HABSelect

Statistical Analysis Plan

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Abstract

Background: *Intra-Cytoplasmic Sperm Injection is a procedure in Assisted Reproduction Technologies, where, instead of the egg being the final arbiter for selection, the 'right' single sperm is selected for each egg by the embryologist. Recent clinically relevant studies suggest, however, that using a hyaluronic acid based selection procedure may increase live birth rates as well as having a number of other positive effects. The HABSelect trial aims to evaluate the effectiveness of this sperm selection procedure on increasing live birth rate. Other secondary measures will also be evaluated.*

Methods/Design: *The trial is a parallel group, two arm, multicentre, blinded, randomised controlled efficacy clinical trial with mechanistic evaluation. A total of 3266 couples will be randomised. The primary outcome is live birth rate beyond 37 weeks gestation. Secondary outcome measures are also presented and the proposed statistical analysis for all clinical outcome measures are outlined in detail in this paper.*

Conclusion: *The HABSelect trial investigates the effect of using a hyaluronic acid based sperm selection approach in Intra-Cytoplasmic Sperm Injection procedures. Clinical outcomes from the HABSelect trial will be analysed according to this pre-specified statistical analysis plan.*

Trial Registration: *HABSelect is registered in ISRCTN under ISRCTN99214271*

Keywords: *HABSelect Trial; Intra-Cytoplasmic Sperm Injection; Live Birth Rate; Polyvinylpyrrolidone; Hyaluronic Acid; Hyaluronan; Sperm Selection; Assisted Reproduction Technologies; Clinical Pregnancy; Miscarriage; Male Fertility; Randomised Controlled Trial; Statistical Analysis Plan*

Trial Overview and Design

Please refer to accompanying protocol for detailed aspects.

Overview: The HABSelect trial is a parallel group two arm multi-centre blinded randomised controlled efficacy clinical trial, with mechanistic evaluation, comparing the use of an HA (hyaluronic acid) selection step prior to ICSI with standard ICSI for treatment of male infertility, with the objective of increasing live birth outcomes and reducing miscarriage rates.

Study Population: The study population for randomisation represents couples undergoing ICSI procedure with the ability to provide informed consent. The following inclusion criteria are also imposed:

Women:

- BMI: 19.0 – 35.0 kg/m^2
- FSH level: 3.0 – 20.0 miU/ml and / or AMH ≥ 1.5 pmol/L
- Age: 18-43

Men:

- Age: 18 – 55
- Able to produce freshly ejaculated sperm for the treatment cycle

The exclusion criteria for the trial are as follows:

- Couples who have not consented prior to ICSI will be ineligible
- Couples using non-ejaculated sperm
- Couples using donor gametes
- Men with vasectomy reversal; cancer treatment involving any chemotherapy and / or radiotherapy in the past two years
- Previous participation in the HABSelect trial
- Split IVF / ICSI procedures
- If both FSH and AMH are tested and either of them falls outside the accepted range

There are 15 participating centres. Recruitment rates will be monitored and optional additional centres may be added as required. Centres will be IVF licensed hospitals and must be able to provide appointments in a dedicated clinic in which to see participants.

Consent: Written informed consent will be obtained by the principal investigator, or by another suitably qualified member of the trial team. This will compromise of a written consent form, and will be obtained for each couple before enrolment in the trial. Patients have the right to refuse consent and / or withdraw from the study at any time without giving reasons and without prejudicing any further treatment.

Randomisation Procedures: Couples are randomised in a ratio of 1:1 to either intervention (PICSI) or non-intervention (PVP-ICSI) arms. Randomisation is stratified by four criteria:

- Maternal age (<35, ≥35)
- Paternal age (<35, ≥35)
- Number of previous miscarriages (0,1-2, >2)
- Hormonal indicator of ovarian reserve: FSH (<6.0, ≥6.0 miU/ml) or AMH (<17.0, ≥17.0 pmol/L) when FSH is not available.

Minimisation factors are balanced separately within each centre.

