HABSelect: Selection of sperm for Assisted Reproductive Treatment by prior hyaluronic acid binding: increasing live birth outcomes and reducing miscarriage rates – multicentre randomised controlled, blinded trial.

Short title/Acronym: HABSelect – **H**yaluronic **A**cid **B**inding **S**perm s**elect**ion

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Chief Investigator:

Dr David Miller
Reader in Molecular Andrology
Reproduction and Early Development Group
University of Leeds, Institute of Genetics, Health and Therapeutics
Clarendon Way
Leeds, LS2 9JT
Tel +44 (0) 113 343 7804
Mob 0778 717 5765
Fax: 0113 343 6603
Email: d.miller@leeds.ac.uk
www.HABSelect.org.uk

The Sponsor and PCTU accept no responsibility for the accuracy of additional documentation or instructions developed by collaborating or third party organisations, from the content of this protocol.

1. Table of Contents

1. KEY CONTACTS

1.1. HABSelect Trial Management Group

Chief Investigator & Lead Laboratory Investigator
Dr David Miller,
Reader in Molecular Andrology
Leeds Institute of Genetics, Health and Therapeutics
University of Leeds, West Yorkshire,
Leeds, LS2 9JT
Email: d.miller@leeds.ac.uk

Co-Lead Laboratory Investigator
Dr Jackson Kirkman-Brown
Birmingham Women Fertility Centre, Birmingham Women Hospital
Mindelsohn Way, Edgbaston
Birmingham, B15 2TG
j.kirkmanbrown@bham.ac.uk

Trial Design & Operations
Dr Sue Pavitt
Reader in Applied Health Research
Deputy Lead for Research
Leeds Institute for Health Research
University of Leeds
Room 2.32 Charles Thackrah Building
101 Clarendon Road
Leeds, LS2 9LJ
s.pavitt@leeds.ac.uk

Clinical Leads – Co-Applicants

1) Dr Yacoub Khalaf
   Consultant/Senior Lecturer in Reproductive Medicine and Surgery Director
   The Assisted Conception Unit & Centre for Pre-implantation Genetic Diagnosis
   11th Floor, Tower Wing
   Guy's and St Thomas Hospital

London, SE1 9RT
Yakoub.khalaf@kcl.ac.uk

2) Professor Siladitya Bhattacharya
Professor of Reproductive Medicine, Head of Division of Applied Health Sciences
School of Medicine and Dentistry, University of Aberdeen
Aberdeen Maternity Hospital,
Foresterhill
Aberdeen, AB25 2ZD
s.bhattacharya@abdn.ac.uk

3) Professor Arri Coomarasamy
Consultant Gynaecologist and Sub-Specialist in Reproductive Medicine and Surgery
Birmingham Women Fertility Centre, Birmingham Women Hospital
Mindelsohn Way, Edgbaston
Birmingham, B15 2TG
a.coomarasamy@bham.ac.uk

1.2. Trial Statistician

Dr Richard Hooper
Pragmatic Clinical Trials Unit
Centre for Primary Care and Public Health
Yvonne Carter Building
Queen Mary, University of London
58 Turner Street
Whitechapel, E1 2AB
r.l.hooper@qmul.ac.uk

1.3. Mechanistic Evaluation Statistician

Professor Robert West
Professor of Biostatistics
Leeds Institute of Health Sciences
University of Leeds
Room 1.27, Charles Thackrah Building
101 Clarendon Road
Leeds, LS2 9LJ

R.M.West@leeds.ac.uk

1.4. Co-Applicants

1) Professor Khalid Khan

Professor of Women's Health and Clinical Epidemiology
Women's Health Research Unit
Blizzard Institute, Bart's and The London School of Medicine
Queen Mary, University of London
58 Turner Street,
London, E1 2AB
k.s.khan@qmul.ac.uk

2) Dr Allan Pacey

Department of Human Metabolism
Academic Unit of Reproductive and Developmental Medicine
Level 4, the Jessop Wing
Sheffield, S10 2SF
a.pacey@sheffield.ac.uk

1.5. Patient Representative

1) Dr Thomas Kabir

MHRN Service Users in Research Coordinator
Department of Psychology PO77
Institute of Psychiatry
De Crespigny Park
London
SE5 8AF
thomas.kabir@kcl.ac.uk

2) Bonnie Berman

69 Buckstone Oval
Almmodley, Leeds
LS17 5HJ
rollsandbonz@hotmail.co.uk

1.6. Coordinating centre

PCTU – Pragmatic Clinical Trials Unit

Centre for Primary Care and Public Health

Yvonne Carter Building, Queen Mary University of London

58 Turner Street, Whitechapel, E1 2AB

Trial Data Management			
Sandy Smith	Data Manager	Tel Fax Email	0207 882 2557 0207 882 2552 s.l.smith@qmul.ac.uk
Clinical Trial Management			
Ann Thomson	Senior Trial Manager	Tel Fax Email	0207 882 2556 0207 882 6084 n.stevens@qmul.ac.uk
Karolina Witt	Trial Co-ordinator	Tel Fax Email	0207 882 2526 0207 882 2552 k.d.witt@qmul.ac.uk
Anitha Manivannan	Quality Assurance Manager	Tel Fax Email	0207 882 6132 0207 882 2552 a.manivannan@qmul.ac.uk
Amy Hoon	Trial Monitor	Tel Fax Email	0207 882 6132 0207 882 2552 a.hoon@qmul.ac.uk
Clinical Trial Statistics			
Dr Richard Hooper	Senior Statistician	Tel Fax Email	0207 882 7324 0207 882 2552 r.l.hooper@qmul.ac.uk
Vichithranie Madurasinghe	Independent PCTU Statistician	Tel Fax Email	0207 882 6133 0207 882 2552 v.madurasinghe@qmul.ac.uk

Preliminary membership of the Data Monitoring and Ethics Committee and the Trial Steering Committees are listed below. Formal membership and invitations are made by the NIHR EME Secretariat.

1.7. Data Monitoring and Ethics Committee

For interim analyses and response to specific concerns

Independent Chair

Professor Jenny Kurinczuk

Director
National Perinatal Epidemiology Unit
University of Oxford
Old Road Campus
Headington
Oxford, OX3 7LF

jenny.kurinczuk@npeu.ox.ac.uk

Independent Statistician

Dr Paul Seed
Senior lecturer in Medical Statistics
Division of Women's Health, King's College London and King's Health Partners
10th Floor, North Wing, STH
Westminster Bridge Road,
London SE1 7EH
Paul.Seed@kcl.ac.uk

Independent Clinician

Dr Nigel Simpson
Senior Lecturer and Honorary Consultant
Academic Department of Obstetrics & Gynaecology
D Floor, Clarendon Wing, Leeds General Infirmary
Leeds LS2 9NS
n.a.b.simpson@leeds.ac.uk

Independent scientist

Darren Griffin
Professor of Genetics, Deputy Head of School (Research)
School of Biosciences,
Stacey Building,
University of Kent,
Canterbury,
Kent, CT2 7NJ
D.K.Griffin@kent.ac.uk

1.8. Trial Steering Committee

Independent Chair

Nicholas Macklon
Professor of Obstetrics and Gynaecology
Division of Human Development
University of Southampton
Building 85
Life Sciences Building
Highfield Campus
Southampton
SO171BJ
N.S.Macklon@soton.ac.uk

Independent Clinician

Kevin McEleny
Consultant Andrologist
Newcastle Fertility Centre at Life
Biomedicine West Wing
International Centre For Life
Times Square
Newcastle upon Tyne, NE1 4EP
kevin.mceleny@nuth.nhs.uk

Independent Scientist

Dr Michael Carroll
Lecturer in Reproductive Science,
Academic Lead for Reproductive Science
School of Healthcare Science
Manchester Metropolitan University,
John Dalton Building (Room E202),
Chester Street,
Manchester
M1 5GD,
michael.carroll@mmu.ac.uk

Independent Embryologists

1) Susan Pickering
   Consultant Clinical Scientist (Embryology)
   Assisted Conception Unit,
   Edinburgh Fertility and Reproductive Endocrine Centre,
   Edinburgh Royal Infirmary,
   Old Dalkeith Road,
   Edinburgh EH16 4SA
   Sue.Pickering@nhslothian.scot.nhs.uk

2) Jane Cuthbert
   Fertility Centre Manager
   BMI The Priory Hospital
   BMI Healthcare
   Priory Road
   Edgbaston
   B5 7UG
   JCuthbert@bmihealthcare.co.uk

More independent Members can be appointed to TSC Committee as required.

Patient representative

Bonnie Berman
69 Buckstone Oval

Almmodley, Leeds
LS17 5HJ
rollsandbonz@hotmail.com

Dr Thomas Kabir
MHRN Service Users in Research Coordinator
Department of Psychology PO77
Institute of Psychiatry
De Crespigny Park
London
SE5 8AF
thomas.kabir@kcl.ac.uk

Non-independent Members

1) Chief Investigator

Dr David Miller,
Reader in Molecular Andrology
Leeds Institute of Genetics, Health and Therapeutics
University of Leeds, West Yorkshire,
Leeds, LS2 9JT
d.miller@leeds.ac.uk

2) Principal Investigator Representatives

Dr Vinay Sharma
The Leeds Centre for Reproductive Medicine
Leeds Teaching Hospitals NHS Trust
Seacroft Hospital, York Road
Leeds, West Yorkshire, LS14 6UH
Vinay.Sharma@leedsth.nhs.uk

Professor Geraldine Hartshorne
Reproductive Health
Room CSRL 00113
University Hospital
University of Warwick

Clifford Bridge Road
Coventry, CV2 2DX
Geraldine.Hartshorne@warwick.ac.uk

Dr Jackson Kirkman-Brown
Birmingham Women Fertility Centre, Birmingham Women Hospital,
Birmingham Women's NHS Foundation Trust
Mindelsohn Way, Edgbaston,
Birmingham B15 2TG;
Tel.:01216272739
j.kirkmanbrown@bham.ac.uk

2. TRIAL PARTICIPATING SITES AND COLLABORATING PRINCIPAL INVESTIGATORS

LEAD CENTRE	
1. The Leeds Centre for Reproductive Medicine Leeds Teaching Hospitals NHS Trust Seacroft Hospital, York Road Leeds, West Yorkshire, LS14 6UH Tel: 0113 206 3102	Dr Vinay Sharma Vinay.Sharma@leedsth.nhs.uk
OTHER PARTICIPATING CENTRES	
2. The Assisted Conception Unit, Guy's and St Thomas' NHS Foundation Trust; 11th Floor, Tower Wing Guy's Hospital; Great Maze Pond London SE1 9RT Tel: 0207188 2300	Dr Yacoub Khalaf Yakoub.khalaf@kcl.ac.uk
3. Centre for Reproductive Medicine; Barts and The London NHS Trust;	Bonnie Collins Bonnie.Collins@bartshealth.nhs.uk

