**Full title:** Elective Freezing of embryos in assisted conception: a randomised controlled trial evaluating the clinical and cost-effectiveness of a policy of freezing embryos followed by thawed frozen embryo transfer, compared with a policy of fresh embryo transfer in women undergoing in vitro fertilisation

**Statistical Analysis Plan**

Version 1.0

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<tr>
<td>AE</td>
<td>Adverse event</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CACE</td>
<td>Complier-average causal effect</td>
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<td>CI</td>
<td>Chief investigator</td>
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<td>CONSORT</td>
<td>Consolidated standards of reporting trails</td>
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<td>CRF</td>
<td>Case report form</td>
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<td>CTU</td>
<td>Clinical trials unit</td>
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<td>DMC</td>
<td>Data monitoring committee</td>
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<tr>
<td>eCRF</td>
<td>Electronic case report form</td>
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<tr>
<td>FSH</td>
<td>Follicle-stimulating hormone</td>
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<td>g</td>
<td>Grams</td>
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<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<td>HFEA</td>
<td>Human Fertilisation and Embryology Authority</td>
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<td>HTA</td>
<td>Health technology assessment</td>
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<tr>
<td>ICSI</td>
<td>Intracytoplasmic sperm injection</td>
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<td>ITT</td>
<td>Intention to treat</td>
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<td>IU</td>
<td>International units</td>
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<td>IVF</td>
<td>In vitro fertilisation</td>
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<td>kg</td>
<td>Kilogram</td>
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<td>LMS</td>
<td>Lambda-Mu-Sigma</td>
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<td>M</td>
<td>Metre</td>
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<td>mm</td>
<td>Millimetre</td>
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<tr>
<td>NIHR</td>
<td>National Institute for Health Research</td>
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<tr>
<td>NNU</td>
<td>Neonatal unit</td>
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<tr>
<td>NPEU</td>
<td>National Perinatal Epidemiology Unit</td>
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<tr>
<td>OHSS</td>
<td>Ovarian hyperstimulation syndrome</td>
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<td>RCOG</td>
<td>Royal College of Obstetricians and Gynaecologists</td>
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<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
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<tr>
<td>SAE</td>
<td>Serious adverse event</td>
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<td>SAP</td>
<td>Statistical analysis plan</td>
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<td>STAI</td>
<td>State-trait anxiety inventory</td>
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<tr>
<td>TSC</td>
<td>Trail steering committee</td>
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<td>UK</td>
<td>United Kingdom</td>
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1. Introduction

This document details the proposed presentation and analyses for the main paper(s) reporting results from the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) programme funded multicentre randomised controlled trial of fresh versus frozen embryo transfer for women undergoing in vitro fertilisation (E-Freeze).

The results reported in these papers will follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, although they are expected to follow the broad principles described. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

The analysis plan will be available on request when the principal papers are submitted for publication. Suggestions for subsequent analyses by journal editors or referees will be considered carefully and carried out, as far as possible, in line with the principles of this analysis plan.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial. The analysis should be carried out by an identified, appropriately qualified and experienced statistician, who should ensure the integrity of the data during their processing. Examples of such procedures include quality control and evaluation procedures.
2. **Background Information**

2.1 **Rationale**

In vitro fertilisation (IVF) involves several steps. Initially, hormones are used to stimulate a woman’s ovaries to produce eggs which are harvested surgically. Next, embryos are created in the laboratory by mixing eggs with sperm produced by her partner, in conventional IVF; these are grown in culture for a few days before being replaced within the uterus by a process known as fresh embryo transfer. Spare embryos are usually frozen with a view to transfer at a later point in time – especially if the initial fresh transfer does not result in a pregnancy. Despite improvements in technology, IVF success rates remain low with an overall live birth rate of 25% per treatment. Additionally, there are concerns about health outcomes for mothers and babies conceived through IVF, particularly after fresh embryo transfer, including maternal ovarian hyperstimulation syndrome (OHSS) and perinatal morbidity.

It is believed that high levels of ovarian hormones during ovarian stimulation could create a relatively hostile environment for embryo implantation whilst increasing the risk of OHSS. It has been suggested that electively freezing embryos with the intention of thawing and replacing them within the uterus at a later stage (thawed frozen embryo transfer) instead of fresh embryo transfer, may lead to improved pregnancy rates and fewer complications. However, the existing evidence in support of an elective frozen embryo transfer policy, derived from three small randomised trials is inadequate to justify such a radical change in practice.

E-Freeze is a multi-centre randomised controlled trial (RCT) to determine if a policy of freezing embryos, followed by thawed frozen embryo transfer results in a higher healthy baby rate when compared with the current policy of transferring fresh embryos. The trial will recruit 1,086 couples undergoing their first, second or third cycle of IVF/ICSI/Split treatment at fertility centres in the UK.

2.2 **Objectives of the trial**

The primary objective of the trial is to determine if a policy of freezing embryos, followed by thawed frozen embryo transfer results in a higher healthy baby rate when compared with the current policy of transferring fresh embryos.

