HABSelect

Statistical Analysis Plan

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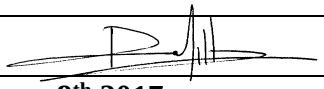
Signature	
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Abstract

Background: *Intra-Cytoplasmic Sperm Injection (ICSI) 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. Clinically relevant studies suggest that using a hyaluronic acid binding based selection procedure (using hyaluronic acid coated plates) increases 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, versus sperm selected on a conventional visual basis prior to ICSI., 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 participants 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 outcome measures are outlined in detail in this paper.*

Conclusion: *The HABSelect trial investigates the effect of using a hyaluronic acid binding 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*

Introduction

In 2008, almost 40,000 couples in the UK alone were treated with assisted reproduction technologies (ART), compromising of 50,687 in vitro fertilisation (IVF) cycles. This number is set to rise in the coming years (1). Currently live birth rates for ART are at an average of 24% per treatment cycle, although live birth rates per couple are higher at 32%, because couples normally receive an average of approximately 1.3 treatment cycles. While it is estimated that more than two thirds of naturally conceived pregnancies end in failure, the limit for improvements in live birth rates following ART may not have been reached.

For all ART procedures including intra-cytoplasmic sperm injection (ICSI), the embryologist seeks to use the best sperm available. Selection is aided by sperm 'washing' techniques using density gradient configuration (DGC) that can enrich for sperm with high motility and good morphology (WHO Manual, 2010) (2). In contrast with standard IVF, where the egg is the final arbiter for selection, ICSI is dependent on the relatively subjective judgment of the andrologist or embryologist to choose the 'right' single sperm for each egg.

Various studies have shown clear inverse relationships between DNA damaged sperm in the ejaculate and clinical pregnancy or live birth rates in standard IVF, but this relationship is less obvious with ICSI cycles (3). We recently reported reductions in levels of sperm DNA fragmentation following density gradient washing of semen and while the values from washed semen were reduced, they were still over twice as high in the non-pregnant (approximately 50%) versus pregnant (approximately 23%) cohorts. These and other data suggest that sperm with poor DNA quality persist in washed sperm preparations from fertile and infertile men (4-13) and unlike IVF, where there is a natural selection by the egg, ICSI could be particularly vulnerable to a poor choice of sperm. By eliminating abnormal sperm from the sample preparation for ICSI, success rates should rise accordingly.

It has been shown that immature sperm with higher rates of DNA damage have a dysfunctional ability to bind to hyaluronic acid (14, 15), which is the major glycosaminoglycan secretion of the cervix. In many clinics, polyvinylpyrrolidone (PVP) is normally used to slow sperm down sufficiently for capture in ICSI procedures (standard-ICSI). However, clinically relevant studies (16, 17) suggest that using hyaluronic acid-selected (using hyaluronic acid coated plates) Physiological Intra-Cytoplasmic Sperm Injection (PICSI) instead of standard-ICSI increases live birth rate and the numbers of grade one embryos.~~.~~ There is also strong evidence that PICSI reduces early pregnancy failure (17). The HABSelect (Hyaluronic Acid Binding Sperm Selection) trial aims to confirm this by comparing the use of a HA (hyaluronic acid) selection step prior to ICSI (PICSI) with standard-ICSI for treatment of male fertility for the treatment of male infertility in a rigorous randomised controlled efficacy trial. A successful conclusion of the study will help provide a more consistent and efficient procedure for ICSI sperm selection which complies with and extends on the National Institute of Clinical Excellence's (NICE) recently called review on fertility guidance (18).

This paper describes the statistical analysis plan (SAP) for the clinical outcomes of the HABSelect trial. A mechanistic evaluation of the action of hyaluronic sperm selection will also be undertaken and included within the final report. However, planning for this aspect of

the trial will be documented separately and only analysis of clinical outcomes are specified within this document. In accordance with good clinical practice, this SAP was drafted without any knowledge of the outcomes by the investigators and this blinding will not be broken before the analysis plan is finalised and signed off.

Trial Overview and Design

Overview: The HABSelect trial is a parallel group two arm multi-centre blinded randomised controlled efficacy clinical trial, with mechanistic evaluation, comparing the use a HA (hyaluronic acid) selection step prior to physiological ICSI (PICSI) 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 (standard-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 visually selected for ICSI with the aid of an inverted microscope. In the interventional arm (PICSI) 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 specifically prepared HA-coated surface 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 / standard-ICSI 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 displayed in Figure 1.