2nd Floor; Kenton and Lucas Wing; St Batholomew's Hospital; Little Britain; London; EC1 7BE Tel: 0203 465 5050	
4. The Department of Reproductive Medicine Central Manchester University Hospitals NHS Foundation Trust, St. Mary's Hospital Manchester M13 9WL Tel: 0161 276 6612	Prof Daniel Brison daniel.brison@manchester.ac.uk
5. Birmingham Women Fertility Centre, Birmingham Women Hospital, Birmingham Women's NHS Foundation Trust Mindelsohn Way, Edgbaston, Birmingham B15 2TG; Tel: 0121 627 2700	Dr Jackson Kirkman-Brown J.Kirkman-Brown@nhs.net
6. Centre for Reproductive Medicine and Fertility The Jessop Wing Sheffield Teaching Hospitals NHS Trust, Sheffield ,S10 2SF Tel: 0114 226 8050	Dr Allan Pacey a.pacey@sheffield.ac.uk
7. Assisted Conception Unit; Ward 35; Ninewells Hospital; NHS Tayside Dundee; DD1 9SY Tel: 0138 263 2111	Katherine Whalley katherine.whalley@nhs.net
8. Aberdeen Fertility Centre; Obstetrics & Gynaecology, University of Aberdeen Aberdeen Maternity Hospital, NHS Grampian Foresterhill, Aberdeen AB25 2ZL; Tel: 0122 455 0567	Prof Siladitya Bhattacharaya s.bhattacharya@abdn.ac.uk

9. Leicester Fertility Centre Assisted Conception Unit Women Hospital Leicester Royal Infirmary Leicester, LE1 5WW	Jane Blower jane.blower@uhl-tr.nhs.uk
10. Homerton Fertility Centre Homerton University Hospital NHS Trust Homerton Row London, E9 6SR	Dr Srikantharajah Arasaratnam arasaratnam.srikantharajah@homerton.nhs.uk
11. Centre for Reproductive Medicine University Hospitals Coventry and Warwickshire NHS Trust, Clifford Bridge Road, Coventry CV2 2DX	Professor Geraldine Hartshorne Geraldine.Hartshorne@warwick.ac.uk
12. IVF Hammersmith Imperial College Healthcare NHS Trust Hammersmith Hospital Du Cane Rd London W12 0HS	Dr Marta Jansa – Perez Marta.JansaPerez@imperial.nhs.uk

MECHANISTIC LABORATORIES:

LEAD CENTRE	
Centre for Human Reproductive Science School of Clinical and Experimental Medicine College of Medical and Dental Sciences University of Birmingham Edgbaston Birmingham, B15 2TT Tel: 0121 627 2739	Dr J Kirkman-Brown – j.kirkmanbrown@bham.ac.uk
OTHER CENTRES	
Reproduction and Early Development Group Institute of Genetics, Health and Therapeutics University of Leeds Clarendon Way Leeds, LS2 9JT Tel: 0113 343 7804	Dr D Miller d.miller@leeds.ac.uk
Centre for Public Health, Institute of Pathology Block 1R.V.H Site School of Medicine, Dentistry and Biomedical Sciences Queen's University of Belfast BT7 1 NN, Northern Ireland Tel: 028 906 33987	Professor Sheena Lewis s.e.lewis@qub.ac.uk
Academic Unit of Reproductive & Developmental Medicine, Department of Human Metabolism University of Sheffield, Level 4 The Jessop Wing Tree Root Walk Sheffield, S10 2SF Tel: 0114 226 8317	Dr Allan Pacey a.pacey@sheffield.ac.uk

3. TABLE 1: STUDY SUMMARY/SYNOPSIS

Title	Selection of sperm for Assisted Reproductive Treatment by prior hyaluronic acid binding: increasing live birth outcomes and reducing miscarriage rates
Short title	HABSelect
Study website	www.HABSelect.org.uk
Protocol Version Number and Date	Version 3.1 dated 10 October 2014
Methodology	A parallel group, two arm, multicentre, blinded, randomised controlled, clinical trial of PICSI (hyaluronic acid coated plates) versus PVP-ICSI procedures for treatment of male infertility
Study Duration	Total 48 months, comprising: 6 months set up 24 months recruitment + 9 months clinical follow-up in two phases (total 36 months across all sites) 6 months data analysis and report/dissemination
Study Centre	At least 10 Assisted Conception or Reproductive Medicine Units where IVF-ICSI is practiced or other relevant clinical settings
Objectives	**Primary Objectives:** • **Clinical** To determine the efficacy of hyaluronan-selected IntraCytoplasmic Sperm Injection (PICSI) versus PVP ICSI in a rigorous randomised controlled clinical trial of participants where the primary outcome measure will be live birth rate (LBR) ≥37 weeks' gestation after ICSI procedure with first fresh embryo transfer. • **Mechanistic** To evaluate if PICSI can compensate for poor sperm quality and investigate HBS score in relation to chromatin integrity and LBR. To evaluate the differences in chromatin architecture in DGC washed and pelleted sperm with high and low HBS including any correlation with DNA damage.

Number of Subjects/Patients	3730 couples (7460 consented subjects)
Main Inclusion Criteria	**Inclusion Criteria** • Couples able to provide informed consent • Couples undergoing ICSI procedure with first fresh embryo transfer. • Women with: - BMI: 19.0 –35.0 kg/m^2 - FSH level 3.0 – 20.0miU/ml and/or AMH level ≥1.5 pmol/L - Age: 18 – 43 • Men: - Age: 18-55 - Able to produce freshly ejaculated sperm for the treatment cycle **Exclusion Criteria** • Couples using non-ejaculated sperm. • Couples using donor gametes. • Men with vasectomy reversal; cancer treatment involving any chemotherapy and/or radiotherapy in the previous two years. • Previous participation in the HABSelect trial. • Split IVF/ICSI • If *both* FSH and AMH are tested and either of them falls outside the accepted range
Statistical Methodology and Analysis	The analysis will be by intention to treat. Outcomes in intervention and non-intervention arms will be compared using multivariable logistic regression, adjusting for the minimisation variables. The mechanistic evaluation will be conducted through a structural equation modelling approach

4. LIST OF ACRONYMS

ACU	Assisted Conception Unit
AE	Adverse Event
AMH	Anti-Mullerian Hormone
AR	Adverse Reaction
ART	Assisted Reproduction Technologies
ASR	Annual Safety Report
BP	Biological pregnancy
CA	Competent Authority
CE	European Conformity marking for European Union approved products
CI	Chief Investigator
CIS	Couple Information Sheet
CLRN	Comprehensive Local Research Network
CP	Clinical pregnancy
CRF	Case Report Form
CRO	Contract Research Organisation
CSG	Clinical Studies Group
DFR	Decapacitation factor receptor
DGC	Density gradient centrifugation
DMC	Data Monitoring Committee
EC	European Commission
FGT	Female genital tract
FSH	Follicle stimulating hormone
GAfREC	Governance Arrangements for NHS Research Ethics Committees
GCP	Good Clinical Practice
HA	Hyaluronic Acid or hyaluronan
HBA	Hyaluronan binding assay
HBS	Hyaluronan binding score
HBRC	Human Biomaterials Resource Centre
HFEA	Human Fertilisation and Embryology Authority
ICF	Informed Consent Form
ICSI	IntraCytoplasmic Sperm Injection
ISRCTN	International Standard Randomised Controlled Trial Number
IVF	In vitro fertilisation

JRO	Joint Research and Development Office
LB	Live births
LBR	Live Birth Rate
MA	Marketing Authorisation
MCR	Miscarriage rates
MFI	Male factor infertility
MS	Member State
Main REC	Main Research Ethics Committee
NHS R&D	National Health Service Research & Development
PCTU	Pragmatic Clinical Trials Unit, QMUL
PI	Principle Investigator
PICSI	Picsi-selected IntraCytoplasmic Sperm Injection
PNZ	Pronucleate zygotes
PPI	Patient public involvement
PVP	Polyvinylpyrrolidone
QA	Quality Assurance
QC	Quality Control
Participant	An individual who takes part in a clinical trial
QMUL	Queen Mary, University of London
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
ROS	Reactive oxygen species
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

5. INTRODUCTION

5.1 Clinical background

Oocyte quality is now recognised as an important determinant of successful pregnancy outcome as donor eggs from younger women seem able to compensate for lower fertility in older women [1]. It is probable, however, that future advances in ART are likely to more speedily benefit from procedures that target selection of higher quality sperm regardless of parental age. While offering universal benefits to the fertility field overall, this approach would offer particular promise for older couples (notably where the female is aged >37 years) and whose oocytes are less efficient at repairing DNA damage in their partners' sperm. These couples are hitherto challenging to treat with current fertility technologies and have the poorest live birth outcomes but they are also the fastest growing group requesting treatment. The relationship of sperm selection, integrity of DNA and pregnancy outcome is precisely what the HABSelect study is designed to evaluate. 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 NICE's recently called review on fertility guidance (http://www.nice.org.uk/newsroom/pressreleases/NICEOutlinesReviewOfFertilityGuideline.jsp).

In 2008, almost 40,000 couples in the UK alone were treated with assisted reproduction technologies (ART), comprising 50,687 IVF cycles. This number is set to rise in the coming years (ESHRE ART fact sheet). Currently, live birth rates (LBRs) 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 ~1.3 treatment cycles. While it is estimated that more than two thirds of naturally conceived pregnancies end in failure, we may not have reached the limit for improvements in LBR following ART. For all ART procedures, including intracytoplasmic sperm injection (ICSI), the embryologist seeks to use the best sperm available. Selection is aided by semen 'washing' techniques using density gradient centrifugation (DGC) that can enrich for sperm with high motility and good morphology (WHO Manual, 2010) [2]. In contrast with standard in vitro fertilisation (IVF) where the egg is the final arbiter for selection, ICSI is dependent on the relatively subjective judgement of the andrologist or embryologist to choose the 'right' single sperm for each egg. Various studies have shown clear inverse relationships between the burden of DNA damaged sperm in the ejaculate and clinical pregnancy (CPR) or live birth (LBR) 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[4]. However, while the values from washed semen were reduced, they were still over twice as high in the non-pregnant (~50%)

versus pregnant (~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. Paternal and maternal genomic integrity must be equally important determinants of successful pregnancy outcome, so to achieve a high LBR in ICSI, the embryologist must have the tools for selecting sperm with either undamaged DNA or with levels of DNA damage that are not beyond the capacity of the egg's natural ability to repair[14-18]. Alternatively, there may be forms of genotoxic DNA damage in the sperm nucleus that are not detected by existing assays, do not prevent fertilisation by either standard IVF or ICSI based procedures but can compromise embryo development and result in higher rates of miscarriages (please see section 5.2.1).