The secondary objectives of the trial are to assess if a policy of freezing embryos, followed by thawed frozen embryo transfer compared with the current policy of transferring fresh embryos results in:

1. Fewer complications associated with IVF/ICSI treatment and pregnancy
2. Greater cost-effectiveness from a health service and broader societal perspective

2.3 **Trial design**

Multi-centre, randomised controlled trial

2.4 **Eligibility**

Inclusion criteria:

- The female partner is ≥ 18 and < 42 years of age at the start of treatment (i.e. start of ovarian stimulation)
- Couples who are undergoing their 1st, 2nd or 3rd cycle of in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatment
- Both partners are resident in the UK
Both partners have provided written informed consent
At least 3 good quality embryos on day 3 following fertilisation

Exclusion criteria:
- Donor gametes are used
- Pre-implantation genetic testing is planned
- Elective freezing of all embryos is planned for medical reasons (e.g. severe risk of OHSS/fertility preservation)
- Couples previously randomised to E-Freeze

2.5 Interventions
Standard care arm: Women will undergo fresh embryo transfer at the cleavage or blastocyst stage according to local protocols.

Intervention arm: All good quality embryos will be frozen according to local protocols. Women will be contacted by their embryologist/research nurse (or delegate) after randomisation and arrangements will be made for frozen embryo transfer (typically after 4 to 6 weeks and always within 3 months of the egg retrieval process). The couples will attend for a clinic visit and additional monitoring visits before thawed frozen embryo transfer is performed.

2.6 Definition of primary and secondary outcomes

2.6.1 Primary outcome
The primary outcome is a healthy baby.

A healthy baby is defined as a live singleton baby born at term (between 37 and 42 completed weeks of gestation) with an appropriate weight for gestation (weight between 10th and 90th centile for that gestation based on standardised charts).

2.6.2 Secondary outcomes
The secondary outcomes are separated by maternal safety, complications of pregnancy and delivery, measures of clinical effectiveness, measures of effectiveness of the process of freezing embryos and health economic outcome measures.

Maternal safety outcome
- Ovarian hyperstimulation syndrome (OHSS) - defined and classified as per the Royal College of Obstetricians and Gynaecologists (RCOG) green top guidelines.

Complications of pregnancy and delivery outcomes
- Vanishing twin or triplet (defined as either: more fetal heartbeats than babies born, more gestational sacs than babies born or more gestational sacs than fetal heartbeats)
- Miscarriage rate (defined as pregnancy loss prior to age of viability i.e. 24 weeks of gestation)
- Ectopic pregnancy
- Termination
- Gestational diabetes mellitus (GDM)
- Multiple pregnancy (defined as more than one fetal heartbeat or more than one gestational sac)
• Multiple births (including live and still births)
• Hypertensive disorders of pregnancy (chronic hypertension, pregnancy induced hypertension, pre-eclampsia and eclampsia)
• Most severe hypertensive disorder (from least to worst: chronic hypertension, pregnancy induced hypertension, pre-eclampsia and eclampsia)
• Antepartum haemorrhage (any bleeding per vaginum after 28 weeks of pregnancy including placenta praevia and placental abruption)
• Onset of labour (spontaneous, induced or planned caesarean section)
• Mode of delivery for each baby (normal vaginal delivery, instrumental vaginal delivery or caesarean section)
• Preterm delivery (defined as delivery at < 37 completed weeks)
• Very preterm delivery (defined as delivery at < 32 completed weeks)
• Low birth weight (defined as weight < 2500g at birth)
• Very low birth weight (defined as weight < 1500g at birth)
• High birth weight (defined as weight > 4000g at birth)
• Large for gestational age (defined as birth weight > 90th centile for gestational age at delivery, based on standardised charts)
• Small for gestational age (defined as birth weight < 10th centile for gestational age at delivery, based on standardised charts)
• Congenital anomaly/birth defect (all congenital anomalies/birth defects identified will be included)
• Perinatal mortality (stillbirth or late as well as early neonatal deaths, up to 28 days after birth)

Measures of clinical effectiveness outcomes
• Live birth rate (this is a live birth episode i.e. multiples will count as one)
• Singleton live birth rate
• Singleton live birth rate at term
• Singleton baby with appropriate weight for gestation
• Pregnancy rate (defined as positive pregnancy test at 2 weeks +/- 3 days after embryo transfer)
• Clinical pregnancy rate (defined as the presence of at least one fetal heartbeat at ultrasound between six and eight weeks gestation; ectopic pregnancy counts as a clinical pregnancy; multiple gestational sacs count as one clinical pregnancy)

Measures of the effectiveness of the process of freezing embryos outcomes
• Total number of embryos frozen, thawed and transferred for all randomised couples
• Proportion of thawed embryos that were then transferred for all randomised couples
• Failure of all embryos to survive after thawing leading to no embryo transfer

Health economic outcome measures
• Cost to the health service of treatment, pregnancy and delivery care
• Modelled long-term costs of health and social care, and broader societal costs

Other secondary outcomes
• Evaluation of emotional state (for both the female and male partners)

2.7 Hypothesis framework
This is a superiority trial and all comparisons will be analysed and presented on this basis.
2.8 Sample size and power

The proposed primary outcome for this trial is novel and is not currently reported by IVF clinics or national regulatory bodies. This means that a number of assumptions have been made in order to determine the expected event rate in the control arm (receiving current standard treatment), which may in turn result in a degree of imprecision in the estimate.