Treatment Procedures: In the non-interventional arm (standard ICSI) density gradient washed and prepared motile sperm and selected for ICSI by adding the sperm suspension to polyvinylpyrrolidone (PVP) on an inverted microscope. Sperm motility is slowed sufficiently to allow capture by the experience embryologist who then immobilises the sperm by crushing its flagellum with the injection pipette. The sperm is then taken up into the injection pipette and injected directly into the egg. In the interventional arm (HA-ICSI) exactly the same procedure is carried out except that the washed and prepared motile sperm are allowed to interact with and become attached to a specially prepared HA-coated surface (PICSI) beforehand. The HA-selection process is henceforward referred to as PICSI

Treatment Blinding: Participants, clinical care providers in IVF licensed units, maternity & neonatal wards and research nurses responsible for participants follow up will be blinded to treatment allocation. The only unblinded group at study sites is going to be the embryologist who performs the PICSI / ISCI procedure, hyaluronic acid binding scoring (HBS) and randomisation. Those within the PCTU who will remain unblinded will be the study data

manager and independent statistician, who will prepare reports for the Data Monitoring and Ethics Committee (DMEC).

An anticipated CONSORT flow chart for the trial is shown in the accompanying protocol document (Figure 1) and detailed in the accompanying CONSORT checklist.

Objectives / Outcome Measures

The main aims of HABSelect are to show 1) that a hyaluronic acid binding step in an assisted reproduction setting can significantly improve live birth rate and 2) assess how the chromatin status of HA-selected versus conventionally recovered sperm corresponds with HA binding score (HBS), clinical pregnancy, live birth rate and pregnancy loss.

Primary Objective: To determine the efficacy of PICSI versus standard ICSI in a rigorous randomised controlled clinical trial of participants where the primary outcome measure will be live birth rate ≥ 37 weeks gestation after first fresh embryo transfer.

Secondary Objectives: To determine the impact of PICSI versus standard ICSI on:

- Increasing clinical pregnancy rate based on detection of fetal heartbeat or presence of fetal sac at 6-9 weeks gestation
- Reducing miscarriage rate defined as pregnancy loss after confirmation of clinical pregnancy
- Increasing live birth rate at <37 weeks gestation

Primary outcome measure: Live birth at ≥ 37 weeks gestation following the first fresh ICSI/PICSI treatment.

Secondary outcome measures:

- Clinical pregnancy rate based on detection of a fetal heartbeat of the presence of fetal sac at 6-9 weeks gestation
- Miscarriage, defined as pregnancy loss after confirmation of clinical pregnancy
- Live birth <37 weeks gestation

General Statistical Considerations

All analyses will be intention-to-treat (ITT). Every attempt will be made to gather data on all women randomised, irrespective of compliance with the treatment protocol. Outliers for continuous variables will be summarised and carefully checked.

All investigators will remain blinded prior to the final analysis so as not to bias the analysis and interpretation of results. An independent statistician employed by the PCTU will provide the DMEC with unblinded summaries and reports using computer code provided by the study statistician. Stata version 12 or higher will be used to code and produce statistical analysis, but other software such as R may be used if appropriate.

Missing data: Every attempt will be made to collect full follow up data on all couples and it is anticipated that missing data will be minimal. The missingness in outcome and baseline data will be summarised, with breakdowns of missingness by trial arm, for example. Where baseline covariates are missing, mean imputation will be used for continuous covariates and a missing indicator will be used for categorical variables. Note that epidemiological arguments against the use of a missing indicator do not apply in randomised trials (64).

If any outcome data are missing we will analyse only those with outcome data, adjusting for baseline covariates. This approach is unbiased if the outcome is 'Missing At Random' (MAR) i.e. missingness for the outcome is related to the observed covariates. If missingness in the primary outcome is >5% then a sensitivity analysis will be conducted to explore the MAR assumption. In this case, a pattern-mixture model estimated by a mean score approach will be adopted, as detailed by Ian White (2).

Results of the sensitivity analysis will be concisely reported with effect sizes and 95% confidence intervals and saying, for example, whether the significance of the main analysis was maintained across departures from the MAR assumption. If it is concluded that inferences about treatment effect are sensitive to departures from the MAR assumption, then missing outcome data will be imputed using multiple imputation. The imputation model will include all variables in the adjusted analysis, plus, as far as computationally feasible, all variables predictive of the missing values themselves and all variables influencing missingness (3). The decision on variables to include will be made post hoc. In this event, this will become the primary analysis.

Statistical Analysis

Please refer to accompanying protocol manuscript for details of sample size considerations.

Post Randomisation Exclusions: Certain exclusions will be made for the analysis, post-randomisation. These will be all women who were enrolled in error or failed to consent. Women withdrew their consent will still be analysed unless it was specified by them that their data should not be used, in which case the data will be safely destroyed and excluded from the trial analysis.