5.2 Summary of current evidence

There is a substantive literature reporting the relationship between sperm DNA integrity and dysfunction. Several key studies show that DNA packaging and fragmentation anomalies influenced by sperm DNA damage are strongly associated with CPR, LBR[8, 19-21] and pregnancy loss in IVF procedures[10, 22,23]. For ICSI, the only clear association is with pregnancy loss[8] supporting the existence of genotoxic damage that is hidden from conventional tests. Hence any improvement in ICSI that allows the selection of sperm with reduced damage is to be encouraged. Additional benefits of increasing success rates include a reduction in the potentially harmful maternal ovarian hyperstimulation protocols that are an integral part of the ICSI cycle (fewer cycles) and a concomitant reduction in the costs of ART procedures. For example, a modest 8% improvement from the current 24% to 32% measured as live births at >34 weeks' gestation *per cycle* would lead to a c*orresponding* 10% improvement in successful live births overall (based on an average of 1.3 cycles per patient) from 30.6% now to 40.6% in future. One effect of this could be to reduce cycles while maintaining current success rates (losing 1 cycle in five overall). Hence, over the current 25,000 ICSI cycles performed per annum in the UK this would represent an annual NHS saving of more than £17.5m (based on average costs of £3500 per ICSI cycle). Maintaining the current cycle average could see even greater longer term savings in relation to the knock-on effects of pregnancy failure to NHS costs.

Sperm genomic integrity is important because work conducted in our and in other laboratories has shown that in human and mouse sperm, the genome is carefully and systematically organised in the nucleus into distinct geographical domains[24-26]. These studies showed that some domains are enriched in histones[27] that can account for their hitherto unexplained

persistence in sperm nuclei alongside the more abundant protamines[28, 29]. Intriguingly, histone-bound sperm chromatin domains are enriched in developmental gene sequences expressed in early embryogenesis[24, 26, 27]. We postulate that these developmentally significant domains are critically relevant for subsequent early embryogenesis and that DNA damage located therein could account for the early pregnancy failure observed after both IVF and ICSI based procedures[24, 26, 30]. We have evidence that the form of paternal DNA damage that may be responsible for such early pregnancy failure involves nucleotide oxidation[30]. This particular form of DNA damage is only revealed after treating the DNA with an enzyme that converts extant oxidised purines (such as 8-OHdG) into DNA strand breaks [31]. Such damage is probably caused by reactive oxygen species (ROS) gaining access to chromatin domains that should normally be protected but are exposed due to anomalous packaging defects at critically important locations. The potential genotoxicity of oxidised nucleotides, however, would be hidden to the investigator because the strand breaks that are normally detected in assays of such damage would only be revealed following gamete fusion.

5.2.1 Is DNA damage the link connecting sperm chromatin integrity and pregnancy failure?

It is highly likely that some important regions in sperm chromatin [20, 31-37] are sensitive to DNA damaging agents [24, 26]. In the most severe forms of DNA packaging defects such as complete absence of protamine (as in mouse knockout models, for example), embryo lethality is the norm[20, 38]. Moreover, even small imbalances in the balance of DNA packaging proteins in sperm have deleterious effects on fertility[33, 38, 39]. Hence, there are clear connections between stoichiometric chromatin imbalance and DNA fragmentation, suggesting that problems with one are reflected by complimentary problems in the other[40-43]. During spermiogenesis, when the paternal genome is being repackaged to fit a much smaller nucleus[44, 45], any deficiencies in the packaging process are likely to leave some DNA sequences more exposed to damaging ROS than others. While it may be the case that we cannot do anything about such types of DNA damage in standard IVF procedures, it should be possible to eliminate these damaged sperm from the pool prepared for ICSI based procedures and by so doing, reducing pregnancy loss and correspondingly increasing LBR.

5.3 Work leading to the study

5.3.1 The potential for hyaluronan binding to discriminate and select for sperm with high chromatin integrity.

In the clinic, wherever possible, ART makes use of sperm isolated through either DGC or swim-up processing (and occasionally both). This helps to obtain the better quality sperm for

subsequent IVF or ICSI (WHO Manual [2]) although even selected sperm are not entirely free of DNA fragmentation as indicated by recent studies [23, 42] see Figure 2B. Hyaluronic acid or hyaluronan (HA) is the major glycosaminoglycan secretion of the cervix and the cumulus-öopherus complex. Sperm reaching these surfaces can bind to HA and subsequent hyperactivation facilitates their penetration to the zona pellucida of the egg. Work by Huszar and colleagues showed that immature sperm with excessive cytoplasm had higher rates of aneuploidy, lowered cytoplasmic maturity and a dysfunctional ability to bind HA[46, 47]. Pelleted sperm are more homogeneously normal in this critical respect. The cytoplasm-rich, poorly HA-binding sperm of DGC interface sperm also have poorer morphology and motility and exhibit higher rates of DNA damage[42, 48-50].

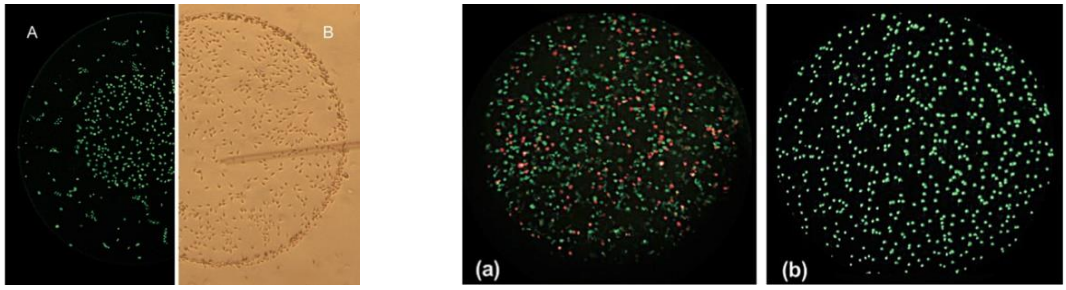

Figure 1. Ability of sperm to bind spots of HA on glass substrates. Panels (a) and (b): fluorescence micrograph of live-dead staining of sperm in contact with HA-coated 'spot' before (a) and after (b) washing to remove non-adherent cells. Panels A and B: fluorescence (A) and brightfield micrograph (B) of adherent sperm. Note the absence of dead sperm in A and the tip of the handling pipette in B. Live-dead assay used cyber green (living) and propidium iodide (dead) in combination. From Huszar, 2007 (Ref 50).

Prinosilova et al [48]obtained an over threefold greater number of strict Tygerberg sperm (a rigorous test for normal sperm morphology) following exposure of highly abnormal semen samples to a HA-coated substrate (Figure 1). Using a similar selection system, Sati et al[49] showed that HA-binding sperm had more compact chromatin, lower DFR and less residual cytoplasm than non-binding sperm.

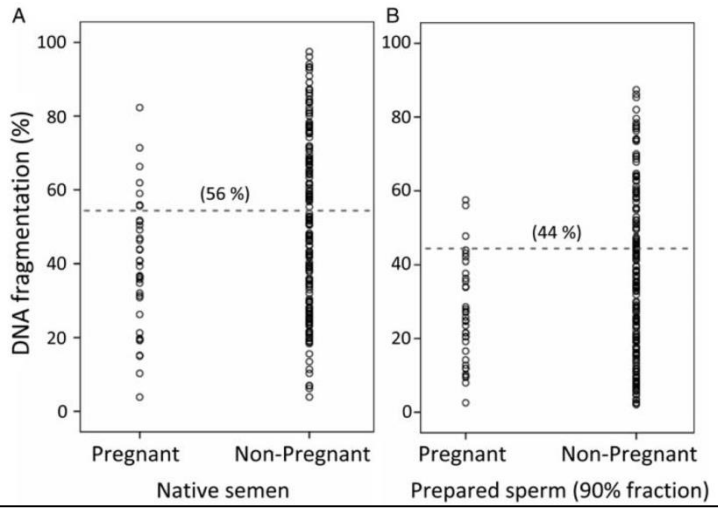

Figure 2 Relationship between DNA fragmentation and CPR in sperm from native semen and semen processed by DGC (90% fraction). From Simon et al, 2010 (Ref 23).

5.3.2 Evidence of the beneficial effect of HA selection on pregnancy outcome.

In many clinics, polyvinylpyrrolidone (PVP) is normally used to slow sperm down sufficiently for capture by the clinical embryologist. Two clinically relevant studies have reported on effects following a HA-selection procedure for ICSI. Parmegiani et al [42] obtained higher numbers of Grade 1 embryos for transfer following ICSI with HA rather than PVP-selected sperm (36% versus 24%) and an improved LBR (23% vs 18%). A more recent and larger randomised study used a fully developed HA based sperm selection (PICSI) versus PVP procedures in 802 ICSI cycles (Table 2 [51] This study showed a 13% increase in CPR [N = 121]) using HA versus PVP selected sperm with a corresponding drop in miscarriage rate (14.1% vs 3.8%; N = 168). Closer examination of the trial data indicates a more general 5-10% improvement in CPR if the data are stratified according to the DGC washed HA binding score (obtained prior to PICSI selection), with lower scores (≤65%) giving the best results. This may explain why CPRs in HA versus PVP arms were balanced before stratification according to the post-DGC washed HA binding score, while pregnancy failure rates fell by 6% (Table 2). The US trial has now reported LBR, and provided strong supporting evidence for HA selection reducing early pregnancy failure [51]. Our trial will seek to confirm this as well as contributing data on LBR, miscarriage rate and notably to a better understanding of the basic underlying mechanistic of action of HA sperm selection.

HBS	Implantation rate (%) at 4 wks	CPR (%) at 6-8 wks	MCR (%) based on **C**P with fetal sac (6 wks) less fetal heartbeat (8 wks)
All scores	32.2 / 33.5[482]	47.8/47.3[482]	10.0 / 4.3[247]
>65%	34.8 / 37.9[357]	51.1/46.2[357]	7.8 / 5.9[188]
≤65%	30.7 / 37.4[121]	37.9/50.8[121]	18.5 / 0[59]

Table 2. Final output from the US clinical trial on PVP (blue) versus PICSI (green) based selection. Sample numbers are shown in superscript. HBS refers to the hyaluronan binding score for DGC washed sperm. From Worrilow et al, [51]. Please see text for more details. Numbers in superscript indicate sample size.

5.4 The study rationale

5.4.1 Mechanistic aspects

Protamines are the principle DNA binding proteins of human sperm chromatin, comprising at least 85% of the mass of the paternal genome [39]. Histones constitute the remaining 10-15%; hence sperm chromatin exists in a complex arrangement of protamine and histone-bound DNA (Figure 3). As indicated above, our work as supported by independent studies has provided strong experimental evidence for differential DNA packaging in sperm nuclei into high (protamine) and low (histone) compaction domains that will have differing susceptibilities to potential DNA damaging agents (Figure 3).

