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Intravenous immunoglobulin and prednisolone to women with unexplained recurrent pregnancy loss after assisted reproductive technology treatment

a protocol for a randomised, double-blind, placebo-controlled trial

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BMJ Open Intravenous immunoglobulin and prednisolone to women with unexplained recurrent pregnancy loss after assisted reproductive technology treatment: a protocol for a randomised, double-blind, placebo-controlled trial

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ABSTRACT

Introduction Recurrent pregnancy loss (RPL), defined as two or more consecutive pregnancy losses in the first trimester, affects around 5% of fertile women. The underlying causes remain unknown in up to 60% of patients; however, most studies point at an immunological pathology in unexplained RPL, and therefore, an effective treatment may be immunomodulatory. This study aims to evaluate the effect of intravenous immunoglobulin (IVIg) and prednisolone on reproductive outcome and the immune system in women with unexplained RPL undergoing assisted reproductive technology treatment.

Methods and analysis This randomised, placebo-controlled trial with double-blinded randomisation to two parallel arms evaluate if immunomodulatory (active) treatment is superior to placebo in increasing the chance of ongoing pregnancy assessed at nuchal translucency scan in gestational weeks (GW) 11–13 after embryo transfer (ET) in 74 RPL patients with ≥ 2 pregnancy losses as its primary objective. The active treatment consists of IVIg (one infusion preferably 1–5 days before ET and in GW 5, 6 and 7) and prednisolone (5 mg/day from first day of menstrual bleeding until ET and 10 mg/day from ET to GW 8+0) while the comparator consists of intravenous human albumin (5%) and placebo tablets. Allocation is concealed for participants, caregivers, and investigators until trial termination and is performed in a 1:1 ratio. The secondary objective is to evaluate treatment safety, and the tertiary objective is exploration of the association between treatment, reproductive outcome after ET, and the lymphocyte subset distribution in peripheral blood collected before and after intravenous infusion(s). Excess biological material is stored in a biobank for future research.

Ethics and dissemination The North Denmark Region Committee on Health Research Ethics (N-20200066) approved this trial. The results will be published in peer-reviewed scientific journals and presented to relevant patient associations, at relevant academic conferences and to key stakeholders.

Trial registration number NCT04701034.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The pragmatic, randomised, double-blinded study design reflecting the contemporary practice strengthens the study results' external validity.
- ⇒ The combination of clinical and immunochemical outcome measures evaluated by blinded clinicians and the randomised study-settings strengthen the current research that evaluate how the immune system is changed during early pregnancy and whether it is related to the reproductive outcome.
- ⇒ The combination of two active drugs in the active treatment group does not allow differentiation of whether one of the active drugs or only the combination possesses the effect on reproductive outcome observed in the study.
- ⇒ Participants are not selected based on a biomarker associated with an aberrant immune function, which was done in some of the previous studies reporting a significant effect of intravenous immunoglobulin on unexplained recurrent pregnancy loss; however, since the evidence of such an association is still sparse, further elaboration on this topic is needed.

INTRODUCTION

Background

Recurrent pregnancy loss (RPL) defined as ≥ 2 consecutive pregnancy losses (PLs)¹ affects around 5% of women in reproductive age.² In Denmark, >25% of all RPL cases happens after assisted reproductive technologies (ART) including in-vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI) and frozen embryo/blastocyst transfer (FET) and >10% children born after 2019 in Denmark are conceived using ART. In up to 60% of RPL patients, no risk factor is found, and for unexplained RPL (uRPL),