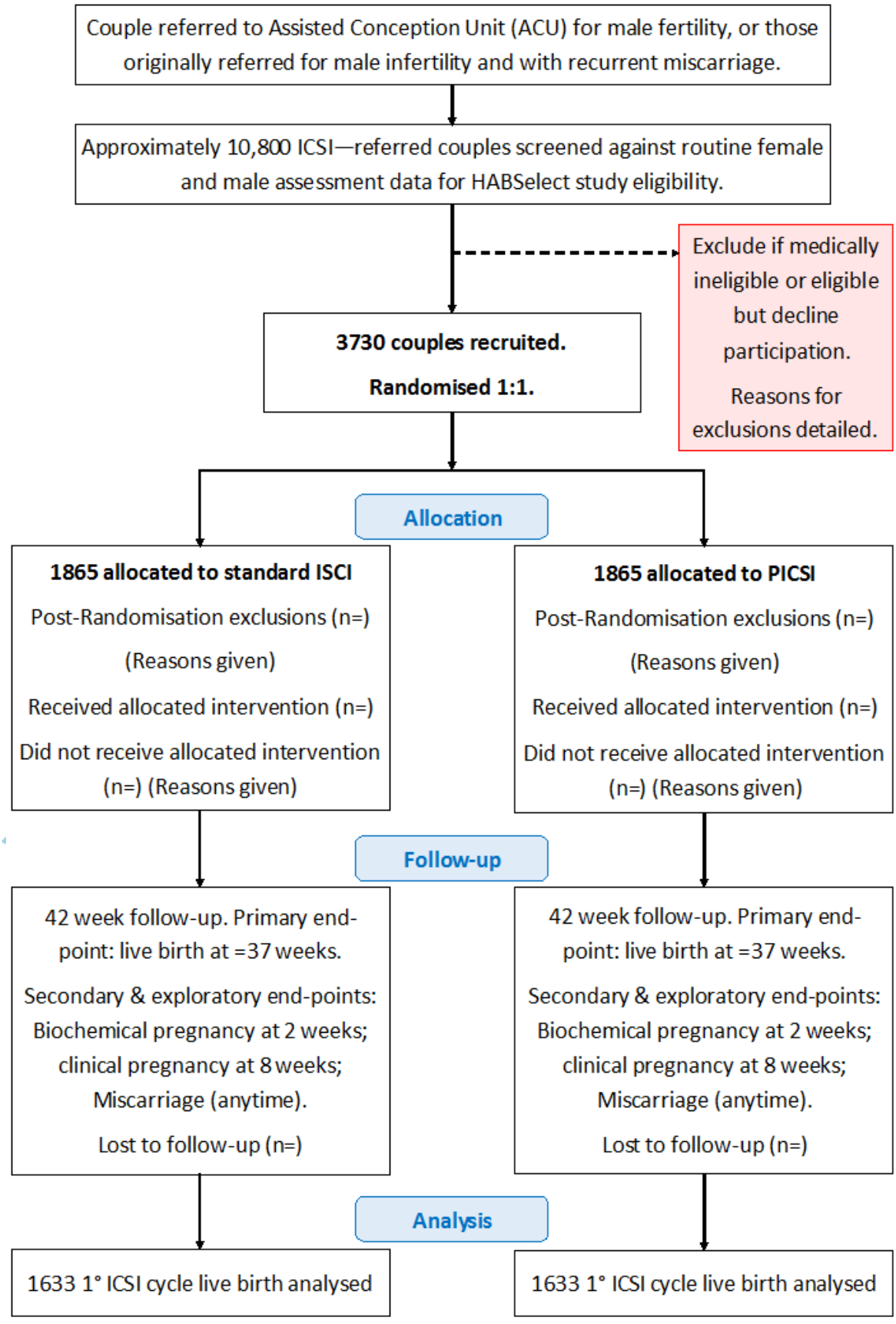

Figure 1: Anticipated CONSORT flow-chart for HABSelect.

Objectives / Outcome Measures

The main aims of HABSelect are to

1. Show that a hyaluronic acid binding step in an assisted reproduction setting can significantly improve live birth rate
2. Assess how the chromatin status of HA-selected versus conventionally recovered sperm corresponds with 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 PISCI 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 PICSI/ICSI treatment.

Secondary outcome measures:

- Clinical pregnancy rate based on detection of a fetal heartbeat or 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

Sample Size

From our study feasibility audit data, we estimate that around 4663 men per annum will be eligible for an ICSI procedure across all 10 participating centres. Given our broad inclusion criteria, we conservatively expect that 40% of the couples to be eligible and willing to consent to the study i.e. 3730 over 21 months. Trial recruitment is based on pro rata targets at each of the participating sites of the Human Fertilisation and Embryology Authority (HFEA) data and the need to recruit at least 3266 couples into the trial to detect a 5% improvement in clinical efficacy with a power of 90%.

For PISCI, average improvements in live birth rate per treatment cycle are likely to be 7.5% based on maternal age and paternal semen profile (16, 17, 19). Older women (≥37) are of particular interest to us because their eggs may have a decreased capacity for repairing sperm

DNA damage in their older partners (20). Lower and higher improvement scores among younger and older women are likely, respectively. Assuming 5% for the former and 15% for the latter, live birth rate will rise from 32.7% to 37.7% (3826 treatment cycles) and 19.3% to 34.3% (358 treatment cycles) in women <35 and women >37, respectively. Because they lie between the more fertile younger and less fertile older age groups, improvements for women aged between 35 and 37 are likely to reflect that of women of all ages, at 7.5%. We assume that miscarriage rate will be inversely correlated with live birth rate and therefore it is unnecessary to repower for it. Clearly, we shall have sufficient recruitment into the study to test outcomes in relation to HBS predictions and parental age. However, lower improvement rates (among younger couples in particular) will incur lower accuracies unless power is relaxed to 80%. Improvement among older women is certainly testable, as those >37 now account for almost 30% of ART procedures, providing 1007 women for the study.

