or cancellation rate, potential biases will be discussed along with any discrepancies between the results of the ITT, PP and per-transfer analyses and conclusions will be drawn accordingly.

**Statistical analysis and interpretation of data**

ITT analyses include dropouts and cancelled cycles, PP analyses include cancelled cycles but not dropouts, and per-transfer analyses exclude both dropouts and cancelled cycles. Differences in LBR will be evaluated by means of risk differences with one-sided 95% confidence intervals (CI) (or equivalently two-sided 90% CI). Non-inferiority will be concluded if the CI excludes a difference of more than 10% in favour of the present standard treatment (postponed FET) in ITT and PP analyses. Difference in LBR per-transfer will be assessed as a secondary outcome by risk difference with 95% CI. Rate of positive hCG, ongoing pregnancy, miscarriage and cancelled cycles will be assessed by risk differences with 95% CI in ITT, PP and per-transfer analyses as
outlined for LBR. Mean day of ovulation and mean levels of hormones will be compared with T-test. Hormone levels known to have a skewed distribution will be log-transformed prior to analysis. Number of ovarian follicular structures >10 mm will be assessed with $\chi^2$-test in a PP analysis. Time-to-pregnancy and live-birth per delivery will be compared in Kaplan-Meier plots and using log-rank test. Rates of pregnancy-related complications and adverse neonatal outcomes per delivery will be assessed using Fisher’s exact test. Data on quality of life and psychosocial status will be obtained in a validated self-reported survey expressed by Likert based 5-scale items and compared by non-parametric Mann-Whitney U-test. Any missing data will be handled using pairwise deletion. Statistical analyses will be performed using R.

**Feasibility**

With an inclusion period of four years, it is feasible to include the desired number of patients. The collaboration between several large trial sites in Denmark, will secure an extensive pool of eligible participants.

**Patient and public involvement statement**

The study was formulated without patient involvement. However, the research question has repeatedly been raised by patients failing to get pregnant after fresh embryo transfer and patients receiving freeze-all. The study results will be disseminated to participants on request by a treating doctor at the fertility clinic.

**Ethics and dissemination**

The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark (J-nr.: H-19086300). Any modifications to the protocol which may impact on the administration, design, conduct or safety of the study will require a formal amendment to the committee. Data will be handled according to Danish law on personal data protection in accordance with the General Data Protection Regulation. Details on data management are given elsewhere in this paper. In order to increase transparency in the research, the study protocol is published and the study is registered at clinicaltrials.gov (NCT04748847).

The safety of the participants of the trial is considered high. The intervention differs from standard treatment solely regarding timing of the first mNC-FET following a fresh IVF/ICSI cycle and we do not anticipate any timing-related risks in performing immediate instead of postponed mNC-FET.
Apart from extra visits; baseline visit for all participants; day 2-5 of treatment cycle for participants randomized to postponed FET; early- and mid-luteal phase for participants enrolled at Rigshospitalet, blood samples (which is not standard clinical practice in FET cycles in most Danish clinics, except for pregnancy testing after transfer) and assessment of ovarian morphology, performed in continuation with the standard care ultrasound scans, there will be no discomfort or harm done to the patients. Treatment with ovulation trigger is according to conventional IVF procedure and the most common side-effects are fatigue, gastro-intestinal discomfort and headache. When drawing blood, patients may experience pain and discomfort and a smaller bruise may appear. There will be no additional financial expenses for study participants, except for transportation costs. Study participants will not receive economical compensation for participating in the study. A potential benefit of participation is that monitoring of endocrinology in the luteal phase may uncover suboptimal conditions for implantation and suggest future changes in the individual treatment strategy.

Results of this study will be disseminated by publication in scientific journals and at clinicaltrials.gov. Results will be presented at national as well as international scientific meetings and published in high-impact peer-reviewed international scientific journals targeting reproductive medicine. Results of common interest will be reported in public press.

A major strength of this study is its multicentre randomized controlled design, focusing on mNC-FET and single blastocyst transfer. To further improve the study method, a double blinded design was considered. However, double blinding would not be possible since the ultrasound appearance immediately after a stimulated cycle differs from that of a natural cycle, a difference that presumably would be recognizable to a fertility doctor. Further, the timing of FET after oocyte pick-up would be apparent to the study participants. Due to these facts, as well as the feasibility in daily clinical practice, it was decided to keep the study non-blinded. Other strengths of this study are the high generalisability of results and the transparency in research.

Robust evidence regarding the optimal timing of FET following ovarian stimulation is yet lacking. As previously discussed, two systematic reviews and meta-analyses regarding timing of FET have recently been published. Both reviews refute the current standard practice of postponing FET for at least one menstrual cycle following oocyte retrieval. The reviews are based on retrospective data;
hence, the presence of selection bias is apparent. Particularly, lack of transparency regarding cancellation rates may increase the risk of selection bias, by means of women with a good prognosis, in favour of immediate FET. To this date, no randomized controlled trials on the subject have been published, the reason why this study is highly relevant. If our hypothesis that immediate mNC-FET is non-inferior to standard postponed mNC-FET is scientifically supported, we can minimize time to transfer, pregnancy and delivery, saving patients from burdensome waiting time. With no delay in time to transfer, patients may be encouraged to choose a freeze-all strategy, thereby reducing the risk of ovarian hyperstimulation syndrome which is one of the most severe side-effects of IVF, often leading to extended hospital admissions. The results of this study can be implemented immediately after publication for the sake and time saving of our patients. Further, storage time of frozen embryos will be reduced, saving costs for fertility clinics. Thus, with this study we hope to set new national as well as international standards in IVF.