no treatment with proven benefit has been found yet.¹ Among women with uRPL, immunological aberrations are thought to be at least partly responsible. For that reason, many treatment regimens used in autoimmune diseases have been tested on uRPL patients. A meta-analysis of RCTs evaluating the efficacy of intravenous immunoglobulin (IVIg) treatment for women with RPL found no overall significant effect on live birth rate; however, the acquired sample size was not obtained. Since subgroup analyses did show a significant effect in secondary RPL, the authors suggested that further RCTs were required to obtain sufficient evidence.³ A pilot study suggested that a combination of prednisone (PRS) and IVIg starting before pregnancy improves the chance of live birth in women with RPL after ART.^{4,5} Additionally, previous studies of PRS supplement to ART treatment have shown improved pregnancy rate, especially for women with immune aberrations.⁶⁻⁹ The underlying mechanism for an improved ART outcome after immunomodulatory treatment may be the regulation of immunological processes occurring particularly in early pregnancy. Some prior studies have suggested different biomarkers for an aberrant immune system in the uterus¹⁰⁻¹⁴ and peripheral blood¹⁵⁻¹⁹ in uRPL patients, which have been associated with unsuccessful pregnancy outcome in some small studies^{11,15,20} but not in others.¹²

Previous studies have observed a counterbalanced lymphocyte distribution in uRPL patients treated with PRS; thus, the treatment re-established the immune system presumed to be beneficial for embryo implantation.²¹⁻²⁴ The same effects have been observed in uRPL patients treated with IVIg.^{22,25-27} For that reason, treatment before pregnancy with PRS and IVIg (PRS&IVIg) is expected to contribute to a favourable immunological environment in peripheral blood and the uterus which is expected to increase the chance of an uncomplicated pregnancy.

STUDY OBJECTIVES

Primary objective

- ▶ To investigate whether treatment with PRS&IVIg before and in early pregnancy improves the reproductive outcome in RPL patients undergoing ART treatment.

Secondary objective

- ▶ To evaluate if PRS&IVIg treatment is associated with decreased incidence of pregnancy complications, negative pregnancy outcome, and negative perinatal outcomes.

Tertiary explorative objective

- ▶ To investigate how PRS&IVIg affect the leucocyte subset distribution (LSD) in peripheral blood and whether a specific LSD can predict which uRPL patients benefit from PRS&IVIg.

METHODS

Study design

In a randomised 1:1 double-blinded, placebo-controlled trial of uRPL patients undergoing ART, the effect of PRS&IVIg treatment is compared with treatment with placebo (placebo tablets and human albumin). The study also possesses an explorative capacity by examining the association between treatment, reproductive outcome and LSD.

Population

The population consists of RPL patients with ≥ 2 consecutive PLs after ART referred to The Centre for Recurrent Pregnancy Loss of Western Denmark (RPL Centre) at Aalborg University Hospital (AaUH). In Danish public clinics, the costs for a couple's first child are covered by tax-financed public health insurance. Also, all Danish citizens with RPL can be referred to The RPL Centre without costs.

The public and private fertility clinics in Denmark have received a detailed description of the RCT to inform their personnel and ask for referral of potentially eligible patients with ≥ 2 PLs to the RPL Centre. All referred patients undergoing ART are screened for eligibility.

Inclusion criteria

1. Women with ≥ 2 consecutive PLs (biochemical pregnancies or clinical miscarriages) before completed gestational weeks (GW 10 after ART with the same partner or with an egg/semen donor*.

Exclusion criteria

1. Body Mass Index (BMI) ≥ 35 .
2. Age ≥ 41 years.
3. Significant uterine malformation(s).
4. Known parental balanced chromosomal translocations.
5. ≥ 2 previous pregnancies with fetuses with known abnormal karyotype
6. Patients with IgA deficiency, IgA-autoantibodies or hyperproliferation
7. Anti-Müllerian hormone (AMH) < 4 pmol/l *
8. Treatment with medication interacting with PRS:
 - a. CYP3A4-inhibitors (eg, erythromycin, itraconazole, ritonavir and lopinavir), CYP3A4-inducers (eg, phenobarbital, phenytoin and rifampicin), loop diuretics, thiazides, amphotericin B, $\beta 2$ -agonists, anti-diabetics (metformin is acceptable), interleukin-2, somatropins, anticholinergics and regular treatment with Non-Steroidal Anti-Inflammatory Drugs .
9. Patients with moderate/severe hypertension, diabetes mellitus, heart insufficiency, severe mental disorders, Cushing syndrome, myasthenia gravis, ocular herpes simplex, pheochromocytoma, systemic sclerosis and moderate/severe renal dysfunction.
10. Patients with a clinical or biochemical profile indicating need for heparin or levothyroxine treatment during pregnancy

11. Previous treatment with IVIg

12. Allergy to PRS and/or IVIg

*or women using egg donation two exceptions occur: first, if the participant plans to use egg donation in the study cycle, the previous two PLs must also have happened with the use of egg donation; however, it is not required to use the same egg donor in all three embryo transfers (ET). Second, a low AMH value is not an exclusion criterion for patients using egg donation.