The most recent data from the HFEA\(^2\), which collects data on all IVF cycles from all clinics in the UK, show that 25% of all women undergoing one episode of IVF treatment involving a fresh embryo transfer have a live birth, and 20% have singleton live births. These figures are for women of all age groups, not necessarily for women fulfilling the inclusion criteria for this trial in terms of the number of good quality embryos in their IVF cycle. No data are available regarding the healthy baby rate (live singletons born between 37 and 42 weeks’ with appropriate weight for gestation), the primary outcome for this study. For our trial population we anticipate that the control arm event rate is likely to be less than 25%, possibly as low as 17%.

To provide relevant information regarding the event rate expected in the control arm, we surveyed 10 IVF centres that expressed an interest in the study, collecting data on the number of live births in women under the age of 42 undergoing their first IVF treatment in 2012. The average live birth episode rate from this survey was 31% with a 95% confidence interval of 25% to 37%. Accurate data on the healthy baby rate in those with at least 3 good quality embryos were not available. Although the live birth rate is expected to be higher in women with at least 3 good quality embryos (likely to have a better prognosis), we anticipate that the healthy baby rate in our trial population will be towards the lower end of the confidence interval, around 25%, taking into account the higher risk of preterm delivery and small for gestational age babies following IVF\(^3\).

The following assumptions have been made for the sample size calculation:

We have assumed a healthy baby rate of between 17% and 25% in women eligible for the trial (age under 42 years with 3 good quality embryos) undergoing standard care (fresh embryo transfer). Taking into account the extra time, effort and potential expense involved in freezing embryos and the delay in embryo transfer of up to three months, a panel of clinicians across the UK agreed that the strategy of freezing embryos would be considered effective if the percentage of women having a healthy baby is increased by at least 8% in absolute terms. With 90% power and using a two-sided 5% level of statistical significance, we will need to randomise a total of 1,086 couples (543 in each group) in order to be able to detect an absolute difference of 8% from 17% to 25% and 9% from 25% to 34% in the healthy baby rate, between fresh embryo transfer and transfer of thawed frozen embryos. The difference detectable differs slightly depending on the event rate in the standard care group, which will be reviewed periodically by the Data Monitoring Committee (DMC).

It is a regulatory requirement for clinics in the UK to report live birth outcomes (including number, weight and gestation) after all embryo transfers i.e. there will be no loss to follow-up. Therefore we have not taken into account loss to follow-up for these sample size calculations.

It is anticipated that a proportion of those consented may not reach randomisation (not having 3 good quality day 3 embryos or requiring all embryos to be frozen for medical reasons), therefore a higher number will need to be consented. As there are no valid data to support the exact proportion, this will be monitored by DMC.

2.9 Intervention allocation
Randomisation will be performed after the creation of embryos, 3 days post egg collection. This will minimise the randomisation-to-intervention time interval as embryos are either transferred at the cleavage or blastocyst stage. Once all eligibility criteria are established (including ensuring that three or more good quality embryos are available), women will be randomised (allocation ratio 1:1) to a strategy of either:

- Fresh embryo transfer
- Elective freezing of embryos followed by thawing and replacement at a later date (typically 4 to 6 weeks later and always within 3 months of egg collection)

Randomisation will be undertaken by the research nurse or a delegated member of the research team using a secure web-based centralised system (with 24/7 telephone backup 365 days/year) hosted by the National Perinatal Epidemiology Unit Clinical Trials Unit (NPEU CTU), University of Oxford (ensuring allocation concealment). The randomisation will employ a minimisation algorithm to balance across the following factors: fertility clinic; woman’s age (at the time of start of treatment i.e. ovarian stimulation): < 35 years, 35 to < 40 years, ≥ 40 years; primary/secondary infertility; self-reported duration of infertility: < 12 months, 12 to < 24 months, 24 to < 36 months, 36 to < 48 months, 48 to < 60 months, ≥ 60 months; method of insemination: IVF, ICSI, Split; and number of previous egg collections (cycles): 0, 1 and 2.

2.10 Data collection schedule
Data for both clinical and economic outcomes will be collected using bespoke electronic case report forms (eCRFs) and entered directly into the study’s OpenClinica electronic database by the centre’s research staff.

After consent and at embryo transfer, the couples will each complete a short paper-based questionnaire asking them how they are feeling. A short questionnaire will be provided for each partner to record details of time and travel expenses accrued during their treatment as part of the economic evaluation. This is to be completed at the time of embryo transfer.
*Routinely collected as part of regulatory requirement

2.11 Interim analyses and stopping rules
An independent Data Monitoring Committee has been established, whose remit will be to safeguard the interests of trial participants, potential participants, their families, their carers, investigators, and the sponsor; to assess the safety and efficacy of the intervention during the trial, and monitor the trial’s overall conduct, and protect its validity and credibility. The terms of reference for the DMC were agreed at their first meeting and the DMC Charter was finalised.

Interim analyses will be supplied, in strict confidence, to the DMC, as frequently as the Chair requests. The DMC will aim to meet in person at least annually, or more often as appropriate, and meetings should be timed so that reports can be fed into the Trial Steering Committee (TSC).

In the light of interim data and other evidence from relevant studies, the DMC will inform the TSC if, in its view, there is proof beyond reasonable doubt that the data indicate that the trial should be terminated. A decision to inform the TSC of such a finding will in part be based on statistical considerations. Appropriate proof beyond reasonable doubt cannot be specified precisely. A difference of at least 3 standard errors in the interim analysis of a major endpoint may be needed to justify halting or modifying the study prematurely. Unless modification or cessation of the trial is recommended by the DMC, the TSC, investigators, collaborators and administrative staff (except those who supply the confidential information) will remain ignorant of the results of the interim analysis. Collaborators and all others associated with the study may write to the DMC to draw attention to any concern they may have about the possibility of harm arising from the treatment under study. The Trial Statistician will provide the DMC with interim reports unblinded to allocation.