A 10% loss to follow-up (the envisaged worst-case scenario) will still ensure outcomes for 3357 primary treatment cycles, which is sufficient to power the study at even 5% improvement per couples undergoing a fresh ICSI treatment cycle. It is anticipated, however, that compliance with follow-up will be high, given the lateness of randomisation and the routine nature of collecting pregnancy outcome data in this population (refer to anticipated CONSORT flow-diagram – Figure 1).

General Statistical Considerations

All analyses will be intention-to-treat (ITT): all couples randomised, with the exception of those enrolled in error or for who consent was not obtained, with a recorded outcome will be included in the analysis, and analysed according to the arm to which they were randomised (21). Every attempt will be made to gather data on all women randomised, irrespective of compliance with the treatment protocol.

Post Randomisation Exclusions: Certain exclusions will be made for the analysis, post-randomisation. These will be all couples who were enrolled in error or because consent was not obtained. Women whose BMI calculated baseline height and weight to be greater than or equal to 18 or less than or equal to 36 will be included in the trial to allow for rounding errors in BMI. Women with BMI below 18 or above 36 will be excluded from the trial as they do not meet the eligibility criteria and will be considered to be enrolled in error. Women who are found not to meet any other eligibility criteria will be excluded from the analysis. Women who withdraw 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 excluded from the trial analysis.

For the primary analysis, all secondary analysis and sensitivity analysis we will report an odds ratio for the effect of the intervention with a 95% confidence interval and two sided P-value.

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 provided 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 (22).

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 (23). We will model the primary outcome using logistic regression, adjusting for maternal age, paternal age, number of previous miscarriages, and hormonal indicator of ovarian reserve in the same way as the primary analysis. Centre will be accounted for using clustered sandwich variance estimator. We will vary the informative 'missingness' odds ratio between 1/3 and 3, i.e. the probability of a missing outcome being a live birth ≥ 37 weeks is between 1/3 and 3 times as likely as an observed outcome.

Statistical 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 and median with interquartile range (IQR) for continuous variables. Mean and standard deviation may also be used to summarise continuous variables where appropriate.

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 (standard-ICSI) with data recorded 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. Please see Appendix 1 for a description of the variables to be used in the derivation of the primary outcome.

Differences in the proportion between treatment arms will be assessed using mixed effects logistic regression model. The analysis will adjust for the minimisation variables: maternal

age, paternal age, number of previous miscarriages, and hormonal indicator of ovarian reserve. Centre will be included as a random intercept. Maternal age and paternal age will be adjusted for using restricted cubic splines with three knots (knot locations based on Harrell's recommendations) (24, 25). Number of previous miscarriages, and hormonal indicator of ovarian reserve will be adjusted for as categorical variables. Number of previous miscarriages will have three categories 0,1-2, >2. Hormonal indicator of ovarian will have two categories FSH <6.0, ≥6.0 miU/ml or AMH <17.0, ≥17.0 pmol/L when FSH is not available.

Sensitivity Analyses: If there is evidence that the secondary outcome clinical pregnancy rate differs between the treatment arms, then as a sensitivity analysis, the primary outcome will be reanalysed taking only women who experience a clinical pregnancy as the denominator. This analysis will be carried out using the same mixed effects logistic regression described for the primary analysis with an analysis population of all couples included in the primary analysis who experienced a clinical pregnancy.

As an additional sensitivity analysis, the primary outcome will be reanalysed using a mixed effect logistic regression, including centre as a random intercept, adjusting for the minimisation variables as described in the primary analysis model and adjusting for additional factors believed to be potentially prognostic or associated with the outcome. Additional factors to be adjusted for in this analysis are:

- Female partner BMI (adjusted for using restricted cubic splines with three knots (knot locations based on Harrell's recommendations) (24, 25))
- Female partner ethnicity (adjusted for using four categories: White / Asian or Asian British / Black or Black British / Other)
- History of previous pregnancy (adjusted for using two categories: yes/no)
- Female partner smoking status (adjusted for using two categories: current smoker/not current smoker)
- Stimulation treatment (adjusted for using three categories: long agonist / short agonist / antagonist)

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

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

- 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 may also analyse treatment effect bFy a very low HBS sub-group, depending on numbers available (≤25%) versus a low HBS sub-group (>25%, ≤65%)

The subgroup analysis will be carried out using the same model as the primary analysis including a subgroup by treatment 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 secondary outcomes: clinical pregnancy rate, miscarriage, and live birth <37 weeks gestation will be analysed using mixed effects logistic regression models. Each secondary outcome will have as its denominator the number of women who are randomised to either intervention (PICSI) or non-intervention (standard-ICSI) with data recorded for the outcome. All secondary analyses will include centre as a random intercept and adjust for maternal age, paternal age, number of previous miscarriages, and hormonal indicator of ovarian reserve. Maternal age and paternal age will be adjusted for using restricted cubic splines with three knots (knot locations based on Harrell's recommendations) (24, 25). Number of previous miscarriages, and hormonal indicator of ovarian reserve will be adjusted for as categorical variables. Number of previous miscarriages will have three categories 0,1-2, >2. Hormonal indicator of ovarian will have two categories FSH <6.0, ≥6.0 miU/ml or AMH <17.0, ≥17.0 pmol/L when FSH is not available.