Allocation, randomisation and blinding

Investigators at the RPL Centre screen for inclusion and exclusion criteria deliver the written and oral study information and obtain a signed written consent form (see online supplemental material 1). The recruited participant is assigned an ID-number, which is given in continuous order, and she receives a tablet container with treatment corresponding to the randomisation list and labelled with the matching ID-number.

Also, a written information folder is given after randomisation with a summary of known adverse reactions (AR) for study drugs, boxes in which the participant can describe her ARs and adverse events (AE), a description on how to respond to such ARs/AEs and a telephone number available 24/7. It also contains a checklist with ticking boxes to tick off each day the participant takes her tablet(s). It describes when to change the dose and serves as a reminder improving treatment compliance. The folder is examined at every appointment to aid high compliance and safety.

To ensure blinding of participants, investigators, outcome assessors and care providers, a computer-generated simple randomisation list is created by the Hospital Pharmacy North Denmark Region (HPNDR) and kept confidential. The list contains 74 ID-numbers with an allocation ratio 1:1. It is made before the first patient is included and located at the HPNDR. Only the personnel at the Department of Clinical Immunology (DCI) preparing intravenous (IV) infusion medicine have a copy. No personnel with access to the randomisation list will be in contact with participants nor be involved in data collection. The allocation sequence is concealed as ID numbers are assigned to participants in consecutive order by the blinded chief investigator.

Randomisation is in blocks of different sizes arranged to ensure a 1:1 ratio when conducting the interim analysis. For every participant who does not meet the criteria for per-protocol analysis, an extra patient will be included. The additional ID-numbers on the randomisation list will be generated by the HPNDR in correspondence with the number of participants missing in each treatment group.

The investigator will authorise the pharmacist to break the code in order to reveal the participant's allocation group in case of serious illness and the code will only be broken if it is of substantial importance to a participant's health or functional capacity and only the chief investigator and sponsor can make this decision.

The randomisation code will be disclosed when the primary outcome is collected on 74 participants with no protocol deviations and a preanalysis blind review on all outcomes described in the SAP has been performed which is expected to happen in November 2023.

Study medicine preparation

PRS and the placebo comparator are in tablet form with identical appearance and prepacked for each participant by the HPNDR in identical containers labelled with ID numbers corresponding to the randomisation list. The chief investigator informs the DCI personnel about when and to whom they should prepare intravenous infusion medicine for. Within 4 hours before administration, the relevant volume and content is transferred into a transparent, yellow (UV-protected) ethylene vinyl acetate (EVA) bag labelled with volume and ID number. Human albumin is chosen as IVIg comparator due to the similar physical form and appearance for example, both drugs form foam. The yellow EVA bags and intravenous sets make visual distinction even more difficult.

Intervention

Within the first 3 days of the participant's menstrual cycle, she starts administration of one tablet daily (5 mg of prednisolone or placebo) until ET on which day the dose increases to two tablets daily. At the time of the ET (from five working days before to two working days after), the participant receives the first intravenous infusion of study medicine and have the first study-specific blood sample taken. Participants with a prepregnancy body weight (BW) ≤ 70 kg will receive 250 mL, participants with BW 70–85 kg will receive 300 mL and participants with a BW ≥ 85 kg will receive 350 mL in each infusion. Around 14 days after ET, plasma-human chorionic gonadotropin (hCG) is measured twice with 24–48 hours interval. If plasma-hCG increases sufficiently (here defined as $\geq 30\%$ per 24 hours), the treatment continues (see figure 1).