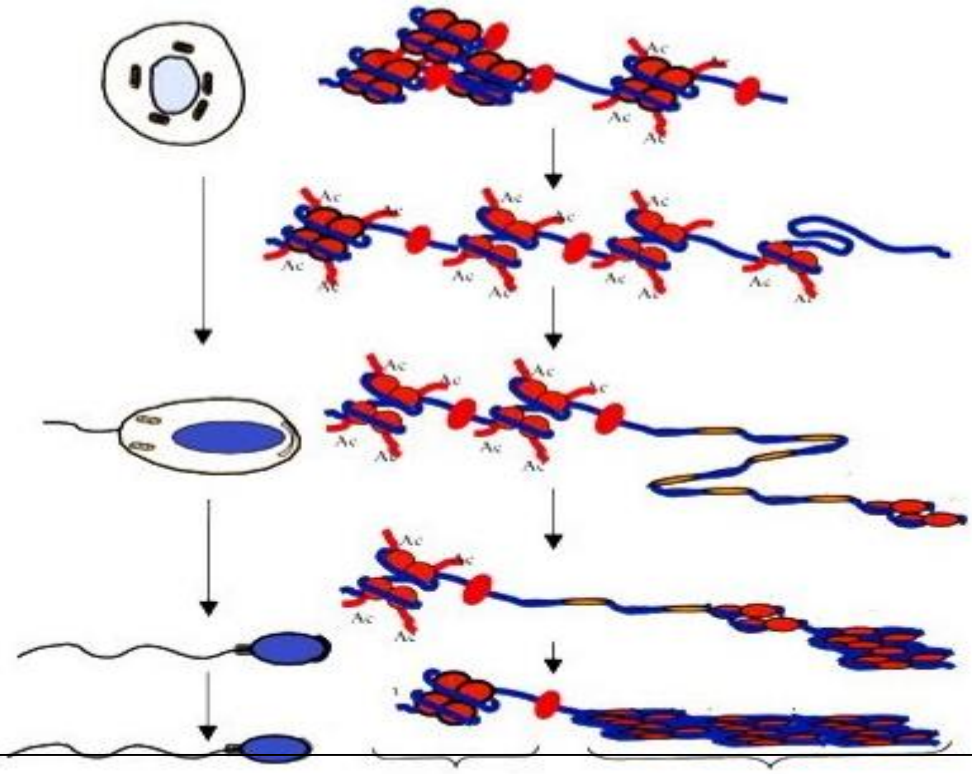

Figure 3. Shift from nucleosomal (histone) to steroidal (protamine) based chromatin via transition proteins during spermatogenesis. Histones are acetylated (Ac) prior to their removal. Although it is not known whether the remaining histones are there by design or as a residue of this shift, their presence introduces a 'weakness' into the overall chromatin structure that may be more vulnerable to naturally or iatrogenically induced damage. From Oliva , 2006 (Ref 36).

Our evidence suggests that the less compact and hence more susceptible domains are enriched in regulatory sequences for genes that are important in early embryonic development[24, 26, 27]. As DNA is differentially packaged into domains that reflect a clear organisational framework, then we hypothesize that sperm DNA fragmentation reflects alterations in the packaging of sperm chromatin that leaves some critical DNA sequences more exposed to oxidative damage than others. We aim to test the hypothesis that PICSI more robustly selects for sperm with good chromatin integrity, and correspondingly low DNA damage than manual selection normally permits. Although this suggests that PICSI (or other HA-based selection procedures) may best be applied among semen samples that are of particularly low quality, there is no reason why it could not be applied more widely in IVF-ICSI if the evidence from this study supports its efficacy. While not the purpose of this study, HA-based sperm

selection could potentially be extended into standard IVF procedures if methods are developed to restore the fertilisation potential of pre-HA bound sperm.

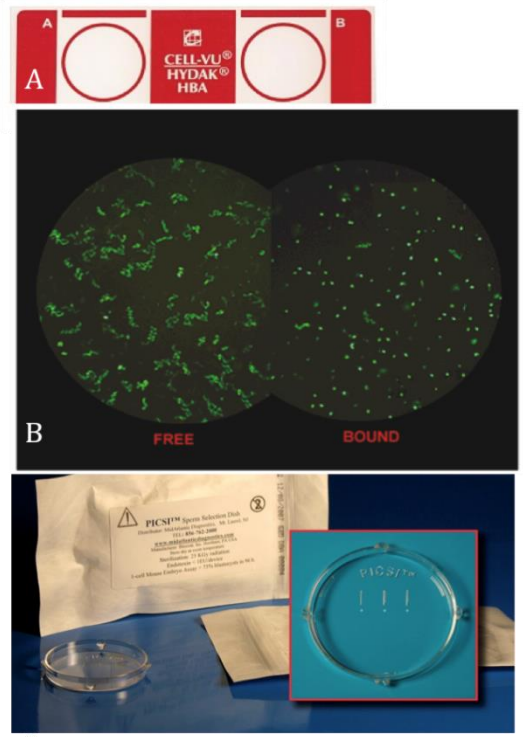

Figure 4. A Hydak HBS slide showing twin chambers. **B**. Photomicrographs of time lapse image from sperm added to chamber at 0 and at 30s. Sperm stained with Cyber green. The degree of 'wiggle' reflects sperm motility. **C**. PICSI plate showing channels into which sperm suspensions are introduced. Sperm migrate towards the hyaluronic coated areas at one end of each channel where they bind. From Biocoat original datasheets.

5.4.2 Interventional Aspects

The 2010 WHO manual[2] on semen analysis has altered the definition of a 'normal' fertile sample because the relationship between sperm 'normality' and the ability to achieve a pregnancy following 12 months of unprotected intercourse is far from obvious. The emerging consensus based on some older observations that remain just as valid today is that the morphology of sperm recovered from the endocervix or zona pellucida is a better indicator of their functionality than is morphology based on raw semen analysis[52-55]. Hence, the emphasis now is on identifying those sperm in the ejaculate that can progress through the female genital tract to reach the endocervical mucus and beyond to the egg. Using the WHO guidelines, the range of percentage 'normal' values for both fertile and infertile men is likely to be between 0–30%, with few samples exceeding 25% normal spermatozoa[52,56]. Such low values inevitably produce low thresholds. For example, limits and thresholds as low as 3–5% normal forms have been found in studies of in-vitro fertilisation[57], intrauterine insemination[58] and in-vivo fertility[59]. Similarly, the range of percentage motile sperm found in even 'pristine' spermatozoa in the ejaculates of fathers can be very wide (8–25%)[60]. Hence, none of the aforementioned parameters are particularly helpful in providing a useful definition of sperm 'normality'. What seems to count most is the sperms' ability to reach the egg's zona, which supports the contention that a prior binding to the HA matrix of the cumulus is a prerequisite. This is why sperm selection for IVF in general and ICSI in particular needs improved standards that do not rely on or at least minimise possible adverse effects of subjective decisions. In clinical practice, both the PVP and PICSI processes make use of special chambers into which DGC or swum-up processed sperm are introduced (Figure

4c). Neither process is inherently any more difficult to perform than the other and an embryologist used to PVP based processing can be quickly trained to use PICSI instead.

5.4.3 Risks and benefits

Hyaluronan is a natural polymeric secretion of the cervical mucus and cumulus-oopherus complex and so poses no known risks to the egg or zygote. PICSI – HA based selection system has been CE approved for use and no risks have been identified by the manufacturer. However as a precaution against possible adverse effects of intervention such as early pregnancy loss or preterm labour we will conduct a safety monitoring interim analysis. PVP is an inert synthetic polymer that is used extensively in the food and pharmaceutical industries with no known adverse side effects. It is used in almost every ACU as a viscous retardant of sperm motility, making it easier for a skilled practitioner to isolate and prepare a single sperm for ICSI. The advantage of switching to hyaluronan is likely to be threefold.

Firstly, sperm bind to HA, effectively immobilising them long enough to be picked up. **Secondly**, HA is thought to work by selectively binding sperm of a higher quality allowing the embryologist to wash away non-adherent sperm before the choice of sperm for pick-up is made.

Thirdly, although the trained embryologist can be very good at selecting the 'right' sperm for injection HA should remove any subjective operator selection and allow consistent objective selection of the 'right' sperm for injection.

The main benefits expected of switching to HA are a decrease in early pregnancy loss and a subsequent increase in LBR at normal term. We consider that HA selection will be beneficial to couples where semen quality is too poor for IVF and may also have a significant benefit for older women with poorer quality eggs that have a decreased potential to repair sperm DNA damage. Couples participating in this research study will benefit the community by increasing the knowledge base of clinical and scientific improvements in the effectiveness of ICSI procedures.

5.4.4 Justification

- There is a need to increase LBR at term for IVF and IVF-ICSI patients by reducing fertilisation failure and miscarriage rate.
- Male fertility is thought to be declining through a reduction in sperm counts across the globe since WWII [61].

- The number of IVF and ICSI procedures is rapidly increasing and in particular, ICSI is being increasingly used for reasons other than treating male infertility (it now accounts for >50% of all IVF cycles), hence the selection of high quality sperm becomes a more urgent priority.
- Average LBR for IVF and IVF-ICSI have reached a plateau at 24% (although success rates vary widely across clinics and younger couples are generally more successful).
- Lower rates of fertilisation and higher rates of pregnancy loss following ICSI procedures are likely to generate higher costs as its use widens beyond treatment for male infertility. Wider use of ICSI without appropriate and adequate safeguards could lead to a future increase in the incidence of deleterious gene lesions in the wider population[8].
- The largest clinical trial so far, involving nine US centers has shown efficacy for PICSI in increasing CPR (10%) and a corresponding reduction in miscarriage rate (10%)[51]. A smaller Italian trial reported an encouraging 5% improvement in LBR following HA-based selection (using a non-optimised HA containing solution).
- Of the two commercially available HA-based selection systems, PICSI can be introduced into the ART procedure with minimal training and without any additional intervention and is the only product shown so far to have clinical efficacy in improving CPR.
- HA-based selection overcomes the highly subjective assessment of sperm quality used by the practicing embryologist to choose the ‘right’ sperm for injection.

6. AIMS AND OBJECTIVES

The main aims of this EME proposal are two-fold:-

- To show that the substitution of the usual polyvinylpyrrolidone (PVP) based sperm selection step with a hyaluronic acid (HA) binding step (PICSI) in an assisted reproduction setting can significantly improve the LBR over conventional ICSI procedures.

- To assess how the chromatin state of PICSI selected versus PVP selected sperm corresponds with HBS, CPR, LBR and pregnancy loss.

6.1 Objectives

6.1.1 Primary Objectives

6.1.1.1 *Clinical:*

To determine the efficacy of hyaluronan-selected IntraCytoplasmic Sperm Injection (PICSI) versus PVP ICSI in a rigorous randomised controlled clinical trial of participants where the primary outcome measure will be live birth rate (LBR) ≥37 weeks' gestation after ICSI procedure with first fresh embryo transfer.

6.1.1.2 *Mechanistic:*

Evaluate if PICSI can compensate for poor sperm quality and investigate HBS in relation to chromatin integrity and LBR. Evaluate the differences in chromatin architecture in DGC washed and pelleted sperm with high and low HBS including any correlation with DNA damage.