Other Data summaries

Serious adverse events (SAEs)

The number of SAEs will be summarised for each treatment group.

Other follow up data and intermediate outcomes

Follow up data collected which is not for primary or secondary outcomes will be summarised by treatment group by the mean and standard deviation or median and interquartile range for continuous variables, and the number and percent for categorical variables. Differences between groups will not be presented and no statistical tests will be performed on this data. This will include summaries of:

- Oocytes collected (per couple)
- Fertilisation rate (number of two pronuclei stage eggs per injected egg)

- Number of embryos created
- Number of single and double embryo transfers
- Biochemical pregnancy rate (bHGC test)
- Multiple pregnancy rate
- Multiple birth rate

Mechanistic Evaluation

As indicated in the protocol, a mechanistic evaluation will also be undertaken. Planning for these analyses will 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 agreeing 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 standard-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.

Abbreviations

IVF: In Vitro Fertilisation; ART: Assisted Reproduction Technologies; ICSI: Intra-Cytoplasmic Sperm Injection; DGC: Density Gradient Configuration; PVP: Polyvinylpyrrolidone; PISCI: PISCI (hyaluronic acid coated plated) Selected Intra-Cytoplasmic Sperm Injection; HABSelect: Hyaluronic Acid Binding Sperm Selection; NICE: National Institute of Clinical Excellence; SAP: Statistical Analysis Plan; HBS: Hyaluronic Acid Binding Score; DMEC: Data Monitoring and Ethics Committee; HFEA: Human Fertilisation and Embryology Authority; ITT: Intention To Treat; MAR: Missing At Random; SD: Standard Deviation; IQR: Inter-quartile Range; FSH: Follicle Stimulating Hormone; AMH: Anti-Mullerian Hormone

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Appendix 1:

Primary Outcome

The primary outcome of live birth rate ≥ 37 weeks will be met for the couple in question if the following conditions are satisfied:

1. Pregnancy end reason is 'Live Birth' for any registered fetus (Variable 'PregEnd' = 6)

   AND

2. Gestational age for the corresponding baby is ≥ 37 weeks (Variable 'NOGAge' ≥ 37)

Secondary Outcomes

Clinical pregnancy will be met if:

1. Clinical pregnancy is confirmed in USG (Variable 'Cpregn' = 1)

OR

2. The first USG for clinical pregnancy is "non-diagnostic" and clinical pregnancy is confirmed in second USG scan (Variable 'Cpregn' = 2 & 'Cpregn2' =1)

Miscarriage, defined as pregnancy loss after confirmation of clinical pregnancy will be met if:

1. Pregnancy end is 'miscarriage' for any registered fetus (Variable 'PregEnd' = 1)

   AND

2. Clinical pregnancy (as described above) is met

Live birth <37 weeks gestation will be met if:

1. Pregnancy end reason is 'Live Birth' for any registered fetus (Variable 'PregEnd' = 6)

   AND

2. Gestational age for the corresponding baby is < 37 weeks (Variable 'NOGAge' < 37)

Barts and The London School of Medicine and Dentistry

PCTU

Statistical report: Clinical Outcomes

Version: 1.0
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1. INTRODUCTION

The purpose of this document is to report the results from the analysis of the clinical analysis of HABSelect described in the HABSelect statistical analysis plan (SAP). The analyses reported in this document were pre-specified prior to the unbinding of the trial statisticians and trial chief investigator to the allocations. This document does not detail the results from the mechanistic analysis or any data collected only for the mechanistic part of the study.

The analysis was carried out by Gordon Forbes under the supervision of Richard Hooper.

This document is based on the HABSelect SAP v2.0 (17th August 2017)

1.1. Quality control

The results of the primary analysis were reproduced by an independent statistician, Brennan Kahan. The derivation of variables used in the analysis was checked by Richard Hooper. All data presented in this report has been checked against STATA log files produced when conducting the analysis.

1.2. Changes from planned analysis in the SAP

- We only included people in subgroup analysis with complete outcome data and complete data for the subgroup variables. This was not specified in the SAP.
- Subgroup analysis include the subgroup variable as a separate covariate even if it was not specified. This affects the HBS subgroup and semen concentration subgroup analysis
- Likelihood ratio tests are used to give p-values for interaction for the HBS low, medium high subgroup analysis.
- All subgroup analysis were repeated examining miscarriage as an outcome. In the SAP it was only specified that subgroup analysis would be carried out on the primary outcome.