IV infusions containing the same volume is repeated in GW 5, 6 and 7, respectively; however, no intravenous infusion is administered after a negative pregnancy test or a PL. The tablet treatment discontinues gradually over 4 days when the participant has a negative pregnancy test, a PL, or when she reaches GW 8+0, whichever comes first.

Before each infusion, a plasma-hCG measurement or ultrasound scan is performed to confirm that the pregnancy continues as expected. Initial infusion rate of 0.3 mL/kg BW/hour and if well tolerated, the infusion rate may gradually increase. Blood pressure and pulse are monitored before, during and after the treatment. In case of anaphylaxis, the treatment is discontinued, epinephrine (0.1 %) is administered and the participant is excluded.

On the day of the first intravenous infusion and again about 4 weeks later (corresponding to GW 6, ie, the day of her third intravenous infusion), a blood sample is taken.

After the last infusion, routine monitoring at the RPL Centre or at her local hospital is offered. A nuchal

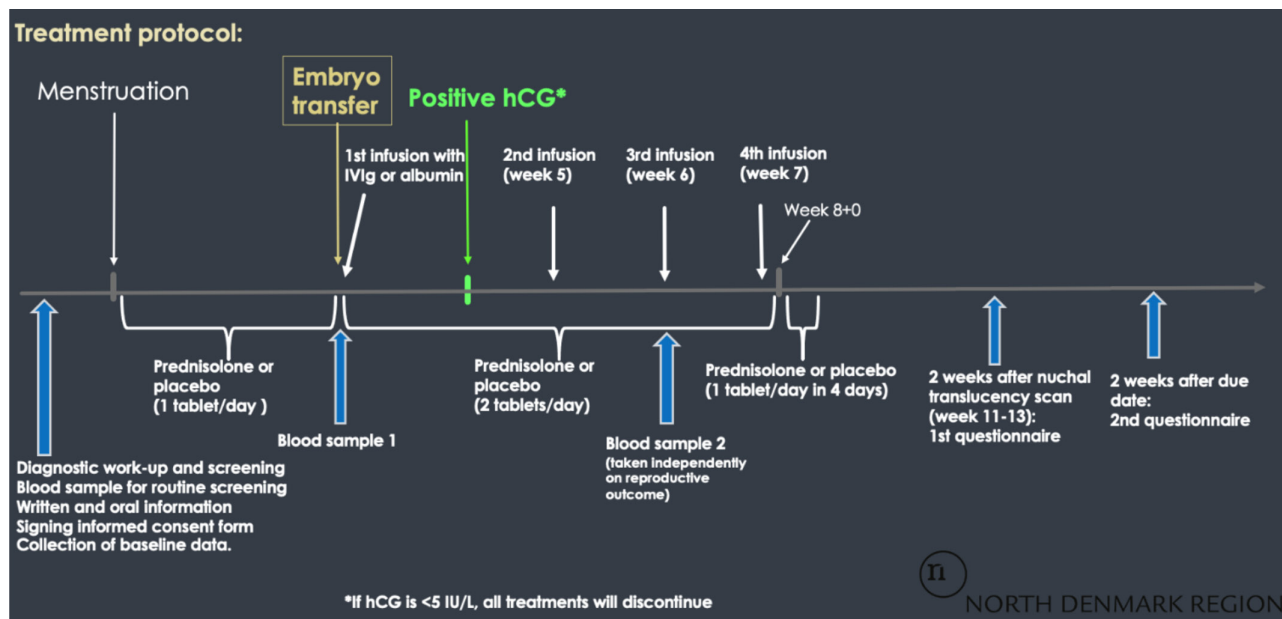


Figure 1 A flow diagram on the treatment for each participant. hCG, human chorionic gonadotropin; IVIg, intravenous immunoglobulin.

translucency (NT) scan is offered in GW 11–13. The participant receives an e-mail with a unique link to a questionnaire 2 weeks after the NT scan and 2 weeks after her due date. After GW 8, the participant is offered the same treatment and follow-up as any other Danish pregnant women except from two additional fetal growth scans in the third trimester due to her history of RPL.

Active treatment

Prednisolone: 5 mg of prednisolone per tablet. One tablet daily rising to two tablets daily after ET.