2.12 Trial reporting
The trial will be reported according to the principles of the CONSORT statement.
3. Protocol non-compliances
A protocol non-compliance is defined as a failure to adhere to the protocol such as the wrong intervention being administered, incorrect data being collected and documented, errors in applying inclusion/exclusion criteria or missed follow-up visits due to error.

All protocol non-compliances will be listed in the final report. Non-compliances are defined below.

3.1 Major
The following are pre-defined major protocol non-compliances with a direct bearing on the primary outcome:

- Data considered to be fraudulent

3.2 Minor
The following will be defined as minor protocol non-compliances:

Participants randomised in error
These include couples:

- where the female partner is not ≥ 18 and < 42 years of age at the start of treatment (i.e. start of ovarian stimulation)
- who are not undergoing their 1st, 2nd or 3rd cycle of IVF or ICSI treatment
- who are not both resident in the UK
- for whom written informed consent is not provided from both partners
- who have less than three good quality embryos and/or the quality of embryos is assessed before or after day three following fertilisation
- for whom donor gametes are used
- for whom pre-implantation genetic testing is planned
- for whom elective freezing of all embryos is planned for medical reasons (e.g. severe risk of OHSS/fertility preservation)
- who have previously been randomised to E-Freeze

Participants who do not receive their intervention as allocated
These include couples:

- randomised to the freezing of embryos followed by thawing and replacement at a later date (within three months of the date of egg retrieval) but who do not receive frozen embryo transfer or receive it after three months of the date of egg retrieval (defined as three calendar months)
- who switch from their randomised trial arm to the other arm
- who do not receive any embryo transfer within three calendar months of the date of egg retrieval

Other minor protocol non-compliances
These include couples:

- randomised before or after day three after egg collection (where day zero is defined as the day of egg collection)
- for whom a pregnancy test was carried out outside two weeks +/- 3 days after embryo transfer
- for whom an ultrasound was performed before six weeks’ or after eight weeks’ gestation
- contacted by telephone outside 12 weeks’ gestation +/- 14 days
- contacted by telephone outside 28 weeks’ gestation +/- 14 days
4. Adherence to the intervention
Data will be collected on women allocated to frozen embryo transfer who received fresh embryo transfer or no embryos, and women allocated to fresh embryo transfer who received frozen embryo transfer or no embryos. Reasons for not receiving the allocated intervention will also be collected.

A sensitivity analysis will be conducted to compare the effects in compliers and non-compliers. See section 7.5.

The date of egg collection and embryo transfer will be collected to derive the time to embryo transfer, and non-adherence to the maximum of 3 months will be reported.

5. Analysis populations

5.1 Post-randomisation exclusions
Exclusions to the analysis post-randomisation are defined as any of the following:

- couples for whom written consent forms from both partners were not received
- couples for whom consent to use their data was withdrawn by one or both of the partners
- couples for whom an entire record of fraudulent data was detected (should fraudulent data be detected, consideration will be given to excluding all data for the site where such data were found)

The numbers of post-randomisation exclusions will be reported by randomised treatment group in a figure presenting the flow of participants, and reasons summarised.

5.2 Population definitions

5.2.1 Intention to treat population
The intention to treat (ITT) population will be all couples randomised, excluding post-randomisation exclusions.

5.2.2 Interim analysis population
Baseline characteristics will be presented for all couples with available data at the time of the database snapshot, excluding known post-randomisation exclusions. Secondary outcomes will be presented for all couples with available outcome data and women who have delivered at the time of database snapshot, excluding known post-randomisation exclusions.

5.2.3 Safety population
All couples randomised, excluding post-randomisation exclusions. Since the post-randomisation exclusions are for couples for whom no written consent has been obtained or who have withdrawn permission for us to use their data, the safety population must exclude these couples.

6. Descriptive analyses

6.1 Representativeness of trial population and participant throughput
The flow of participants through each stage of the trial will be summarised by randomised group using a figure presenting the flow of participants. This will describe the numbers of couples:

- Consented
- Not randomised (with reasons)
- Randomised
- Allocated to frozen embryo transfer (with reasons if did not receive allocation)
  - Received frozen embryo transfer
  - Did not receive frozen embryo transfer
    - Received fresh embryo transfer (with reasons)
    - No embryos transferred (with reasons)
- Allocated to fresh embryo transfer (with reasons if did not receive allocation)
  - Received fresh embryo transfer
  - Did not receive fresh embryo transfer
    - Received frozen embryo transfer (with reasons)
    - No embryos transferred (with reasons)
- Randomised in error
- Withdrawn (consent/no consent to use data)
- Included in the ITT population
- Delivered (no. of women and no. of babies)
- Post-randomisation exclusions (with reasons)

6.2 Baseline comparability of randomised groups
Demographic and clinical characteristics at trial entry will be described for all couples in the ITT population by randomised group, and separately for couples who delivered (in the analysis report only). The following characteristics will be described:

- Fertility clinic
- Woman’s age at ovarian stimulation (years)
- Woman’s ethnicity
- Woman’s smoking status
- Woman’s BMI (kg/m²)
- Type of infertility
- Woman’s previous pregnancies
- Woman’s previous live births
- Main cause of infertility
- Duration of infertility (months)
- Endoscratch performed
- Stimulation regimen used
- Total stimulation dose of FSH (IU)
- Adjuvants used
- Trigger injection used
- Total number of eggs collected
- Method of insemination
- Number of eggs fertilised normally (two pro nucleus)
- Time lapse used
- Good quality embryos created on day three
- Number of previous egg collections
- Number of previous embryo transfers
Clinical characteristics of the embryo and the endometrium, which are collected post-randomisation, will also be described for the ITT population by randomised group, and separately for couples who delivered (in the analysis report only):

- Method of embryo freezing
- Number of embryos frozen
- Time from egg collection to embryo freezing
- Number of embryos thawed
- Stage of embryo at transfer
- Number of embryos transferred
- Number of remaining frozen embryos after transfer
- Method of endometrial preparation for transfer
- Endometrial appearance
- Endometrial thickness (mm)

The number and percentage will be presented for binary and categorical variables. The mean and standard deviation or the median and the interquartile range will be presented for continuous variables, or the range if appropriate. There will be no tests of statistical significance performed nor confidence intervals calculated for differences between randomised groups on any baseline variable.

### 6.3 Loss to follow-up

Although there is no follow-up planned for this trial, the primary outcome is collected some time after trial entry. It can be expected that there may be some data missing due to the delay in data collection. The number and percentage of missing outcome data among couples will be reported for the two trial arms in the results tables. All maternal and infant deaths will be reported separately.

As it is a regulatory requirement for clinics in the UK to report live birth outcomes after all embryo transfers, there will be no loss to follow-up for the primary outcome. Therefore, a sensitivity analysis will not be conducted to impute missing data.

### 6.4 Description of adherence to intervention

Adherence to the allocated intervention will be presented for the ITT population. Adherence will be summarised by randomised group in a figure presenting the flow of participants.

The number of women allocated to frozen embryo transfer who received fresh embryo transfer or no embryos will be reported, along with the reason if provided. This will include the number of embryos failing to survive after thawing.

The number of women allocated to fresh embryo transfer who received frozen embryo transfer within three calendar months from the date of egg collection, or no embryos will be reported along with the reason if provided.

In addition, the number of women receiving frozen embryo transfer (whether allocated or not) within three months of egg collection will be presented, along with the mean and standard deviation or median and interquartile range of the time from egg collection to embryo transfer for those receiving frozen embryo transfer. The number of women receiving fresh embryo transfer (whether allocated or not) will also be presented.
7. Comparative analysis

Couples and babies will be analysed according to their allocation regardless of the intervention they actually received. The fresh embryo transfer group will be used as the reference group in all analyses.

Since we are following women up from prior to pregnancy until after pregnancy, this raises the question of the most appropriate denominator for outcomes that can only occur in particular states. There will be some outcomes that cannot be reported for some women (e.g. miscarriage for women who do not become pregnant, birthweight of babies for women who had a miscarriage). In order to perform the analyses for all outcomes on the ITT analysis population, these will not be considered as missing data and for these outcomes all women will be included in the denominator. This means that regardless of whether a pregnancy or live birth occurs, the woman will be included in the denominator once for all outcomes. Where this is a perinatal outcome, but there is no baby for the outcome to occur in, the woman will still be included once in the denominator.

Outcomes will be summarised with counts and percentages for categorical variables, means and standard deviations for normally distributed continuous variables, or median and interquartile range for other non-normally distributed continuous variables.

Risk ratios and confidence intervals will be calculated using log binomial regression, and if a model fails to converge a log Poisson regression model with a robust variance estimator will be used. Analyses will be adjusted for all minimisation factors where possible (i.e. fertility clinic, woman’s age at the time of start of treatment (i.e. ovarian stimulation), primary/secondary infertility, self-reported duration of infertility, method of insemination and number of previous egg collections). Fertility clinic will be treated as a random effect in the model, and all other factors as fixed effects. Both crude and adjusted risk ratios will be presented, but the primary inference will be based on the adjusted estimates.

For neonatal secondary outcomes, the unit of analysis in the ITT analysis will be the mother. In cases of multiple pregnancy where the infants’ outcomes differ, the worst outcome will be reported.

Analysis of secondary outcomes will be clearly delineated from the primary outcomes in any statistical reports produced.

The emotions questionnaires at randomisation and post-embryo transfer capture responses from the State-Trait Anxiety Inventory (STAI). The response at randomisation will be used as a covariate in an analysis of covariance (ANCOVA) model. Hypothesis testing will investigate if there is a post-embryo transfer difference in the means of the two treatment groups having adjusted for responses at randomisation. To avoid bias, maximise the power of the study and to obey the intention-to-treat principle, the missing-indicator method will be used to replace missing baseline scores. This method replaces all missing baseline observations with the same value and an extra indicator variable is added to the model to indicate whether the value for that variable is missing. Models will be fitted separately for both the female and male partner.