2. Trial Outcomes

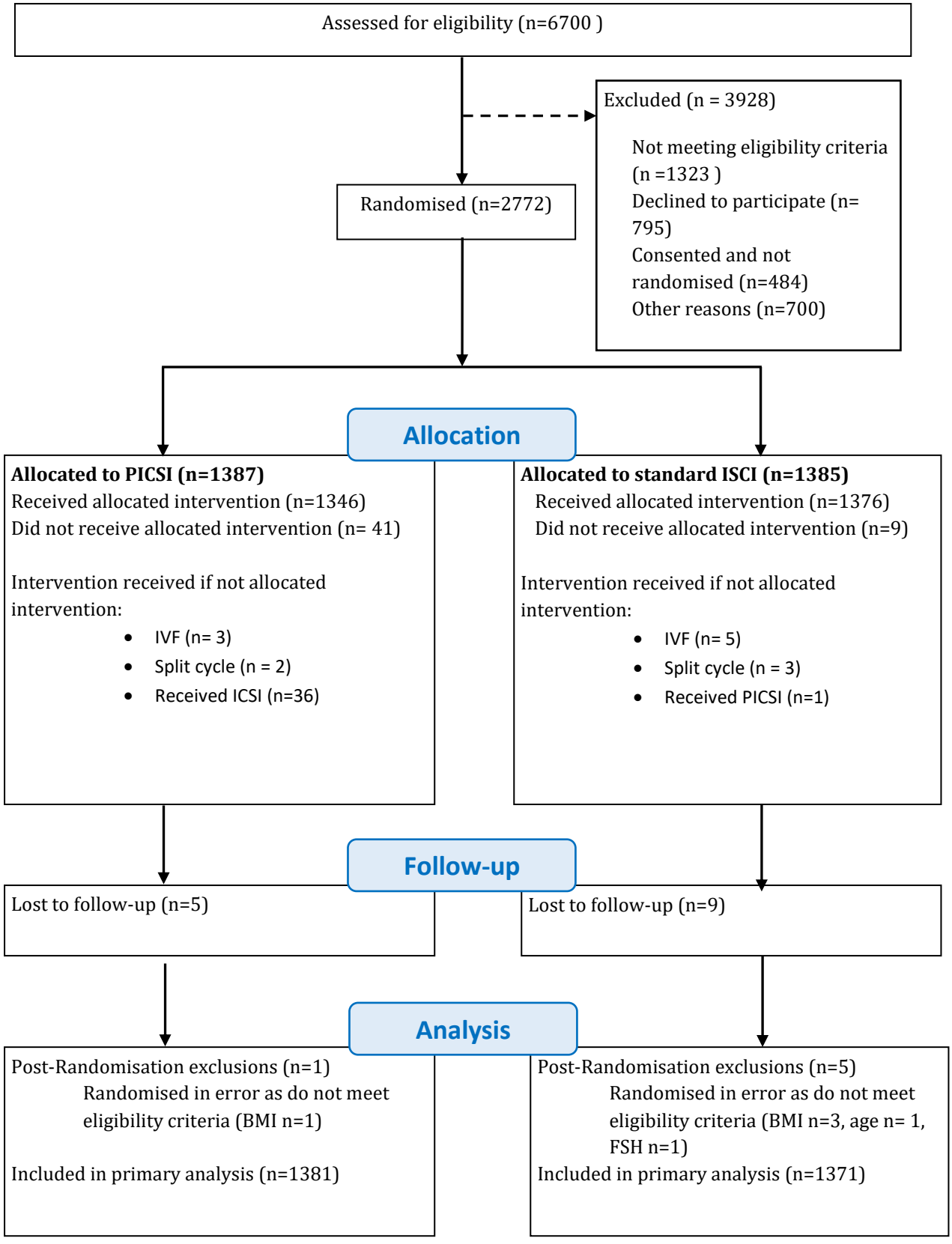
2.1. Primary outcome

- Live birth at ≥ 37 weeks gestation following the first fresh PICSI/ICSI treatment.

2.2. Secondary outcome measures

- Clinical pregnancy rate based on detection of a fetal heartbeat or 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

2.3. Consort Diagram



3. Tables

Table 1. Baseline characteristics

Figures are mean (SD) unless stated otherwise.