Evaluation of Demographics and Baseline Covariates: Numbers of couples who are eligible, recruited and followed up will be recorded in a CONSORT flow-chart. Baseline characteristics of couples in each arm will be summarised with counts (percentages) for categorical variables, mean with standard deviation (SD) for normally distributed continuous variables or median with interquartile (IQR) or entire range for other continuous variables. Intermediate outcomes, such as pregnancy test results and confirmation of clinical pregnancy will also be summarised.

Primary Analysis: The primary outcome measure is the proportion of women randomised who experience a live birth ≥ 37 weeks. This proportion has as its denominator the number of women who are randomised to either intervention (PICSI) or non-intervention (PVP-ICSI). and as its numerator the number of women who conceive and proceed to have a live birth ≥ 37 weeks as a result of their first fresh ICSI cycle This is because we believe that, if effective, the impact of the intervention will be evident in the first fresh study cycle. Differences in the proportion between treatment arms will be assessed using logistic regression. Univariable and multivariable logistic regression models will be used to estimate crude and adjusted odds ratios with 95% confidence intervals. A two sided P-value will also be reported in each case. The adjusted analysis will adjust for the minimisation variables, as well as including centre as a random effect.

Sensitivity Analyses: If there is evidence that the clinical pregnancy rate differs between the treatment arms, then as a sensitivity analysis, the analysis will be redone taking only women who experience a clinical pregnancy as the denominator.

As an additional sensitivity analyses, the analysis will be redone adjusting for other factors believed to potentially prognostic or associated with the outcome, as well as for centre (as a random effect) and the minimisation variables. Factors to be included in this additional analysis are

- Female partner BMI (Normal: 19≤BMI<25, Overweight: 25<BMI≤30, Obese: 30≤BMI≤35)

- Female partner ethnicity (White / Asian or Asian British / Black or Black British / Other)
- History of previous pregnancy (Yes/No)
- Maternal age (18-29, 30-34, 35-39, 40+)
- Smoking status
- Oocytes collected (<5, 5-9, 10-14, >14)
- Number of embryos transferred
- Stimulation treatment (long agonist / short agonist / antagonist)

In all cases, results of the primary analysis will be given more weight than those of any secondary analyses.

Subgroup Analysis: The following subgroup analyses will be performed for the primary outcome:

- Analysis of treatment effect by embryos transferred (single vs. double or more)
- Analysis of treatment effect by HBS (high (>65%) versus low (≤65%))
- Analysis of treatment effect by maternal age (≤35 years verses >35)
- Analysis of treatment effect by number of previous miscarriages (0 versus >0)
- Analysis of treatment effect by Follicle stimulating hormone (FSH) hormone level (<6.0miU/ml versus ≥ 6.0miU/ml) or Anti-Mullerian Hormone (AMH) hormone level (<17pmol/L versus ≥ pmol/L) where FSH testing is not available
- Analysis of treatment effect by sperm concentration (<15mml versus ≥ 15mml)
- We will also analyse treatment effect by a very low HBS sub-group (≤25%) versus a low HBS sub-group (>25%, ≤65%)

These subgroup analyses will be undertaken by statistically testing for an interaction term. Subgroup specific estimates (for planned and exploratory analyses) will be reported with 95% confidence intervals and displayed graphically. All subgroup analyses will be hypothesis generating and findings will be treated with caution. Hence there will be no corrections made for the issue of multiplicity.

Secondary Analysis: The proportions of each secondary outcome will be compared between treatment arms using univariate and multivariable logistic regression models to estimated crude and adjusted odds ratios. A 95% confidence interval and two sided P-value

will be reported in each case. The adjusted analysis will adjust for the same factors as the primary analysis.

Mechanistic Evaluation

As indicated in the accompanying protocol, a mechanistic evaluation will also be undertaken. Planning for these analyses are covered in the accompanying protocol with a more detailed description to be documented separately.

Conclusion

With this SAP we present the analyses that will be published in the primary publication for the clinical aspect of the trial. By publishing this SAP prior to unblinding of any investigators, we avoid any bias that may arise from knowledge of outcome and data-driven results.

The aim of the HABSelect study is to compare the use of PICSI to ISCI procedures for treatment of male fertility. With the publication of this paper pre-specifying the analyses to be used, we hope that the results from the HABSelect trial will be as transparent as possible.

References

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