6.1.2 Secondary Objectives

6.1.2.1 *Clinical:*

To determine the impact of PICSI-selected IntraCytoplasmic Sperm Injection (PICSI) versus PVP ICSI on:

- increasing clinical pregnancy (CP) 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 LBR at <37 weeks' gestation.

6.1.2.2 *Mechanistic:*

The primary mechanistic evaluation will be extended to selected sperm collected from the 45:90 (or 40:80) interface of DGC washed samples. We wish specifically to determine the relationship between chromatin compaction and nuclear integrity in these interface samples with the HBS of their corresponding pellets.

7. TRIAL DESIGN

A parallel group, two arm, multicentre blinded, efficacy clinical trial with mechanistic evaluation.

7.1 Setting

Assisted Conception or Reproductive Medicine Units where IVF-ICSI is practiced and other relevant clinical settings.

7.2 Allocation

Randomisation into PVP-ICSI (non-intervention) or PICSI (intervention) groups

7.3 Main Endpoint

LBR at ≥37 weeks' gestation following a ICSI treatment with first fresh embryo transfer cycle in randomised couples.

7.4 Intervention Model

Parallel Assignment

7.5 Masking

Blinded –participants, clinical care providers in IVF-licensed unit, maternity and neonatal wards, research nurse responsible for participants follow-up will be blinded. The only unblinded group at study sites is going to be embryologists who perform ICSI/PICSI and HBS procedures and who will be also responsible for couple randomisation; in PCTU they will be study Data Manger and independent statistician who will prepare reports for the DMEC. HBS in relation to arm allocation will be known to scorer only.

7.6 Primary Purpose

Increasing LBR ≥37 weeks' gestation following ICSI.

8. ELIGIBILITY

8.1 Centre eligibility

Participating centres will be IVF licensed hospitals.

The centre must be able to provide appointments in a dedicated clinic in which to see participants. There are 10 planned participating centres as described in Section 13.4.1. Recruitment rate will be monitored and optional additional centres may be added as required once the necessary approvals have been obtained.

8.2 Inclusion criteria for randomisation

- Couples able to provide informed consent
- Couples undergoing ICSI procedure
- 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

8.3 Exclusion criteria for randomisation

- 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

9. RECRUITMENT, ENROLMENT AND RANDOMISATION

9.1 Recruitment process

Participants will be recruited from multiple research sites within the United Kingdom (see section 13.4.1). Research sites will be required to have obtained all necessary local management approvals, sign the model Agreement for Non-Commercial research (mNCA) and undertake a site initiation meeting prior to the start of recruitment into the trial.
The recruitment target across all sites is 3730 participants over a period of 21 months.

9.2 Eligibility and Informed Consent Process

9.2.1 Identifying and approaching of potential participants

Process of identifying potential participants and inviting them to the study will be individualized for each participating centre and adapted to their routine practice. Potential trial participants will be identified in several ways:

1. Approached during standard IVF Fertility Centre visits, either during individual appointment with a clinician or a Patient Evening/Meeting.
2. From waiting lists, registries or review of case records. Participants identified by these means may be sent the personalised HABSelect Invitation letter inviting them to take part. This letter will include a brief introduction to the study and also a copy of the Couple Information Sheet and Informed Consent Form (CIS-ICF). Patients will be invited to contact their local research clinician to find out more information and to make an appointment to discuss the study further.
3. Self-referral after accessing information from the study website, which we will endeavour to link to other similarly-themed websites or from the posters displayed in each participating centre.

Couples will be identified as candidates for the HABSelect study by local IVF-ICSI licensed Fertility Centre staff if they have opted for or been advised to make use of ICSI based procedures. Normally, routine NHS assessment of ejaculate semen quality is sufficient for men to be selected for ICSI procedures over IVF. The clinical team will then check that the couple meets the inclusion and exclusion criteria (see section 8.2 & 8.3). Only couples meeting these criteria will be approached to provide consent to participate in the HABSelect study. Details will be recorded on the trial screening log.

9.2.2 Informed Consent Procedures

The assessment of eligibility and the informed consent process will be undertaken by the principal investigator, or another suitably qualified member of the trial team who has received appropriate training and has been approved by the principal investigator as detailed on the delegation of responsibilities log. All staff involved in taking informed consent to the study will have a thorough knowledge and experience of Good Clinical Practice, issues around consent and will be fully conversant and trained in the study protocol. Informed, written consent for entry into the trial must be obtained prior to participant enrolment to the study.

The verbal explanation of the trial and CIS-ICF will be provided by the attending clinic staff for the patient to consider. This will include detailed information about the rationale, design and personal implications of the trial. They will be clearly advised that participation in the study is entirely voluntary with the option of withdrawing from the study at any stage, and that non-participation will not affect their usual care. Couples will be informed that they will be randomly allocated into standard (PVP-ICSI) or interventional (PICSI) groups, but both they and their clinical care staff will be unaware which group they are randomised to.

Following information provision, patients will have as long as they need to consider participation (a minimum of 24 hours is recommended) and will be given the opportunity to discuss the trial with their family and other healthcare professionals before they are asked whether they would be willing to take part in the trial. Assenting patients will be invited to provide informed, written consent. Where the patient has capacity to provide informed consent but is unable to sign or otherwise mark the consent form, we will follow the same procedure which the clinic adopts for such cases in regards to signing main HFEA consent forms for fertility treatment. If a translator is needed, the HABSelect research nurse will endeavour to make available the provision of a translation service* in the spoken language of the participant per standard local NHS Trust arrangements to try to be as inclusive as possible to potential participants. The right of the patient to refuse consent without giving reasons will be respected. Further, the participant will remain free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment.

* It is difficult to predict the languages required as some of the NHS Trusts are located in areas with a high influx of asylum seekers from a multitude of ethnic background - all common languages will be endeavoured to be covered but we will not be translating our material as the NHS sites have reported that it is not uncommon that one member of the couple translates for the other. As there are no participant-completed questionnaires in this study we have taken advice and conclude that the provision of a translator is sufficient and indeed the best option to maximize inclusion. All other data collected on the participant is from routine collected data and is not affected by the language of the participant.

A record of the consent process detailing the date of consent and all those present will be recorded in the participants' hospital notes. The original consent form will be filed in the

hospital notes (as per local practice), a copy retained in the Investigator Site File or study file containing patient identifiable data and a second copy of the consent will be given to the participants. With the couple's consent, the participant's GP will be notified using the REC approved GP letter provided. Once consent had been provided, the participants will be registered and recorded on the trial enrolment log.

Where valid, informed consent is obtained from the participants and if the participants subsequently becomes unable to re-affirm their consent by virtue of mental incapacity, the consent previously given is not enduring. Participants who lose capacity after informed consent has been obtained will be withdrawn from the trial without any further follow up or data collection.

No associated risks of the investigative procedure have been identified by the manufacturer of the interventional product. However if any further safety information which may result in significant changes in the risk/benefit analysis is identified, the CIS and Informed Consent Form (ICF) will be reviewed and approved accordingly. All participants who are actively enrolled on the study will be informed of the updated information and given a revised copy of the CIS/ICF to sign, confirming their wish to continue in the study. Copy of the consent forms will be given to the participant and the original added to the hospital notes.

9.2.3 Consent for the donation of residual semen samples for Biomedical Research

Patients who are eligible to take part in the trial will also be eligible to have any residual semen samples remaining after the ICSI procedure and mechanistic evaluations donated to the Human Biomaterials Resource Centre (HBRC) Biobank, University of Birmingham. Participation within the HBRC Biobank will be discussed with couples at the same time as discussing their participation in the HABSelect trial. Verbal and written details (the Donation of Human Tissue for Research Patient Information Sheet) will be provided to patients. Following information provision, patients will be given as long as they need to consider participation (a minimum of 24 hours is recommended) and will be given the opportunity to discuss the biobanking of any residual semen sample after all the HABSelect procedures are completed with their family and other healthcare professionals.

Patients who wish to have residual semen samples stored for the HBRC Biobank will be asked to sign The University of Birmingham Donation of Human Tissue for Research consent form. As for the main trial, a record of consent to this biobanking, detailing the date of consent and all those present will be kept in the patients notes. One copy (University of Birmingham Donation of Human Tissue for Research) of consent form will be transferred to the HBRC, one copy filed within the Investigator Site File and the third copy of the consent form will be given

to the participant.

9.3 Screening

Participating research sites will be required to complete a screening log of all couples who were recommended ICSI procedure as a method of choice for egg fertilisation during their fertility treatment. The screening log will be used to indicate whether couples are eligible for the trial based on their assessment according to trial inclusion/exclusion criteria or if they decline participation. Anonymised information will be collected including:

- age
- date screened
- which treatment cycle they are currently undergoing

  AND
- reason if not eligible for trial participation

  OR
- reason for declining participation despite eligibility (only with participants agreement)

  OR
- other reason for non-enrolment

Screening information will be entered into the secure electronic database or sent directly to PCTU trial coordinator when requested.

9.4 Enrolment

Following written informed consent, participants will be enrolled into the study by a delegated member of staff at the trial research site. **At the point of enrolment the couple will be issued a unique ID number and recorded on the trial enrolment log.**

Participants may only be enrolled into the trial by an authorised member of staff at the trial research site, as detailed on the Site Research Staff Delegation Log.

A unique ID number will consist of the trial site code (site ID) and followed by the consecutive screening number starting with 001 (please also refer to section 11.10.3 and Appendix 2).

After trial registration the research site will:

- Complete the trial enrolment log
- Add the unique couple participant ID number to all CRFs
- Ensure that participants are notified of their appointment dates.

9.5 Randomisation Procedures:

Following screening and formal enrolment onto the study, confirmation of eligibility and completion of baseline assessments the female participant will commence egg stimulation and the couple will enter the IVF clinical care pathway. At the couples' visit for the IVF procedure, approximately 2-8 weeks following enrolment, couples will be randomised into the trial. The time interval between enrolment and randomisation is centre dependent as they will be following local practice for egg simulation which precedes IVF. Couples will be randomized 1:1 to interventional (PICSI) and the non-interventional (PVP-ICSI) arms. Randomisation into the study will be typically on the day of the ICSI/PICSI and will be performed by an authorised member of staff at the research site, using the web 24-hour automated randomisation system developed by PCTU. Each centre will be provided with a unique set of log-in usernames and passwords for the study staff to do this. Online randomisation is available 24 hours a day, 7 days a week apart from short periods of scheduled maintenance. The randomisation system will generate intervention arm allocation to the couple which will be recorded on the Randomisation and Intervention Form.

9.6 Randomisation method and stratification variables

Randomisation will be achieved using a computer-generated minimisation algorithm that includes a random element to ensure treatment groups are well-balanced for the following participant characteristics, details of which will be required.

- maternal (<35, ≥35) age,
- paternal (<35, ≥35) age,
- number of previous miscarriages (0,1-2, >2),
- hormonal indicator of ovarian reserve: FSH (<6.0, ≥6.0miU/ml) or AMH (<17.0, ≥17.0 pmol/L) when FSH is not available

Due to logistical constraints minimisation will *not* include HBS.
Minimisation factors will be balanced separately within each centre.