IVIg: priven 100 mg/mL (10 %) (CSL Behring). The three optional volumes to be administered depend on BW as previously described; thus, the dose corresponds to approximately 0.4 g IVIg per kg BW.

Placebo

Prednisolone comparator: placebo tablets contain 85 mg lactose monohydrate, 86 mg potato starch, 8.1 mg talc, 3 mg gelatine and 0.9 mg magnesium stearate. One tablet daily rising to two tablets daily after ET.

IVIg comparator: human albumin 50 mg/mL (5%) (CSL Behring).

Other medication

The study medicine does not affect the participant's ART treatment decided solely by her fertility clinic; however, no immunotherapy is accepted. Thus, hormone-replacement-therapy and natural cycles with fresh or FETs are accepted.

Data collection and management

Study-relevant data are recorded in an electronic case report form (eCRF) managed in REDCap; an electronic data capture tool hosted at AaUH. The information is obtained from electronic medical records, the

information folder and directly from the participant. Demographic and medical baseline data are collected before randomisation during the diagnostic workup. After randomisation, data related to the index ART cycle are collected including information about fertilisation method, use of fresh or frozen embryos, transfer of cleavage stage embryos or blastocyst and possible performance of PGT-A. When the treatment is terminated, remaining tablets and the information folder are returned which enables the investigators to assess compliance and drug safety. The remaining tablets from each participant are counted by the unblinded DCI personnel. If a participant experiences a missed abortion, the investigators will apply for a uterine evacuation with chromosomal examination of fetal/placental tissue.

The two questionnaires collect data after NT scan and birth, respectively, about pregnancy complications, AEs/ARs, the participant's and her fetus/baby's well-being and perinatal outcome. The second questionnaire also requests a copy of the hospital's delivery record.

The chief investigator is the primary data manager responsible for the validity of data in the eCRF. An audit trail ensures data traceability and validity to maintain transparency and accountability. During the study period, continuous review and evaluation of the audit trail and the data accuracy are performed by the chief investigator and the Good Clinical Practice (GCP) board. The eCRF have range checks, description of codes and valid options of choices where applicable. After 1 week, it automatically sends a reminder to participants who have not replied to the questionnaires and notifies the investigators. Personal identifier variables are marked which allows anonymous data export. The same data are collected in participants with and without major protocol deviations, and the reason for any protocol deviation is described. Data are

protected according to the GDPR (the General Data Protection Regulation), the Danish Data Protection Act and the Danish Health Act.

Specific authorised persons from The North Denmark Region Committee on Health Research Ethics or the Danish Medicines Agency have unrestricted access throughout the trial to monitor, audit and inspect the source data regarding the study participants.

Blood sample analysis

The study-specific blood sample analyses are performed by the DCI, and the results will be shared with the investigators after the randomisation code is disclosed. In each fresh EDTA blood sample, the total leucocyte cell count, and the fractions of T helper cells (Th) 1, Th2, Th17, cytotoxic T cells (Tc), regulatory T cells (Tregs), B cells and natural killer (NK) cells (CD56^{dim}CD16⁺ and CD56^{bright}CD16) are measured with flow cytometry. A TruCulture analysis is performed on both blood samples from only 25 participants as this RCT serve as a pilot study exploring the relevance of measuring stimulus-specific cytokine production in RPL patients treated with immunomodulatory medicine. The appendices contain a detailed description of the methods of the flow cytometric and TruCulture analyses used (see online supplemental appendix A,B).

Remaining biological material is saved in a research biobank for later analysis of small extracellular vesicles with different phenotypes and for other future studies on immunological risk factors to RPL. The biological material is stored at -80°C and consists of three 6 mL collection tubes containing serum, EDTA plasma and citrate plasma, respectively.