The following secondary outcomes will be described only and no formal statistical analysis comparing groups will be conducted:

- Chronic hypertension, pregnancy induced hypertension, pre-eclampsia, and eclampsia
- Most severe hypertensive disorder (from least to worst: chronic hypertension, pregnancy induced hypertension, pre-eclampsia, and eclampsia)
### 7.1 Detailed definition of outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Unit of analysis</th>
<th>Primary analysis denominator</th>
<th>Secondary analysis denominator (clinically relevant population)</th>
<th>Details of derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
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<tr>
<td>Healthy baby</td>
<td>Woman</td>
<td>ITT population</td>
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<td>A healthy baby is defined as a live singleton baby born at term (between 37 and 42 completed weeks of gestation) with an appropriate weight for gestation (weight between 10th and 90th centile for that gestation based on standardised charts).</td>
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<tr>
<td></td>
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<td></td>
<td>The LMS method(^7) will be used to calculate birthweight centile.</td>
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<tr>
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<td></td>
<td>Gestational age at birth is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the date of birth. This is divided by 7 to give the age in weeks.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
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<tr>
<td><strong>Secondary outcomes</strong></td>
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<tr>
<td>Maternal safety outcome</td>
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<tr>
<td>Ovarian hyperstimulation syndrome (OHSS)</td>
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<tr>
<td>Complications of pregnancy and delivery</td>
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Date printed: 23/07/2019 13:33:00
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<th>Secondary analysis denominator (clinically relevant population)</th>
<th>Details of derivation</th>
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<td>Vanishing twin or triplet (defined as either: more fetal heartbeats than babies born, more gestational sacs than babies born, or more gestational sacs than fetal heartbeats)</td>
<td>Woman</td>
<td>ITT population</td>
<td>Total number of women with multiple gestational sacs and/or multiple fetal heartbeats</td>
<td>Gestational age at a given date is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the given date. This is divided by 7 to give the age in weeks. Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
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<td>Miscarriage rate (defined as pregnancy loss prior to age of viability i.e. 24 weeks of gestation)</td>
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<td>ITT population</td>
<td>Total number of women with a positive pregnancy test at 2 weeks +/- 3 days after embryo transfer</td>
<td>Gestational age at a given date is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the given date. This is divided by 7 to give the age in weeks. Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
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<td>Ectopic pregnancy</td>
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<td>Gestational diabetes mellitus (GDM)</td>
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<td>Multiple pregnancy (defined as more than one fetal heartbeat or more than one gestational sac)</td>
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<td>Total number of women with a positive pregnancy test at 2 weeks +/- 3 days after embryo transfer</td>
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<tr>
<td>Multiple births (including live and still births)</td>
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<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
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<tr>
<td>Outcome</td>
<td>Unit of analysis</td>
<td>Primary analysis denominator</td>
<td>Secondary analysis denominator (clinically relevant population)</td>
<td>Details of derivation</td>
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<td>Hypertensive disorders of pregnancy (chronic hypertension, pregnancy induced hypertension, pre-eclampsia, and eclampsia)</td>
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<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
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<tr>
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<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
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<tr>
<td>Antepartum haemorrhage (any bleeding per vaginum after 28 weeks of pregnancy including placenta praevia and placental abruption)</td>
<td>Woman</td>
<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
<td></td>
</tr>
<tr>
<td>Onset of labour (spontaneous, induced or planned caesarean section)</td>
<td>Woman</td>
<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
<td></td>
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<tr>
<td>Mode of delivery for each baby (normal vaginal delivery, instrumental vaginal delivery or caesarean section)</td>
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<td>ITT population</td>
<td>Total number of infants of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
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<td>Preterm delivery (defined as delivery at &lt; 37 completed weeks)</td>
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<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
<td>Gestational age at birth is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the date of birth. This is divided by 7 to give the age in weeks. Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
</tr>
<tr>
<td>Very preterm delivery (defined as delivery at &lt; 32 completed weeks)</td>
<td>Woman</td>
<td>ITT population</td>
<td>Total number of pregnant women with an ongoing pregnancy resulting in delivery (live or stillbirth)</td>
<td>Gestational age at birth is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the date of birth. This is divided by 7 to give the age in weeks.</td>
</tr>
<tr>
<td>Outcome</td>
<td>Unit of analysis</td>
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</tr>
<tr>
<td>Low birth weight (defined as weight &lt; 2500g at birth)</td>
<td>Infant</td>
<td>ITT population</td>
<td>Total number of babies born</td>
<td>Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
</tr>
<tr>
<td>Very low birth weight (defined as weight &lt; 1500g at birth)</td>
<td>Infant</td>
<td>ITT population</td>
<td>Total number of babies born</td>
<td>Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
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<tr>
<td>High birth weight (defined as weight &gt; 4000g at birth)</td>
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<td>ITT population</td>
<td>Total number of babies born</td>
<td>Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
</tr>
<tr>
<td>Large for gestational age (defined as birth weight &gt; 90th centile for gestational age at delivery, based on standardised charts)</td>
<td>Infant</td>
<td>ITT population</td>
<td>Total number of babies born</td>
<td>Using LMS method for birthweight centiles. Gestational age at birth is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the date of birth. This is divided by 7 to give the age in weeks.</td>
</tr>
<tr>
<td>Small for gestational age (defined as birth weight &lt; 10th centile for gestational age at delivery, based on standardised charts)</td>
<td>Infant</td>
<td>ITT population</td>
<td>Total number of babies born</td>
<td>Using LMS method for birthweight centiles. Gestational age at birth is calculated as the difference between the expected date of delivery and 40 weeks, subtracted from the date of birth. This is divided by 7 to give the age in weeks.</td>
</tr>
<tr>
<td>Congenital anomaly/birth defect (all congenital anomalies/birth defects identified will be included)</td>
<td>Infant</td>
<td>ITT population</td>
<td>Total number of babies born</td>
<td>Expected date of delivery is 38 weeks after egg collection for fresh transfer. For frozen transfer, the time between freezing and thawing is added on to this.</td>
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<tr>
<td>Outcome</td>
<td>Unit of analysis</td>
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<tr>
<td>Perinatal mortality (stillbirth or late as well as early neonatal deaths, up to 28 days after birth)</td>
<td>Infant</td>
<td>ITT population</td>
<td>Total number of babies born</td>
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<tr>
<td><strong>Measures of clinical effectiveness outcomes</strong></td>
<td></td>
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<tr>
<td>Live birth rate (this is a live birth episode i.e. twins will count as one)</td>
<td>Woman</td>
<td>ITT population</td>
<td></td>
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<tr>
<td>Singleton live birth rate</td>
<td>Woman</td>
<td>ITT population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton live birth rate at term</td>
<td>Woman</td>
<td>ITT population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton baby with appropriate weight for gestation</td>
<td>Woman</td>
<td>ITT population</td>
<td>The LMS method will be used to calculate birthweight centiles, which will be between the 10th and 90th centile.</td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate (defined as positive pregnancy test at 2 weeks +/- 3 days after embryo transfer)</td>
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<td>ITT population</td>
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<tr>
<td>Outcome</td>
<td>Unit of analysis</td>
<td>Primary analysis denominator</td>
<td>Secondary analysis denominator (clinically relevant population)</td>
<td>Details of derivation</td>
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<tr>
<td>Clinical pregnancy rate (defined as the presence of at least one fetal</td>
<td>Woman</td>
<td>ITT population</td>
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<tr>
<td>heartbeat at ultrasound between six and eight weeks gestation; ectopic</td>
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<tr>
<td>pregnancy counts as a clinical pregnancy; multiple gestational sacs</td>
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<tr>
<td>count as one clinical pregnancy)</td>
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</table>