	Summary		Missing data	
	PICSI (N=1386)	**ICSI (N=1380)**	**PICSI no. (%)**	**ICSI no. (%)**
Male partner				
Age (years)	36.1 (5.5)	35.9 (5.4)	0 (0.0)	0 (0.0)
≥35 - no. (%)	812 (58.6)	803 (58.2)	0 (0.0)	0 (0.0)
BMI (weight(kg)/height^2(m^2))	27.3 (4.6)	27.0 (4.2)	816 (58.9)	831 (60.2)
Ethnicity - no. (%)			0 (0.0)	0 (0.0)
White	1047 (75.5)	1078 (78.1)		
Asian	193 (13.9)	166 (12.0)		
Black	49 (3.5)	45 (3.3)		
Other	36 (2.6)	45 (3.3)		
Not stated	61 (4.4)	46 (3.3)		
Current smoker - no. (%)	68 (5.0)	65 (4.8)	21 (1.5)	27 (2.0)
If yes, how many cigarettes/day	8.0 (5.5)	8.5 (5.2)	5 (0.4)	6 (0.4)
Drink alcohol - no. (%)	771 (59.1)	791 (60.8)	82 (5.9)	80 (5.8)
If yes, how many units/week	7.7 (6.3)	7.7 (6.8)	47 (3.4)	51 (3.7)
Recreational drug use - no. (%)	7 (0.5)	6 (0.5)	83 (6.0)	94 (6.8)
Semen assessment				
Sperm concentration (x10^6/ml) - median (IQR)	11.0 (3.5-29.5)	11.0 (3.6-31.0)	51 (3.7)	42 (3.0)
Based on semen assessment ICSI recommended - no. (%)	1268 (96.1)	1245 (95.0)	66 (4.8)	70 (5.1)
Female partner				
Age (years)	33.6 (4.4)	33.7 (4.3)	0 (0.0)	0 (0.0)
≥35 - no. (%)	618 (44.6)	617 (44.7)	0 (0.0)	0 (0.0)
BMI (weight(kg)/height^2(m^2))	24.7 (3.5)	24.4 (3.5)	18 (1.3)	20 (1.4)
Ethnicity - no. (%)			0 (0.0)	0 (0.0)
White	1029 (74.2)	1049 (76.0)		
Asian	214 (15.4)	189 (13.7)		
Black	45 (3.2)	46 (3.3)		
Other	52 (3.8)	55 (4.0)		
Not stated	46 (3.3)	41 (3.0)		
Current smoker - no. (%)	31 (2.3)	20 (1.5)	11 (0.8)	12 (0.9)
If yes, how many cigarettes/day	6.4 (3.3)	6.3 (3.6)	3 (0.2)	0 (0.0)
Drink alcohol - no. (%)	646 (48.2)	673 (50.7)	46 (3.3)	52 (3.8)
If yes, how many units/week	5.1 (4.3)	5.1 (4.7)	32 (2.3)	39 (2.8)

Recreational drug use - no. (%)	1 (0.1)	1 (0.1)	69 (5.0)	78 (5.7)

	Summary		Missing data	
	PICSI (N=1386)	**ICSI (N=1380)**	**PICSI no. (%)**	**ICSI no. (%)**
Pre-treatment hormonal assessment				
FSH level (miU/L)	7.1 (2.3)	7.1 (2.3)	477 (34.4)	458 (33.2)
AMH level pmol/L	22.6 (18.7)	22.0 (18.5)	571 (41.2)	585 (42.4)
FSH < 6.0 miU/ml or AMH < 17.0 pmol/L where FSH testing is not available - no. (%)	292 (21.1)	274 (19.9)	0 (0.0)	0 (0.0)
Length of menstrual cycle (days)	30.3 (11.0)	30.7 (12.9)	97 (7.0)	79 (5.7)
Type of menstrual cycle - no. (%)			12 (0.9)	8 (0.6)
Regular	1176 (85.6)	1170 (85.3)		
Irregular	187 (13.6)	189 (13.8)		
Not known	11 (0.8)	13 (0.9)		
Previous fertility and pregnancy history				
Previous natural pregnancy - no. (%)	302 (21.8)	313 (22.7)	0 (0.0)	0 (0.0)
Live birth following natural pregnancy - no. (%)	47 (3.4)	57 (4.2)	14 (1.0)	19 (1.4)
Previous IVF/ICSI fertility treatment cycle - no. (%)	411 (29.7)	401 (29.1)	0 (0.0)	0 (0.0)
Live birth following previous IVF/ICSI fertility treatment - no. (%)	82 (6.0)	74 (5.4)	14 (1.0)	13 (0.9)
Previous miscarriage - no. (%)			0 (0.0)	0 (0.0)
0	1190 (85.9)	1174 (85.1)		
1-2	187 (13.5)	193 (14.0)		
>2	9 (0.6)	13 (0.9)		
Gynaecological disorders				
Polycystic ovaries - no. (%)	216 (15.6)	208 (15.1)	0 (0.0)	0 (0.0)
Fibroids - no. (%)	60 (4.3)	80 (5.8)	0 (0.0)	0 (0.0)
Endometriosis - no. (%)	98 (7.1)	109 (7.9)	0 (0.0)	0 (0.0)
Other - no. (%)	109 (7.9)	122 (8.8)	0 (0.0)	0 (0.0)
Pelvic surgery				
Myomectomy - no. (%)	15 (1.1)	18 (1.3)	0 (0.0)	0 (0.0)
Endometriosis surgery - no. (%)	52 (3.8)	48 (3.5)	0 (0.0)	0 (0.0)
Salpingectomy - no. (%)	45 (3.2)	37 (2.7)	0 (0.0)	0 (0.0)
Caesarean - no. (%)	24 (1.7)	22 (1.6)	0 (0.0)	0 (0.0)
Other - no. (%)	180 (13.0)	201 (14.6)	0 (0.0)	0 (0.0)
Hormonal treatment				
Type of hormonal cycle - no. (%)			2 (0.1)	1 (0.1)
Long agonist	697 (50.4)	692 (50.2)		
Short agonist	147 (10.6)	122 (8.8)		

Antagonist	533 (38.5)	550 (39.9)		
Other	7 (0.5)	15 (1.1)		

Table 2. Describing the intervention

Figures are mean (SD) unless stated otherwise.

