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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 randomised 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.

Methods and analysis Multicentre randomised 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 randomised 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 Good Clinical Practice (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.

Trial registration number NCT04748874; Pre-results.

Strengths and limitations of this study

- This is the first randomised 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 generalisability 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, securing 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 (OHSS).[2] Today, it is standard practice to postpone FET in a modified natural cycle (mNC) 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 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. NCs are subdivided into true NCs (tNCs) or human choriogonadotropin (hCG) triggered 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 time point at which, in the majority of women, the luteinizing hormone (LH) surge is induced in the NC.[10 11] Reproductive outcomes in tNC-FET 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 to 1.03)) and live birth rate (LBR) (RR 0.94 (95% CI 0.85 to 1.03)) while Bergenheim *et al* found a slightly higher CPR (adjusted OR (aOR) 1.22 (95% CI 1.07 to 1.39)) and LBR (aOR 1.20 (95% CI 1.01 to 1.44)) in immediate versus postponed FET. Regardless, the standard practice of routinely postponing mNC-FET for at least one menstrual cycle following in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (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 randomised 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 randomised controlled non-blinded trial including fertility clinics in Denmark. All clinics are part of an academic hospital setting performing standardised treatments according to the public healthcare 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 Standard Protocol Items: Recommendations for Interventional Trials recommendations[16] when drafting this protocol.

Eligibility criteria

Inclusion criteria: patients eligible for FET in an mNC; 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 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 (ie, 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 (1) the patient's general condition contradicts participation in the study or (2) the protocol is violated to an extent that influences 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 freshIVF/ICSI cycle that did not result in pregnancy, or after a 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.

Randomisation

Randomisation 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 randomisation programme. Allocation concealment will be ensured, as the service will not reveal the allocation before the randomisation procedure, that is, at the end of the baseline visit. Patients are randomised 1:1 by simple randomisation 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.
2. FET postponed: mNC-FET at least one full menstrual cycle after the fresh embryo transfer or freeze-all cycle, that is, 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 transvaginal ultrasound (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 the same 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 (1) disappearance or typical change in the shape of the leading follicle, (2) 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-related 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

Table 1 Overview of study visits

	Baseline* 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								x†	
Treatment-related data collection	x	x‡	x	x	x§	x	x§	x	x	x
Randomisation	x									
Transvaginal ultrasound scan	x	x‡	x	x					x	
Ovarian morphology TVUS scan	x	x‡		x						
Blood sample	x	x‡		x	x§	x	x§	x		
Quality of life questionnaire	x¶	x‡					x			

*All participants.
†In case of pregnancy custodians sign informed consent regarding access to the future child's records.
‡Participants randomised to postponed FET only.
§Only at Rigshospitalet.
¶Participants randomised to immediate FET only.
FET, frozen embryo transfer; GA, Gestational age; hCG, human choriogonadotropin; TVUS, Transvaginal ultrasound.

phase (hCG trigger +11) and (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 are digitally obtained at time point 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 time points. 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, follicle stimulating hormone (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°C/−80°C 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 this 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 5 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, that is, 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 fetal 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 two- and three-dimensional TVUS at cycle day 2–5 of the treatment cycle and at the day of hCG-trigger. Two-dimensional scans will be performed for all participants. Three-dimensional 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 the Research Electronic Data Capture (REDCap) system; 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 Good Clinical Practice (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 International Committee of Medical Journal Editors 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 this

study's. No data will be shared until 3 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 predefined time points.
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% to be clinically relevant. The power calculation was performed using a computerised algorithm.[18] We expect a LBR of 25% per randomised 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% CI (or equivalently a 90% two-sided CI) will exclude a difference in favour of the standard group of more than 10%.

Drop-outs and cancelled cycles

Drop-outs are defined as randomised women who, at their own initiative, decide to leave the study. Drop-outs 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 randomised 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 drop-out and cancellation will be tabulated for the two treatment groups and descriptive tables will be compiled for comparison of characteristics of drop-outs, cancelled cycles and completers within and between the groups. We anticipate a drop-out rate of at most 5% and a cancellation rate of at most 5%. In case of a differential or larger than expected drop-out 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 drop-outs and cancelled cycles, PP analyses include cancelled cycles but not drop-outs, and per-transfer analyses exclude both drop-outs and cancelled cycles. Differences in LBR will be evaluated by means of risk differences with one-sided 95% 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 χ^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 4 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.

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 randomised to postponed FET; early-luteal 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, gastrointestinal 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 randomised 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 an NC, a difference that presumably would be recognisable 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 randomised 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 minimise 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 OHSS 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.

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Contributors KL, ABP, JLF and SJB participated in the conception, design, writing and editing of the study protocol. SJB wrote the first draft and KL, ABP, SJB, JLF, MS, NP, ECL, NH, MF, JWB, SZ, NLCF, BN, LFA and PH were involved in the critical

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Competing interests ABP and KL has received a research grant from Merck supporting the present study. SJB has received a research grant from Rigshospitalet's research fund supporting the present study. As a partner of Reprounion, ABP has received a grant from Ferring as well as a research grant from Gedeon Richter. ABP 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. NLCF 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, NLCF is head of the steering committee for Danish fertility guidelines.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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