Endpoints

The primary endpoint is the percentage of participants with ≥ 1 normal, viable fetus at NT scan in GW 11–13 among all participants in each treatment group, and subsequently among participants pregnant after ET. Also, the percentage participants with ≥ 1 normal, viable fetus at NT scan among all participants except those pregnant with a fetus having a confirmed chromosomal abnormality will be reported. Furthermore, the relative risk, absolute risk reduction and an adjusted risk ratio for this primary outcome will describe the primary outcome. The primary endpoint is also measured in subgroups based on diagnosis of primary and secondary RPL, respectively, that is, no prior birth or a history of ≥ 1 previous birth after 22 GW. The secondary endpoints are the incidence of AE/ARs and pregnancy complications (miscarriage rate, negative pregnancy rate, pre-eclampsia, gestational hypertension, gestational diabetes mellitus and acute instrumental delivery), and the perinatal outcomes (congenital deformations, prematurity, small for gestational age, low birth weight, admission to neonatal care unit, sex, birth weight and gestational age), while the tertiary endpoints are the distribution of leucocyte subsets. A thoroughly

description can be found in the statistical analysis plan (SAP) (see online supplemental material 3).

Safety considerations, safety monitoring and reporting

An AE is defined as any untoward medical occurrence (including any unfavourable or unintended sign, symptom or disease) in a participant during the study (from the day of randomisation until childbirth or 6 months after last intravenous infusion) and which does not necessarily have a causal relationship with the treatment in contrast to an AR. A serious AE (SAE) is defined as an AE that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity or is a congenital anomaly or birth defect. Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events will also be considered as serious ARs (SARs). A suspected unexpected serious adverse reaction (SUSAR) is defined as an AR, the nature or severity of which is not consistent with the applicable product information (EUR-Lex, CT-3). For each case, it is considered whether it is serious, unexpected and possibly, probably or definitely related to an intervention based on the Summary of Product Characteristics and timing.

Participants are encouraged to describe all AEs in their information folder, and they are followed-up on each appointment at the RPL Centre. The questionnaires follow-up on AEs in the participants and their foetuses/children after treatment cessation. If a new AE is described in the questionnaire, an investigator contacts the participant to assess the AE's character and whether follow-up consultations or therapy is required. Non-pregnant participants are informed to contact the investigators in case of AEs after treatment cessation. All AEs are reported from the investigator to the sponsor immediately (<24 hours) and described in detail in a follow-up report to the sponsor. The sponsor reports SUSARs to The National Board of Health and The North Denmark Region Committee on Health Research Ethics during the entire study period within 7 days. In addition, an annual report on SAE will be sent to these authorities. Thus, the study follows the EudraLex Clinical Trials Guidelines Chapter II—safety reporting. Some AEs linked to pregnancy, ART or RPL are expected and includes amenorrhea, vaginal haemorrhage, weight gain, backpain, pelvic pain, drowsiness, nycturia, increased hunger, nausea and vomiting. These AEs are not recorded unless they are serious; likewise, hospitalisation due to symptoms of a threatened miscarriage, induced abortion or surgical abortion (evacuation), is not assessed as a SAR/SAE.

Sample size

Based on previous studies of similar patients^{4,5} reporting an live birth rate (LBR) of 36.5% and 34.2% after IVlg and prednisolone treatment, respectively, and an expectation of similar treatment effects in women with 2 and ≥ 3 PLs, a minimum LBR of 40% in the active treatment