The person randomising the participant will have to enter the couple's unique ID number, allocated at trial enrolment onto randomisation service website in order to receive the intervention allocation for that couple.

10. TRIAL INTERVENTION

10.1 Trial intervention with PICSI dishes and Hydak slides

The investigational products are the PICSI sperm-selection dish and the Hydak, hyaluronan binding score slides, both marketed (in the UK) by Biocoat, USA. Both products are CE marked and approved for clinical use. Full details can be found on the Origio and the Biocoat web sites. Regardless of the randomised allocation, HBA scores (HBS) will be obtained from all semen samples from both the interventional (PICSI) and the non-interventional (PVP-ICSI) arms using the Hydak slide. Only the interventional arm will make use of the PICSI plates.

10.2 Application

The protocol makes no additional requirements of the couple undergoing IVF treatment. Normally, density gradient washed and prepared motile sperm are selected for ICSI. This is achieved by adding the sperm suspension to PVP on an inverted microscope. Sperm motility is slowed sufficiently to allow capture by the experienced 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, exactly the same procedure is carried out except that the washed and prepared motile sperm are allowed to interact with the PICSI substrate to immobilise the sperm first. There are no other interventions.

10.3 Packaging, and supply of PICSI plates, Hydak slides and PVP

As already indicated, the PICSI plates and Hydak slides will be sterile packed and supplied by Origio (UK) Ltd, directly to the participating clinic on a pro-rata basis based on estimated recruitment targets and under the auspices of the TMG. Reserve stocks will also be held at the sponsor site for distribution as and when required.

At study initiation, the central trial team in collaboration with site staff will arrange an initial supply (equivalent to three months) of the products under test to the trial sites. The receiving clinics will confirm receipt of the products.

10.4 Participant Intervention

Couples will follow the guidance and procedures required for IVF preparation according to the centre involved (which as NHS units follow standard ICSI procedures – with the quality control assessment provided by UKNEQAS). For standard ICSI, it is normal practice for eggs to be

injected with sperm a few hours after egg collection. The embryologist or andrologist will prepare the semen samples by density gradient (DG) washing. Couples will then be randomised into one of the two arms and the prepared sperm will be processed through either modified (PICSI) or standard (PVP-ICSI) procedures by the embryologist. The operating procedures for PICSI and PVP-ICSI will be standardised across centres and integrated into their SOP Quality Management Systems as required by Human Fertilisation and Embryology Authority (HFEA). Prepared (pelleted) sperm samples will be used to obtain hyaluronan binding score (HBS). Injected eggs will be incubated overnight to allow pronucleate zygotes (PNZ) to form by Day 1. Once all ICSI procedures, including HBA scoring have been completed on Day 0, any remaining sperm sample will be processed according to Section 11.8.1 for the mechanistic research evaluation.

10.5 Subject Withdrawal

By consenting to participate in the trial, couples are consenting to the initial baseline screening for eligibility, trial intervention, follow-up and data collection. Couple or individual partner participants may withdraw consent from the trial at any time without explanation. Alternatively, participants may be withdrawn if in the opinion of the investigator or the care providing clinician or clinical team, it is medically necessary to do so. If a couple or either partner explicitly states their wish to withdraw from the trial, Pragmatic Clinical Trial Unit (PCTU) should be informed by completion of End Report CRF.

10.5.1 Participant withdrawal before intervention

i.e. post enrolment and prior to randomisation will not receive the trial intervention and will resume standard treatment/care. Participants who withdraw prior to randomisation or trial intervention will be replaced with another couple on the trial.

10.5.2 Participant withdrawal after intervention

Withdrawal from follow-up is the decision of the participant. As there is no difference in the follow-up monitoring of intervention versus non-intervention arms, we do not anticipate couples choosing to withdraw following embryo transfer. However we acknowledge withdrawal from the follow-up is the decision of the participant and occasionally such situations may occur.

Participant withdrawal post randomisation (after intervention) is categorised as follows:

Withdrawal of consent but the couple/ participant is willing for clinical data to be collected on pregnancy outcome but not for any further mechanistic assessments to be undertaken. Data collected to this point may be used.

- Withdrawal of consent for the trial follow-up schedule but the participant is willing

to have any information already collected to be utilised.

- Withdrawal of consent for follow-up information to be used and refusal of data already collected to be utilised.

Study personnel will make every effort to obtain and record information about the reasons for discontinuation and to follow-up the women for all safety and efficacy outcomes, as appropriate. To make a clear distinction as to exact participants' preferences we will use a withdrawal of consent form. If a participant explicitly withdraws consent to have any data recorded their decision will be respected and recorded on the electronic data capture system. All communication surrounding the withdrawal will be noted in the participant's records and no further Case Report Forms (CRFs) will be completed for that participant.

11. SCHEDULE OF ASSESSMENT, SAMPLE & DATA COLLECTION

PROCEDURE	**Pre-treatment (CRF1)**	**Hormonal treatment phase (CRF2)**	**Treatment phase (CRF3-4)**			**Pregnancy follow-up & outcome (CRF5-7)**			**Sperm mechanistic assessment**
TIMELINES	**Referral to Fertility Centre Referral – Day 21 of female menstruation**	**21 days after the day 1 of female menstruation – 36 hours after hCG stimulation**	**Day 0**	**Day 1**	**Day 2-5**	**Day 7--14**	**Week 6-9**	**End of pregnancy**	**6 months after study recruitment commencement**
Screening									
Eligibility assessment	**X**								
Informed Consent and enrolment to the study	**X**								
Baseline assessments for both partners	**X**								
Female - history of fertility treatment, gynaecological disorders and previous pregnancies	**X**								
Male – history of previous fertility treatment; general medical history and current medication	**X**								
Hormonal profile (AMH/FSH)	**X**								
Type of hormonal treatment		**X**							
Gametes collection & randomisation to the study			**X**						
Sperm profile (volume, sperm concentration, % forward progressive motility, % abnormal cells)			**X**						

HBS			X						
Number of aliquots			X						
ICSI / PICSI			X						
Number of pronucleozygotes				X					
Number of embryos transferred					X				
Biochemical pregnancy assessment (hCG level)						X			
Clinical pregnancy							X		
Fetal sac presence & number or fetal heartbeat							X		
Miscarriage (gestational age)								X	
Live Birth (gestational age)								X	
Adverse Events								X	
Sperm analysis									
Cytology									X
Comet									X
Tunel									X
HALO									X
Acridine Orange									X
CMA3									X
Aniline Blue									X

11.1 Clinical Assessments

11.1.1 Completion of Baseline Assessments (refer to Schedule of Assessment table)

Baseline Assessment will consist of the following information:

BOTH PARTNERS

1. Formal trial eligibility criteria assessment (inclusion/exclusion criteria)
2. Baseline demographic data (age, height, weight, BMI and ethnicity)

3. History of smoking, alcohol, recreational drugs use

FEMALE

4. Pre-treatment hormonal assessment
5. History of previous fertility treatment
    a. number of treatment cycles
    b. year
    c. partner (if different)
    d. type of treatment
    e. outcome of treatment (pregnancy outcome if any)
6. History of previous natural pregnancies (if available) including:
    a. year
    b. partner (if different)
    c. biochemical and clinical pregnancy assessment
    d. pregnancy outcome
7. Gynaecological & general medical history
    a. gynaecological disorders (i.e. polycystic ovaries syndrome, endometriosis etc.)
    b. gynaecological surgeries (if any)
    c. general medical history and current medication

MALE

8. Male semen assessment prior to current treatment (if available).
9. General medical history and current medication

11.1.2 Completion of CRF related to semen characteristics and gamete transfer.

At the end of Day 0, Intervention CRF will be completed on the online database by the delegated staff member. CRF will capture semen sample assessment (that details the sperm sample characteristics such as: sample sperm concentration before processing, % forward progressive motility, % abnormal forms etc.). The Embryo Transfer CRF will be completed on day 2-5 after embryo transfers have been completed to capture pertinent data (per couple) on whether PNZs formed, whether a transfer took place and how many embryos were transferred. The PCTU trial coordinator and CI will have access to the whole online database and will monitor CRF completion.

11.2 Clinical Assessments post intervention

Following the IVF procedure couples will resume standard IVF/ante-natal care and have no further scheduled trial-specific follow up. However the couples participating in the HABSelect trial will have their unique ID number linked to the female partner's patient records so that the routine fetal/pregnancy outcome data can be periodically captured and entered onto the CRF. IVF follow-up is relatively standard throughout the country and concerns the mother only who will be tested for biochemical pregnancy 7-14 days after embryo transfer. If the biochemical pregnancy test is positive, a scan will be performed looking for a fetal sac or heartbeat at 6-9 weeks after embryo transfer. These two outcomes are normally recorded in the patient notes and will be recorded on the trial CRF. If clinical pregnancy is confirmed the woman will be transferred from IVF to standard antenatal care for all further follow up of pregnancy. Beyond this point, patient records will be monitored as required to capture any pregnancy loss (a routinely recorded event), or record of a live birth; these outcomes will be transferred onto the appropriate HABSelect CRF by the research nurse or another appropriately trained staff member allocated to the study as they become available.

11.3 Outcome Data Collection:

- Biochemical pregnancy rates based on the detection of hCG in the mother's blood or urine.
- Clinical pregnancy rates based on ultrasound scanning (fetal sac and/or detection of heartbeat).
- For the primary clinical outcome: successful live birth ≥37 completed weeks since embryo transfer or last menstrual period.
- For the secondary clinical outcomes:
    - miscarriage, defined as pregnancy loss after confirmation of clinical pregnancy
    - live birth <37 weeks

All of the above will be based on data gathered from the ICSI cycle with first fresh embryo transfer per couple.

11.4 End of Study Definition

The trial will end when outcome measures from the last participant entered are known.

11.5 Procedures for unblinding

We do not anticipate any need to unblind participating couple during the trial. However, if emergency unblinding is required for patient safety reasons there will be a staff member at each site and an independent statistician at the PCTU with access to the arm allocation. In the event of any unblinding the CI and trial statistician will be immediately notified. The DMEC will have access to unblinded data throughout the trial, and can recommend that the trial be terminated at any time on safety grounds.

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11.6 Early Termination of Study

The Sponsor, Chief Investigator, Research Ethics Committee (REC) and Regulatory Authorities independently reserve the right to discontinue the study at any time for safety or other reasons. This will be done in consultation with the Sponsor where practical. In the occurrence of premature trial termination or suspension, the above mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (with the exception of the sponsors responsibility for notifying the Regulatory Authorities). After such a decision, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interest. The investigator must review all participating subjects as soon as practical and complete all required records.