Measures of the effectiveness of the process of freezing embryos outcomes

<table>
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<tr>
<th>Measures of the effectiveness of the process of freezing embryos outcomes</th>
<th>Unit of analysis</th>
<th>Primary analysis denominator</th>
<th>Secondary analysis denominator (clinically relevant population)</th>
<th>Details of derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of embryos frozen, thawed and transferred for all</td>
<td>Couple</td>
<td>Total number of randomised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>randomised couples</td>
<td></td>
<td>couples</td>
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<tr>
<td>Proportion of thawed embryos that were then transferred for all</td>
<td>Couple</td>
<td>Total number of randomised</td>
<td></td>
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<td>randomised couples</td>
<td></td>
<td>couples</td>
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<tr>
<td>Failure of all embryos to survive after thawing leading to no embryo</td>
<td>Couple</td>
<td>ITT population</td>
<td></td>
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<tr>
<td>transfer</td>
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Other secondary outcomes

<table>
<thead>
<tr>
<th>Other secondary outcomes</th>
<th>Unit of analysis</th>
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<td>state (for both the female and male partners)</td>
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<tr>
<td></td>
<td>Woman</td>
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</tbody>
</table>
7.2 Primary analysis
The primary analysis for the primary outcome and all secondary outcomes will be conducted on the ITT population.

7.3 Secondary analyses
There are a number of other clinically relevant populations of equal importance to the ITT population in the interpretation of results. In order to assess the treatment effect for the clinically relevant populations (e.g. effect on live birth rate for women who become pregnant), secondary analyses of specific outcomes will be performed with respect to clinically relevant denominators. These are as follows:

Per total number of women with a positive pregnancy test at 2 weeks +/- 3 days after embryo transfer:
- Miscarriage rate (defined as pregnancy loss prior to age of viability i.e. 24 weeks of gestation)
- Multiple pregnancy (defined as more than one fetal heartbeat or more than one gestational sac)

Per total number of pregnant women with an ongoing pregnancy resulting in delivery:
- Gestational diabetes mellitus
- Multiple births (including live and still births)
- Hypertensive disorders of pregnancy (chronic hypertension, pregnancy induced hypertension, pre-eclampsia and eclampsia)
- Most severe hypertensive disorders (from least to worst: chronic hypertension, pregnancy induced hypertension, pre-eclampsia and eclampsia)
- Antepartum haemorrhage (any bleeding per vaginum after 28 weeks of pregnancy including placenta praevia and placental abruption)
- Preterm delivery (defined as delivery at < 37 completed weeks)
- Very preterm delivery (defined as delivery at < 32 completed weeks)
- Onset of labour (spontaneous, induced or planned caesarean section)
- Mode of delivery for each baby (normal vaginal delivery, instrumental vaginal delivery or caesarean section)

Per total number of babies born:
- Low birth weight (defined as weight < 2500g at birth)
- Very low birth weight (defined as weight < 1500g at birth)
- High birth weight (defined as weight > 4000g at birth)
- Large for gestational age (defined as birth weight > 90th centile for gestational age at delivery, based on standardised charts)
- Small for gestational age (defined as birth weight < 10th centile for gestational age at delivery, based on standardised charts)
- Congenital anomaly/birth defect (all congenital anomalies/birth defects identified will be included)
- Perinatal mortality (stillbirth or late as well as early neonatal deaths, up to 28 days after birth).