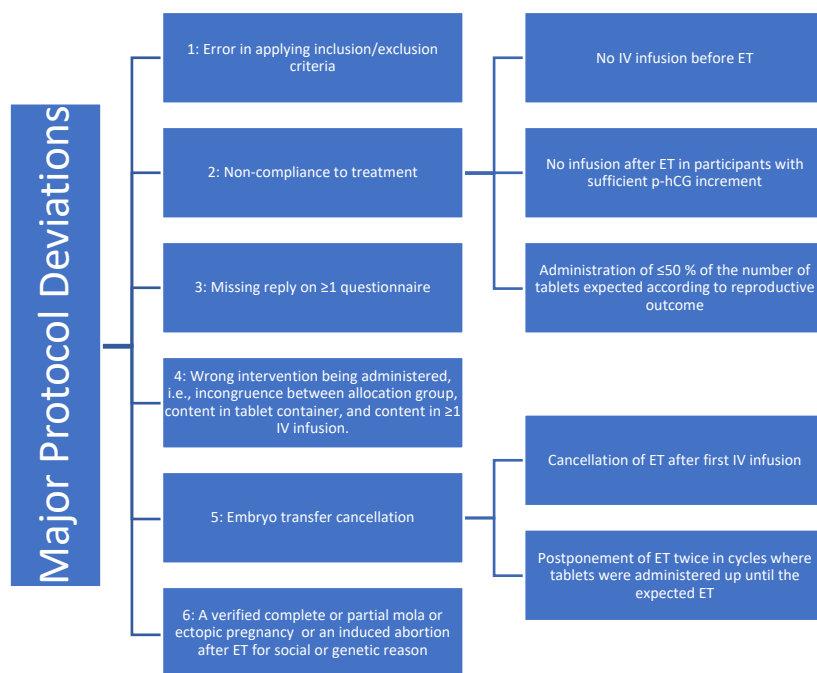


Figure 2 Diagram over the criteria for major protocol deviations. ET, embryo transfer; IV, intravenous.

group is anticipated. In the RCT by Stephenson and Fluker,²⁸ an LBR of 12% in IVF/ICSI patients with a mean of 3.2 previous failed ETs receiving placebo treatment was reported. The patients admitted to the RPL Clinic within the last 3 years, who would have been eligible for the study, have had a mean of 6.4 previous failed ETs, and we therefore think that a reasonable estimate of the LBR in those of our patients allocated to placebo will be similar to the observed 12%. Based on these expectations, a type I error of 0.05, and a type II error of 0.20, the study will need a sample of 74 participants. Based on experience from previous trials, <2% dropouts and <10% protocol deviations (see [figure 2](#)) are expected.

Statistical analysis plan

The statistical analyses will be carried out by the investigators in collaboration with professional statisticians. A $p \leq 0.05$ is considered statistically significant. All randomised participants will be included in the intention-to-treat (ITT) population, while the PP population will only include participants with no major protocol deviations listed on [figure 2](#).

When the primary outcome is collected in 38 participants, who fulfil criteria for the PP analysis, an interim analysis will be conducted by an independent group of statisticians who get access to data on the primary outcome; SARs and SUSARs; and a simple-coded (A/B) randomisation list. The statisticians will not get data on ARs/AEs, since it will increase the risk of breaking the code. The interim analysis will provide information of differences in efficacy and safety between groups. If a difference of ≥ 3 SUSARs or SARs between groups is observed, the study will discontinue. The study will not terminate in case of futility, and it will not implement any

modifications to trial procedures. Therefore, no correction of the reported p value will be performed in the final analysis. The sponsor has the ultimate authority to stop the study.

After completion of the study inclusion and before breaking the blind, a preanalysis blind review on data will be carried out by the statisticians to perform data validation, detect outliers, assess distribution of variables and measure the outcomes. Afterwards, the unblinded data analysis will proceed.

The analyses on primary outcome will be performed as an ITT and a PP analysis, while the secondary outcomes are performed on the ITT population, and the tertiary outcomes are performed on the PP population. A χ^2 test will compare the primary, secondary and tertiary categorical outcome variables between treatment groups. Fisher's Exact Test will be used when less than five participants are expected in one group. An adjusted relative risk for a normal viable pregnancy at NT scan between the two treatment groups is estimated using Poisson regression and is adjusted for relevant confounders including BMI, smoking and age.

For the analysis of the tertiary outcomes, including the concentration and fraction (continuous) of leucocyte subsets, an unpaired two-sample Mann-Whitney U test or t-test will be used for between group comparisons depending on data distribution, while paired Wilcoxon signed-rank test or paired t-test will be used for within group comparisons. When comparing more than two groups, that is, subgroups according to reproductive outcome after ET or percentile subgroups, an analysis of variance or Kruskal-Wallis test will be used.

The analyses will compare

1. The outcomes from blood sample 1 with blood sample 2 within each two treatment groups, respectively.
2. The outcomes from each two blood samples, respectively, between treatment groups
3. The outcome changes from blood sample 1 to 2 between treatment groups and between subgroups based on pretreatment cell fraction (three percentile subgroups).