11.7 Long-term follow-up

The developmental function of the infants born to couples in the HABSelect Trial is of interest but outside the scope and time-frame for the Trial as it currently stands. Should further funding become available, a new observational protocol will be developed, approval gained and potential participants traced through Fertility Clinics. Informed consent will have to be obtained.

11.8 Samples

11.8.1 <u>In-clinic samples collection, processing and labeling</u>

In the IVF centre the semen samples will be assessed for HBA score (HBS) on fresh samples (i.e. prior to freezing). Some samples will be also HBS tested after thawing in the mechanistic research laboratories to test continuity. In all cases, HBS scores will be linked to the samples' respective unique ID.

Remaining semen samples following ICSI/PICSI will be used for the mechanistic evaluations. Although not mandatory, we recommend that droplets for ICSI/PICSI are returned to the

remaining sperm pellet prior to centrifugation. Clinics will indicate on the appropriate CRF the semen characteristics and HBS. Samples from patients with certain conditions (i.e. chronic viral infections) may be excluded from further mechanistic evaluations. The decision to exclude these samples will be taken by Chief Embryologist in consultation with project Laboratory Leads. Processed sperm aliquots (250 µl) will be barcoded with the labels provided by the Human Biomaterials Resource Centre, University of Birmingham, prior to freezing and storage in LN_2. Each aliquot will be clearly identified with the couple's unique ID number. All samples processing will be performed according to the approved standard operating procedures.

11.8.2 In-clinic samples logging and storage

Samples will be stored in the designated freezer or storage cask assigned for that purpose by the participating centre and a storage log maintained. All effort will be made to ensure the samples are stored separately from any other samples kept for clinical treatment or for research purposes in the participating centres. At the end of each day samples and sample information for each participant centre will be logged by the delegated staff member in the online Database.

11.8.3 HBS pre and post freezing Continuity assessment.

The HBS from each of the participating centres will be regularly monitored. Scores will be harvested from the on-line database every four months and continuity tested to estimate and if necessary, reduce inter-centre variation. The information will be used to alert the data manager if any centre appears to be deviating from the expected range. If necessary, additional training may be provided to counteract the effect if it is marked. However, because the *same* sample will be sent to each mechanistic lab *and* can be tracked as such, inter-centre HBS variation should not adversely affect subsequent downstream analyses

11.8.4 Post-clinic sample transport to the central storage

Every participating centre will store frozen samples and transfer samples in batches by courier to the Human Biomaterials Resource Centre, University of Birmingham that will act as the central repository for all HABSelect semen samples. Shipping of the samples will be arranged on a periodic basis by the trial coordinator in collaboration with the participating centres and central storage provider. Samples will be shipped to the central storage on dry ice by the dedicated service acting on behalf of Birmingham biobank and contracted by them for that purpose. Prior to being sent, samples will be checked to validate the exact content of each shipment. Each consignment will include shipping logs, courier consignment notes and temperature audit to ensure the samples are delivered in the intact condition. On arrival the

central storage facility staff will check that the physical integrity of the samples have not been compromised in the transit. Study CI, sponsor and trial coordinator will be informed if such situation arises. Laboratory staff will ensure all samples are accounted for as per shipping log. All samples received will be logged in an accountability log.
All samples received into HBRC will remain there until required. At the end of the HABSelect trial any surplus semen samples from trial participants will either be biobanked at HBRC or destroyed in accordance with the couples' instructions on their consent forms.

11.9 Selection of Samples for Laboratory Mechanistic Assessments

The Birmingham Mechanistic Laboratory will thaw out single aliquots and cytology will be performed on all or as many of the 3730 trial samples as possible. Cytology provides an important primary benchmark on the integrity of the semen samples
The mechanistic evaluations will be performed following PCTU stratification of the clinical HBS values (three scoring strata: <50%, 50-65%, >65%, HBS). The routine cytology will then determine if there is sufficient sample available; it is anticipated that a significant proportion of the samples will have insufficient sperm for further mechanistic evaluation. However, the stratification will generate a balanced sampling framework from each trial arm across the three HBS strata. Those samples identified by PCTU from their HBS for the mechanistic evaluation will be communicated to the HBRC central sample repository. The HBRC will then coordinate sample transfer to the four mechanistic laboratories to perform the hierarchy of mechanistic test evaluations as described in Figure 5 and Table 3**.**

In summary, the PCTU will select the HABSelect semen samples to be used for mechanistic analysis based on HBS and communicate this to the HBRC who will arrange shipment to the appropriate Mechanistic Laboratories. This process can be reiterated until ~900* samples have been tested with each assay across the three, <50% 50-65%, >65%, HBS strata. It is anticipated that access to screening the full complement of 3730 trial samples in order to achieve this may be required. With dynamic PCTU and Birmingham monitoring of sample selection for analysis, it will be possible to obtain high quality information for each test in each stratum even if only a minority of samples is amenable to global examination by *all* tests. Local statistical support is confident that the information can be integrated. Shipment of samples to the mechanistic labs will follow the same operating procedures as transport of the samples from the participating centres to the central storage bank.

** Belfast will take 450 samples for analysis but will analyse them using two distinct variations of the comet assay.*

11.9.1 Mechanistic assessments

Four basic science laboratories will perform mechanistic evaluation of the collected semen samples according to the following order:

- Birmingham – cytology, TUNEL, Acridine Orange (AO) stain
- Belfast –Comet
- Sheffield – HALO, Aniline Blue
- Leeds –TUNEL, HALO, Aniline Blue, CMA3

*

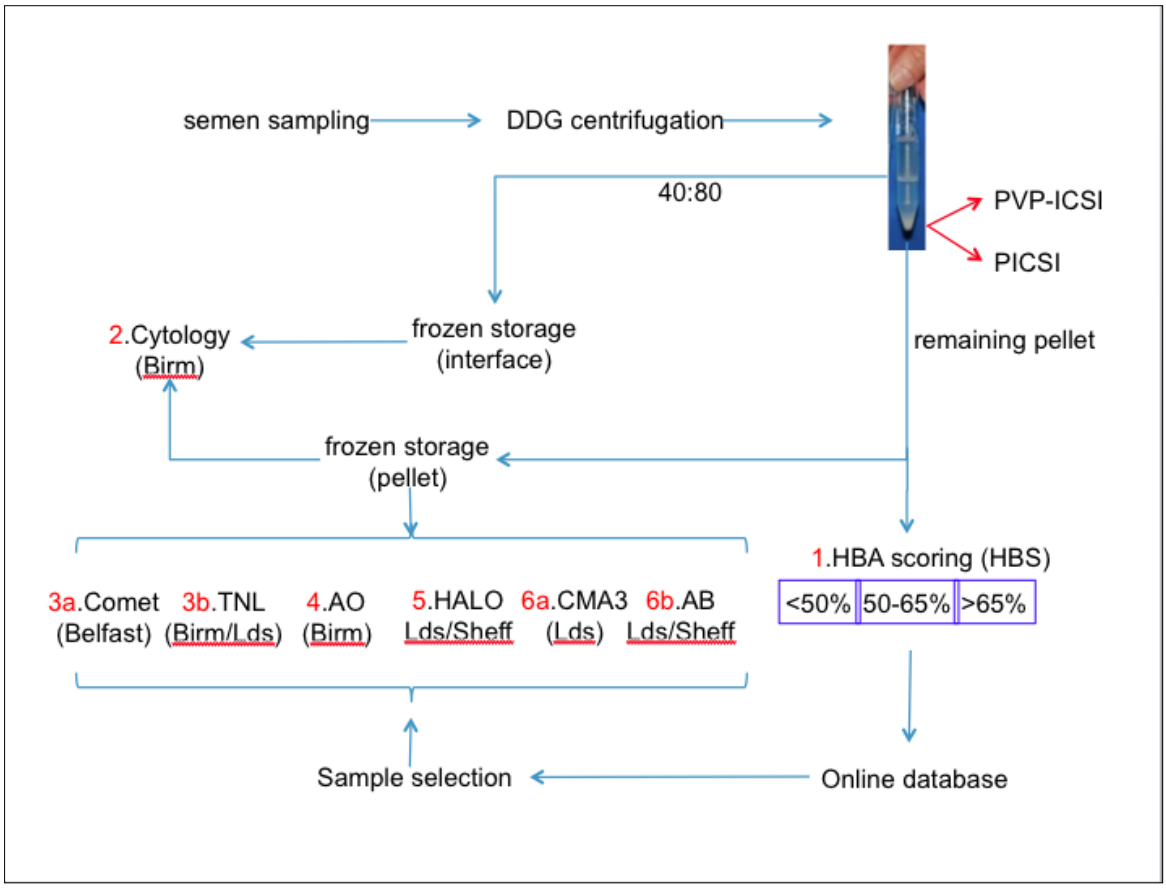

Figure 5. Schematic of mechanistic protocol. There is a hierarchical priority of testing, with the number carried out depending on the number (Table 2, **N**) of available sample aliquots after HBA[1] scoring. The testing priority is 1, 2, 3a AND/OR 3b, 4, 5, 6a AND/OR 6b. If four aliquots are available, all tests can be carried out across the four centres. If three aliquots are available, going to three centres, it will not be possible to replicate tests 5 and 6 in both Lds and Sheff. If two aliquots are available, going to two centres the same restrictions will apply and additionally, only one of tests 4, 5 or 6 will be possible. All samples will be stored centrally in Birmingham HBRC; hence they can begin to process and assess samples for distribution as soon as they are ready. If following cytology (1), there is only sufficient sample for analysis at one centre, it will be Birm who carries out any additional tests. PCTU will assist in maximising the use of available samples by all mechanistic labs. Collection of sperm from the 40:80 interface will occasionally be undertaken on CI request for the mechanistic studies.

Table 3. Mechanistic assays are subject to a hierarchical order of importance as follows: 1. HBS^{CL} > 2. $Cytology^{BI}$ > 3. ($aComet^{BL}$ = $bTunel^{BI}$) > 4. AO $stain^{BI}$ > 5. $HALO^{SH,\ LE}$ > 6. ($aAniline\ Blue^{SH,\ LE}$ = $bCMA3^{SH,\ LE}$). It is assumed that the HBS (1) will be carried out on all samples by the clinics prior to aliquoting.

Equivalent available aliquots (N)‡	**Test**
4	2, 3a, 3b, 4, 5, 6a, 6b
3	2, 3a, 3b, 4, 5*, 6*
2	2, 3a, 3b, 4 OR 5* OR 6*
1	2, 3b, 4

CL, clinical labs; BI, Birmingham; BL, Belfast; LE, Leeds; SH, Sheffield.

* Sent to either of the two centres carrying out these assays. To balance the numbers, tests

may be alternately carried out (OR) and in alternate centres.

‡All semen samples will be aliquoted in the IVF clinic into four equal volume samples. The cytology examine will determine the sufficiency of the sperm in each aliquot and adjustment will be made of the number of equivalent aliquots available for the mechanistic evaluation, e.g. where there is a low sperm count in the semen sample the 4 combined aliquots may only provide 1 equivalent aliquot and therefore only a limited range of mechanistic testing is available and priority will be given following a rank order of priority. In addition, Leeds and Sheffield will establish manual processes for quantifying the results of their tests and a proportion of the manually processed slides will be sent to Birmingham for automated quantitative analysis. This will satisfy continuity requirements for all other assays.