For neonatal secondary outcomes, where the unit of analysis is the baby, the method of analysis will account for the anticipated correlation in outcomes between multiples.
7.4 Pre-specified subgroup analysis
The consistency of the effect of type of embryo transfer across specific subgroups of couples will be assessed for the primary outcome using the statistical test of interaction, in addition to the adjusted model. Results will be presented on forest plots with risk ratios, 95% confidence intervals and the results of the interaction test.

Pre-specified subgroup analyses are:
- Fertility clinic
- Woman’s age (at the time of start of treatment i.e. ovarian stimulation): < 35 years, 35 to <40 years, ≥ 40 years
- Blastocyst versus cleavage
- Single versus multiple embryo transfer
- Number of previous embryo transfers (0, 1 – 3, ≥ 4).

In addition, for those receiving frozen embryo transfer, the following subgroups will be described using numbers and percentages:
- Natural versus hormone replacement cycles
- Vitrification versus slow freezing.

7.5 Sensitivity analysis
To assess the impact of non-compliance to the randomised allocation, i.e. women randomised to the frozen arm receiving fresh embryo transfer (non-compliers), a complier-average causal effect (CACE) analysis will be conducted. This analytic technique provides a robust estimate of the treatment effect amongst compliant participants.\(^9\)

The baseline characteristics of women randomised to the frozen arm will be reported by compliance status, and the unadjusted event rate for the primary outcome will be calculated for the observed compliers and non-compliers in the frozen arm. CACE analysis assumes that the proportion of would-be non-compliers in the fresh arm (i.e. couples in the fresh group who would not have complied had they been randomised to frozen) is the same as the proportion of non-compliers in the frozen group. It also assumes that the event rate among the non-compliers in the frozen arm is the same as the event rate among the would-be non-compliers in the fresh group. Applying these two assumptions, the unadjusted event rate for the primary outcome will be calculated for the would-be compliers and would-be non-compliers in the fresh arm. The unadjusted CACE risk ratio plus 95% confidence intervals for the primary outcome will be calculated using the event rates for compliant groups only (i.e. the observed compliers in the frozen arm and the would-be compliers in the fresh arm). The confidence intervals for the CACE estimated risk ratio will be calculated using bootstrapping methods.\(^10\)

If an entire record is considered fraudulent, this will be excluded from all analyses (see section 5.1). However, if any individual data is considered fraudulent, a sensitivity analysis will be conducted on outcomes where any fraudulent data is detected excluding data considered fraudulent.

7.6 Significance levels and adjustment of p-values for multiplicity
For all analyses of the primary outcome, a 95% confidence interval will be calculated. To take account of the number of hypothesis tests performed, 99% confidence intervals will be used for all analyses of the secondary outcomes.
7.7 Missing data
Missing data will be described, for example, by presenting the number of individuals in the missing category. All data collected on data collection forms will be used, since only essential data items will be collected.

For any partially completed self-evaluated STAI questionnaires in the Emotions Questionnaire CRF, the following strategy for estimation of total and subscale scores will be employed where items are missing:

- If one or two items are omitted then the prorated full-scale score can be obtained by: Determining the mean weighted score for the scale items to which the individual responded, multiply this value by 20 and then round the product to the next higher whole number.
- If three or more items are omitted then the validity of the questionnaire must be questioned.\(^5\)

7.8 Statistical software employed
The statistical software Stata/SE will be used for all analyses.

8. Safety data analysis
Serious adverse events will be listed by allocation.

9. Additional exploratory analysis
The following exploratory analyses will be conducted on the primary outcome:

- A restricted per protocol analysis, excluding couples who did not receive the allocated intervention as randomised.
- An as-treated analysis, grouping couples according to the allocation they received.

Analysis methods as used for the primary analysis will be employed.

Any other additional exploratory analyses not specified in the analysis protocol will be exploratory in nature and a 1% two-sided significance level will be used with 99% confidence intervals. All such analyses will be approved by the Co-investigator Group.

10. Deviation from analysis described in protocol
None yet.

11. References

11.1 Trial documents

Dummy tables can be found in K:\NPEUDATA\Current trials\E-Freeze\Statistics\Dummy Tables\Final Analysis

The Data Derivation spreadsheet can be found in K:\NPEUDATA\Current trials\E-Freeze\Statistics\Data derivation
11.2 Other references


12. Approvals

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<tr>
<th>Senior Statistician</th>
<th>Name: Dr Louise Linsell</th>
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<tr>
<th>Chief Investigator</th>
<th>Name: Dr Abha Maheshwari</th>
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<tr>
<th>Chair of Trial Steering Committee (or delegate)</th>
<th>Name: Professor Richard Anderson</th>
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<tr>
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Document history

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<th>Edited by</th>
<th>Comments/Justification</th>
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<td>Minor comments and edits</td>
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<td>20 Mar 2017</td>
<td>PH</td>
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<td>PH/JB</td>
<td>Updates after discussions between PH and JB on 21/3/17. Now includes table of outcomes specifying analysis units, denominators and derivations for each outcome.</td>
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<td>Updated section 7.1, with mind amendments and clarifications to</td>
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<td>Sensitivity Analysis &amp; Additional Exploratory Analysis sections updated. Comments removed</td>
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<td>Minor changes following comments from TSC. Clarification to section 7.5 Sensitivity Analysis, and addition of references.</td>
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