	Summary		Missing data	
	PICSI (N=1386)	**ICSI (N=1380)**	**PICSI no. (%)**	**ICSI no. (%)**
Male partner semen pre-preparation assessment				
Sperm volume	2.9 (1.4)	3.0 (1.5)	48 (3.5)	48 (3.5)
Sperm concentration (x10^6/ml) - median (IQR)	14.7 (4.0-35.0)	16.0 (5.0-36.4)	150 (10.8)	157 (11.4)
% of forward progressive mobility	39.5 (20.1)	40.8 (20.3)	170 (12.3)	182 (13.2)
Male partner semen post-preparation assessment				
Sample processing - no. (%)			43 (3.1)	43 (3.1)
Swim up	18 (1.3)	19 (1.4)		
Density gradient	1044 (77.7)	1028 (76.9)		
Direct centrifugation	191 (14.2)	198 (14.8)		
Other form of processing	89 (6.6)	90 (6.7)		
Sample not processed	1 (0.1)	2 (0.1)		
% of forward mobility	68.6 (28.1)	69.5 (27.5)	225 (16.2)	240 (17.4)
Hyaluronan binding score				
HBA score - no. (%)			423 (30.5)	433 (31.4)
≤25%	86 (8.9)	74 (7.8)		
25% > & ≤ 65%	188 (19.5)	181 (19.1)		
>65%	689 (71.5)	692 (73.1)		
Female partner Oocytes collection				
Number of eggs collected (per couple)*	10.9 (6.3)	10.8 (6.3)	41 (3.0)	43 (3.1)
Number of metaphase II oocytes injected with sperm	8.7 (5.1)	8.5 (5.1)	45 (3.2)	49 (3.6)

*Suggestion to show number of eggs collected as median (IQR) due to skewed data.

Table 3. Fertilisation and biochemical pregnancy

Figures are mean (SD) unless stated otherwise.

	Summary		Missing data	
	PICSI (N=1386)	**ICSI (N=1380)**	**PICSI no. (%)**	**ICSI no. (%)**
Fertilisation				
Fertilisation rate (number of two pro-nuclei stage eggs per injected egg) - mean (sd)	0.66 (0.24)	0.69 (0.24)	64 (4.6)	68 (4.9)
Number of fresh embryos transferred			21 (1.5)	24 (1.7)
0	131 (9.6)	116 (8.6)		
1	712 (52.2)	691 (51.0)		
2	510 (37.4)	535 (39.5)		
3	12 (0.9)	14 (1.0)		
Cryopreserved Embryos				
Number of embryos frozen	1.0 (1.8)	1.1 (2.1)	100 (7.2)	95 (6.9)
Number of embryos frozen in women who had embryos transferred	0.91 (1.6)	0.90 (1.6)	2 (0.2)	1 (0.1)
Biochemical Pregnancy				
Positive biochemical pregnancy (bHGC test)	546 (39.48)	544 (39.51)	3 (0.2)	3 (0.2)

Table 4. Analysis of the primary outcome: live birth ≥ 37 weeks gestation

	Number included in the analysis		Summary			
	PICSI - no.	**ICSI - no.**	**PICSI - no. (%)**	**ICSI - no. (%)**	**Odds ratio (95% CI)**	**p-value**
Primary* analysis	1381	1371	379 (27.4)	346 (25.2)	1.12 (0.95, 1.34)	0.18
Sensitivity analysis**	1379	1370	379 (27.5)	346 (25.3)	1.13 (0.95, 1.34)	0.17

*The primary analysis adjusts for maternal age (restricted cubic splines), paternal age (restricted cubic splines), number of previous miscarriages (categorical), and hormonal indicator of ovarian reserve (categorical).

**The sensitivity analysis was conducted to check the robustness of the primary analysis to different covariate adjustment. In addition to the covariates adjusted for in the primary analysis, the sensitivity analysis adjusts for female partner BMI (restricted cubic splines), female partner ethnicity (categorical) history of previous pregnancy (categorical), female partner smoking status (categorical), female partner smoking status (categorical), and hormonal treatment (categorical).

For full details on covariate adjustment see the HABSelect SAP v2.0.