Author’s contributions
KL, AP, JF and SB participated in the conception, design, writing and editing of the study protocol. SB wrote the first draft and KL, AP, SB, JF, MS, NP, ECL, NH, MF, JWB, SZ, NCF, BN, LFA and PH and SZ were involved in the critical revision of this paper. All authors have approved the final version of the manuscript prior to submission.

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Disclaimer
The funding sources had no role in the design of this study and will not have any role during its execution, analyses, interpretation of data, or decision to submit results. Merck KGaA, Darmstadt, Germany reviewed the manuscript for medical accuracy only before journal submission. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

Competing interest’s statement
AP and KL has received a research grant from Merck supporting the present study. SB has received a research grant from Rigshospitalet’s research fund supporting the present study. As a
partner of Reprouion, AP has received a grant from Ferring as well as a research grant from Gedeon Richter. AP has received consulting fees from Novo Nordisk, honoraria from Merck and Ferring, honoraria as well as support for attending meetings from Gedeon Richter. KL has received honoraria from pharmakon and support for attending meetings from Gedeon Richter. PH has received unrestricted grants from Merck, Gedeon Richter, IBSA, Ferring and MSD as well as honoraria for lectures from Merck and Gedeon Richter. MF has received a research grant from Gedeon Richter. NCF has received an unrestricted grant from Gedeon Richter, honoraria for lectures from Merck and support for attending meetings by Ferring, Merck and Gedeon Richter. Since 2018, NCF is head of the steering committee for Danish fertility guidelines.
List of abbreviations

AC – Artificial Cycle
FET – Frozen Embryo Transfer
hCG – human Chorionic Gonadotropin
IVF – In Vitro Fertilization
LH – Luteinizing Hormone
mNC – modified Natural Cycle
NC – Natural Cycle
tNC – true Natural Cycle
TVUS – Transvaginal Ultrasound

Definitions

Live birth - delivery of a live born child ≥22+0 weeks of gestation
Positive hCG - serum β-hCG >5 IU/L
Ongoing pregnancy - visualization of an intrauterine gestational sac containing a foetus with heartbeat, by TVUS at 7-8 weeks of gestation

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

**Instructions to authors**

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

| Reporting Item | 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

Trial registration:  #2b  All items from the World Health Organization Trial data set Registration Data Set

Protocol version  #3  Date and version identifier

Funding  #4  Sources and types of financial, material, and other support

Roles and responsibilities:  #5a  Names, affiliations, and roles of protocol contributors

Roles and responsibilities:  #5b  Name and contact information for the trial sponsor

Roles and responsibilities:  #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

Roles and responsibilities:  #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)

Introduction

Background and rationale

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

Background and rationale: choice of comparators

Explanation for choice of comparators

Objectives

Specific objectives or hypotheses

Trial design

Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)

Methods:

Participants, interventions, and outcomes

Study setting

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
| Eligibility criteria | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Interventions:       | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered |
| Interventions:       | 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) |
| Interventions:       | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) |
| Interventions:       | Relevant concomitant care and interventions that are permitted or prohibited during the trial |
| Outcomes             | 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 |
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)

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

Recruitment  #15  Strategies for achieving adequate participant enrolment to reach target sample size

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

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

Allocation:  #16c Who will generate the allocation sequence, who will implement enrolment, and who will assign participants to interventions

Blinding (masking)  #17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how

Blinding (masking):  #17b If blinded, circumstances under which unblinding is emergency permissible, and procedure for revealing a participant’s unblinding allocated intervention during the trial

Methods: Data
collection, management, and analysis

Data collection plan  #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
Data collection plan: #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

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

Statistics: outcomes #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

Statistics: additional analyses #20b Methods for any additional analyses (eg, subgroup and adjusted analyses)

Statistics: analysis population and missing data #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)

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.

Data monitoring: #21b Description of any interim analyses and stopping
interim analysis guidelines, including who will have access to these
interim results and make the final decision to terminate
the trial

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

Auditing #23 Frequency and procedures for auditing trial conduct, if
any, and whether the process will be independent from
investigators and the sponsor

Ethics and
dissemination

Research ethics #24 Plans for seeking research ethics committee /
approval institutional review board (REC / IRB) approval

Protocol #25 Plans for communicating important protocol
amendments modifications (eg, changes to eligibility criteria,
outcomes, analyses) to relevant parties (eg,
investigators, REC / IRBs, trial participants, trial
registries, journals, regulators)
Consent or assent:  #26a  Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)

Consent or assent:  #26b  Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable

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

Declaration of interests:  #28  Financial and other competing interests for principal investigators for the overall trial and each study site

Data access:  #29  Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators

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

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
Dissemination policy: **#31b** Authorship eligibility guidelines and any intended use of professional writers

Dissemination policy: **#31c** Plans, if any, for granting public access to the full reproducible research protocol, participant-level dataset, and statistical code

**Appendices**

Informed consent **#32** Model consent form and other related documentation given to participants and authorised surrogates

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

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