Also, receiver operation characteristic curve analyses will determine the optimal cut-off values for the Th1/Th2 ratio, Th17/Treg ratio and the NK cell fraction in blood sample 1 to identify participants with a positive reproductive outcome in the active treatment group.

Further information on the statistical analysis is described in detail in the SAP in online supplemental material 3.

Data quality and monitoring

The study protocol, trial master file (TMF) and eCRF have been reviewed by the regional GCP board. The GCP board comprises experts in clinical trials who are not involved in other aspects of the study and have no competing interests. They make systematic inspections including examinations of the TMF, study procedures, safety and data collection, accuracy, completeness and validity to ensure it is performed in accordance with the protocol and comply with the Danish legislation and GCP guidelines. The trial will also be open for inspection from the Danish Health Authority.

Patient and public involvement

The active treatment was given to 10 RPL patients according to a preliminary protocol proposal as well as presented to stakeholders in the field of ART. In a post-treatment semistructured interview with the patients, proposals for optimisations according to treatment plan and compliance as well as motivational factors and barriers were performed. Following this, stakeholders submitted their proposals for optimisation of the feasibility of the treatment protocol, which is highly dependent on cooperation with fertility clinics and their treatments. These proposals were all used to revise the protocol.

DISCUSSION

Ethics and dissemination

The study protocol, participant material and informed consent (see online supplemental material 1) are approved by The North Denmark Region Committee on Health Research Ethics (N-20200066), The National Board of Health (EudraCT number: 2020-000256-35), and the Data Protection Agency (2020-156). It was registered at ClinicalTrials.gov before the study was initiated (NCT04701034). In case of protocol modifications, a formal amendment will be approved by these authorities before implementation and when it is approved, it will be sent by e-mail to all collaborating parties in the study.

The study adheres to the Declaration of Helsinki II, local regulations and GCP guidelines.

Participation is voluntary, and the written informed consent can be withdrawn at any time without consequences for the subsequent treatment at the RPL Centre. Future studies on the research biobank material not already described will require a new approval from ethical committee and if required, an additional consent provision from the participants. The study is covered by the national patient health insurance. Study drugs are given to participants without expenses. None of the study investigators will get any economic or professional advantages before, during or after the study.

The findings will be submitted for publication in internationally acknowledged peer-reviewed journals in obstetrics and gynaecology or general medicine and will be described in at least two scientific papers: one manuscript focusing on the reproductive outcome and minimum one manuscript focusing on changes of immune biomarkers according to treatment and the reproductive outcome. Data will also be presented at national and international congresses. Future research based on the biobank will subsequently be performed and shared in scientific papers. Authorship eligibility will be based on the International Committee of Medical Journal Editors (ICMJE) recommendations 2018. An anonymous data set may be shared on reasonable request after agreement and approval by the sponsor and chief investigator. Study results and the allocation group of each participant will be shared with the participants who requested this information after the complete data analysis. We will publish both positive, negative and inconclusive results. No publication restrictions are imposed.

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Contributors OBC is sponsor of the study. CN-P and OBC made the study design, and planned, conceived, initiated and conducted the study and the clinical implementation. They are both grant holders. CN-P is the chief investigator and wrote the protocol, made the statistical analysis plan and made the eCRF and arranged all data collection, data management, data reporting, data analysis. OBC and USK made critical protocol revisions. KN, LE, MMJ and RS provided detailed information on immunological analyses on blood samples and planned and performed all laboratory analyses on participants' blood samples. USK provided information on ART and supported CN-P and OBC in patient and stakeholder involvement. CN-P decided to submit protocol for publication. CN-P, USK and OBC will interpret data after final data analysis. All authors contributed to refinement of the study protocol and approved the final manuscript.

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Competing interests USK received consulting fees from Merck for Update of patient information leaflet and from IBSA Nordic for Clinical instruction and support for attending meeting/congress from Merck.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting or dissemination plans of this research. Refer to the Methods section for further details.

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