Table 5. Subgroup analysis of the primary outcome: live birth ≥ 37 weeks gestation

	Number included in the analysis		Summary			
	PICSI no.	ICSI no.	PICSI no. (%)	ICSI no. (%)	Odds ratio (95% CI)	p-value*
HBA score						
≤65%	273	254	80 (29.3)	72 (28.3)	1.10 (0.75, 1.61)	0.67
>65%	688	690	178 (25.9)	180 (26.1)	0.99 (0.78, 1.27)	
≤25%	85	74	23 (27.1)	24 (32.4)	0.79 (0.40, 1.58)	0.50
25% > & ≤ 65%	188	180	57 (30.3)	48 (26.7)	1.26 (0.80, 2.01)	
>65%	688	690	178 (25.9)	180 (26.1)	0.99 (0.78, 1.27)	
Maternal Age						
< 35	766	755	239 (31.2)	231 (30.6)	1.03 (0.83, 1.29)	0.22
≥ 35	615	616	140 (22.8)	115 (18.7)	1.29 (0.98, 1.71)	
Previous miscarriage						
0	1186	1165	327 (27.6)	296 (25.4)	1.13 (0.94, 1.36)	0.86
>0	195	206	52 (26.7)	50 (24.3)	1.08 (0.69, 1.71)	
FSH level or AMH level where FSH not tested						
< 6.0 miU/L (< 17.0 pmol/L for AMH)	291	272	78 (26.8)	68 (25.0)	1.08 (0.74, 1.59)	0.82
≥ 6.0 miU/L (≥ 17.0 pmol/L for AMH)	1090	1099	301 (27.6)	278 (25.3)	1.14 (0.94, 1.38)	
Sperm concentration						
<15x10^6/ml	777	763	225 (29.0)	196 (25.7)	1.16 (0.92, 1.46)	0.71
≥ 15x10^6/ml	553	566	141 (25.5)	140 (24.7)	1.08 (0.82, 1.42)	

*P-values are for the interaction term between the subgroup variable and the treatment variable. Where the subgroup variable has more than two levels the p-value given is from a likelihood ratio test comparing the analysis model containing a treatment-subgroup interaction to the same model no including the interaction term.

For details on covariate adjustment see the HABSelect SAP v2.0.

Table 6. Analysis of secondary outcomes

	Number included in analysis		Summary			
	PICSI no.	**ICSI no.**	**PICSI no. (%)**	**ICSI no. (%)**	**Odds ratio**	**p-value**
Clinical pregnancy at 6-9 weeks gestation	1382	1375	487 (35.2)	491 (35.7)	0.98 (0.84, 1.15)	0.80
Miscarriage following clinical pregnancy	1381	1371	60 (4.3)	96 (7.0)	0.61 (0.43, 0.84)	0.003
Live birth <37 weeks gestation	1381	1371	46 (3.3)	45 (3.3)	1.02 (0.67, 1.55)	0.94

Table 7. Subgroup analysis of miscarriage

	Number included in the analysis		Summary			
	PICSI no.	ICSI no.	PICSI no. (%)	ICSI no. (%)	Odds ratio (95% CI)	p-value*
HBA score						
≤65%	273	254	8 (2.9)	16 (6.3)	0.44 (0.18, 1.05)	0.43
>65%	688	690	35 (5.1)	52 (7.5)	0.65 (0.42, 1.01)	
≤25%	85	74	1 (1.2)	2 (2.7)	0.42 (0.04, 4.71)	0.75
25% > & ≤ 65%	188	180	7 (3.7)	14 (7.8)	0.45 (0.18, 1.15)	
>65%	688	690	35 (5.1)	52 (7.5)	0.65 (0.42, 1.01)	
Maternal Age						
< 35	766	755	31 (4.0)	38 (5.0)	0.81 (0.50, 1.32)	0.11
≥ 35	615	616	29 (4.7)	58 (9.4)	0.47 (0.30, 0.75)	
Previous miscarriage						
0	1186	1165	55 (4.6)	83 (7.1)	0.63 (0.45, 0.90)	0.42
>0	195	206	5 (2.6)	13 (6.3)	0.40 (0.14, 1.15)	
FSH level or AMH level where FSH not tested						
< 6.0 miU/L (< 17.0 pmol/L for AMH)	291	272	15 (5.2)	14 (5.1)	1.04 (0.49, 2.20)	0.12
≥ 6.0 miU/L (≥ 17.0 pmol/L for AMH)	1090	1099	45 (4.1)	82 (7.5)	0.53 (0.36, 0.77)	
Sperm concentration						
<15x10^6/ml	777	763	28 (3.6)	53 (6.9)	0.52 (0.32, 0.83)	0.33
≥ 15x10^6/ml	553	566	29 (5.2)	39 (6.9)	0.73 (0.44, 1.19)	

*P-values are for the interaction term between the subgroup variable and the treatment variable. Where the subgroup variable has more than two levels the p-value given is from a likelihood ratio test comparing the analysis model containing a treatment-subgroup interaction to the same model no including the interaction term.

For details on covariate adjustment see the HABSelect SAP v2.0.

Table 8. Women with multiple births and multiple clinical pregnancies – no. (%)

	Summary		Missing data	
	PICSI (N=1386)	ICSI (N=1380)	PICSI no. (%)	ICSI no. (%)
Multiple clinical pregnancies	68 (5.0)	54 (4.0)	16 (1.2)	19 (1.4)
Multiple births	52 (3.8)	29 (2.1)	5 (0.4)	9 (0.7)

Table 9. Serious adverse events

	PICSI (N=1386)	ICSI (N=1380)
Number of serious adverse events	29	27
Number of related SUSAR	1	1
Number of unrelated SUSAR	28	26