11.9.2 Mechanistic Laboratory Data Recording/Reporting

Outputs of the mechanistic studies will be recorded as follows:

1. Initial observations recorded in paper based laboratory notebooks according to standard practice.
2. Digital images associated with experimental outputs to be held locally on portable, encrypted solid-state hard drives.
3. Digital images and experimental outputs uploaded on to a secure web page shared between the four mechanistic labs. Times of data uploads will be logged.
4. Following data analysis and summarisation, all data will be reported firstly to the DMEC and via that committee to the TSC and TMG. Reports will be drafted for internal discussion followed by approved submission of the outputs to peer reviewed journals.

11.10 Data Handling & Record Keeping

11.10.1 Confidentiality

The Chief Investigator is the 'Custodian' of the data and will ensure that information with regards to study participants will be kept confidential managed in accordance with the Data Protection Act 1998, NHS Caldicott Guardian 2010, The Research Governance Framework for Health and Social Care 2005 and Research Ethics Committee Approval.

Personal data and any sensitive information required for the HABSelect Trial will be collected directly from trial participants and hospital notes. Identifiable information to be collected from the participants include, full name, DOB, NHS number for female and contact details after couples consent to the trial. Male NHS number will be collected only if consent for donation of residual sperm sample in HBRC has been given. This information will be entered to Identification Log, which also includes couple study ID and used to contact participants but will not leave the study site. All case report forms (CRFs) will be pseudonymised. All staff involved in the HABSelect share the same duty of care to prevent unauthorised disclosure of personal information.

The trial data will be made available to suitably qualified members of the research team, sponsor representatives, study monitors and auditors, the REC and regulatory authorities as far as required by law.

No data that could be used to identify an individual will be published. The participants will be anonymised with regards to any future publications relating to this study.

11.10.2 Study Documents

The following documents will be used in the HABSelect trial:

- A signed protocol and any subsequent amendments
- PCTU monitoring assessment template for the trial team to follow as detailed by the Monitoring section
- Current/Superseded Couple Information Sheets
- Current/Superseded Consent Forms (as applicable)
- Human Biomaterials Resource Centre, University of Birmingham - Donation of Human Tissue PIS and associated consent form
- GP Letter to inform them of participants inclusion in the HABSelect Trial
- Invitation Letter for potential participants to invite them to the study
- Indemnity documentation from sponsor
- Conditions of Sponsorship from sponsor
- Final R&D Approval
- Signed site agreements and laboratory contracts
- Ethics submissions/approvals/correspondence
- CVs of CI and site staff
- Laboratory accreditation letter, certification and normal ranges for all laboratories to be utilised in the study
- Site Research Staff Delegation log
- Certificates of the training in the study intervention for the site Embryologist(s)/Andrologists(s)
- Participant identification log
- Screening log
- Enrolment log
- Site visit log
- Protocol training log
- Correspondence relating to the trial
- Communication Plan between the CI/PI and members of the study team
- Case Report Forms (CRFs)
- Provisional or conditional R&D letter
- GCP/RGF certificates of the patient-contacting study team member(s)

- Trial specific SOPs
- Adverse Events log

11.10.3 Case Report Forms

For all participating couples, pre-interventional trial data will be recorded in the patient notes which will be completed by the ACU/Fertility clinic team (i.e. clinicians, embryologists). The following data will be abstracted from the notes and recorded on CRF for the trial by the research nurse or another appropriately trained staff blinded to the participant allocation, relying on the research support in each of the recruiting hospitals. Any available missing information will be obtained from trial participant prior to discharge from the Fertility Centre to the community ante-natal care.

Pre-Intervention Phase

- Eligibility criteria for both partners
- Demographic assessments for both partners
- Previous fertility treatment for both partners
- Gynaecological and obstetric history for female
- Hormonal profile for female partner
- Semen profile for male partner

Intervention Phase

- Four equal aliquots obtained from the remainder of the semen sample
- Initial Sperm concentration
- HBS score
- Number of pronucleate zygotes formed
- Number of embryos transferred
- Biochemical pregnancy indicators
- Clinical pregnancy indictors (as confirmed by USG scan)

Post Intervention Phase:

- Successful live birth ≥37 completed weeks since embryo transfer or last menstrual period.
- Clinical pregnancy rate based on detection of fetal heartbeat or presence of fetal sac at 6-9 weeks' gestation
- Miscarriage rates defined as pregnancy loss at any time post confirmation of

clinical pregnancy

- Live births at < 37weeks' gestation.
- Serious adverse events

All of the above will be based on data gathered from the ICSI cycle with first fresh embryo transfer per couple.

Extra

- Additional Information/Note to File CRF
- Final Study Status /Early Withdrawal CRF

CRFs will be pseudonymised using couple unique ID allocated during enrolment. This code will consist of the trial site code followed by consecutive numbers starting at 001. Site codes are documented in Appendix 2.

E.g.: Birmingham Women Fertility Centre (0119), couple number 1 (001): 0119/001

11.10.4 Data collection, processing and monitoring

The PCTU will activate and deploy a web-based trial database. PCTU trial coordinator, monitor, quality assurance manager, data manager, statistician, study CI and Clinical Advisors will have full access to the entire database for monitoring, coordinating, safety reporting and analysis purposes. A set of trial specific CRFs will be created. Participating sites will record the required data item via the internet using secure socket layer encryption technology. Missing and discrepant data will be flagged by the trial coordinator, trial monitor or trial statistician by following data cleaning and quality control procedures with additional data validations raised during monitoring visits. Schedule of Assessment table in first part of Section 11 provides a summary of assessment procedures that will be undertaken during the HABSelect study.

11.10.5 Format

Relevant trial data will be transcribed from participants' notes into CRF. Source data will comprise of the research clinic notes, hospital notes, hand-held pregnancy notes and laboratory results.

Women will be encouraged to report pregnancies, miscarriages and deliveries occurring between clinic visits or presenting at non-participating hospitals to the research nurse or assistant. Self- reports will be verified against clinical notes.

11.10.6 Data Completeness

Compliance with the clinical procedure will be checked at the end of each week by the trial coordinator who will ensure that the participating sites' Day 0 and Day of embryo transfer entries have been uploaded onto the online database as required. The research nurse or assistant will also record the use of the Hydak slides and PICSI plates allowing the Trial Coordinator to check regularly against the delivered inventory of each trial participant. In an effort to improve compliance, sites failing to upload their data or report inventory use will be contacted by telephone or email. Any protocol deviations will be recorded and notified to DMC and any serious breaches of the protocol or GCP will be reported to the Sponsor in line with Sponsor SOP.

12. SAFETY

12.1 Adverse Events (AE)

The IVF intervention under evaluation is limited to the process of fertilising the eggs (Day 0), using a CE marked device in both arms of the trial. There is no on-going intervention or routine follow up planned until the final outcome measure; as a consequence the clinical advisors and sponsor have determined that no additional 'active' monitoring for patient safety and adverse event reporting is required.

Outcomes related to pregnancy success (biochemical and clinical pregnancy) will be captured whilst the patient is still under the clinical supervision of the IVF clinic. If the pregnancy is confirmed the woman will then pass to standard ante-natal care based on her own risk criteria and pregnancy outcome will be reported to the IVF clinic using established local protocols for IVF clinical audit.

12.2 Serious Adverse Event (SAE)

An SAE fulfils at least one of the following criteria:

- Is fatal – results in death (NOTE: death is an outcome, not an event)
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is otherwise considered medically significant by the Investigator

12.2.1 Expected SAEs

It is possible that during their pregnancy, participants will be admitted to hospital for treatment or monitoring of their pregnancy. Expected SAEs will include hospitalisations for the following events.

- routine treatment or monitoring of miscarriage or threatened preterm birth, not associated with any deterioration in condition including :
    - Premature Rupture Of Membranes or suspected PROM
- treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study, and did not worsen including:

- Elective Caesarean Section,

- admission to a hospital or other institution for general care, not associated with any deterioration in condition including:
  - Hospitalisation for rest
  - Hospitalisation for observation or monitoring of pregnancy
  - Hospitalisation for Maternal Discomfort
  - Hyperemesis which is quickly resolved.

Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission will not be treated as an SAE.

Pregnancy related SAEs recorded in the medical notes will be captured by each site as part of the pregnancy outcome and recorded on an SAE log. All SAEs will be notified to the trial coordinator and chief investigator who will maintain a central log of all SAEs for onward reporting. These will be reported according to PCTU standard procedures.

12.3 Notification and Reporting of Serious Adverse Events

The National Research Ethics Service defines related and unexpected SAEs as follows:

- related' – resulted from administration of any research procedure.
- 'unexpected' – this type of event is not listed in the protocol as an expected occurrence

Only Serious Adverse Event (SAEs) that are considered to be 'related' and 'unexpected' are to be reported to the sponsor and PCTU QA manager within 24 hours of learning of the event and to the Main REC within 15 days in line with the required timeframe.

12.4 Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health and safety, in accordance with Regulation 30. The measures should be taken immediately. In this instance, the approval of the Licensing Authority Approval prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor and Main Research Ethics Committee (via telephone) of this event **immediately**.

The CI has an obligation to inform both the Main Ethics Committee **in writing within 3 days**, in the form of a substantial amendment. The sponsor and PCTU QA manager must be sent a copy of the correspondence with regards to this matter.

12.5 Annual Safety Reporting

The CI will send the Annual Progress Report to the main REC using the NRES template (the anniversary date is the date on the MREC “favourable opinion” letter from the MREC) and to the sponsor and PCTU QA manager.

13. STATISTICAL CONSIDERATIONS

The analysis will be by intention to treat. Every attempt will be made to gather data on all women randomised, irrespective of compliance with the treatment protocol.

13.1 Trial Outcomes

13.1.1 Primary outcome measures:

13.1.1.1 *Clinical:*

Live birth at ≥37 weeks' gestation following the first fresh ICSI treatment

13.1.1.2 *Mechanism:*

HBA score will be recorded on the day of PICSI / PVP-ICSI procedure and DGC washed sperm will be examined, retrospectively for disruption of chromatin architecture and DNA damage.

13.1.2 Secondary outcome measures:

13.1.2.1 *Clinical:*

- 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

13.1.2.2 *Mechanism*:

Chromatin disruption in relation to DNA damage and DNA packaging anomalies in 45:90 interface samples and correlation between clinical and post-clinical HBA scores in relation to initial sperm concentration. The relationship between the tests of chromatin and DNA integrity with live birth outcome and miscarriage will be dynamically assessed by statistical modelling (please see section 13.7).

13.2 Endpoint analyses

13.2.1 Primary endpoint analysis

The primary endpoint 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 followed up after their ICSI cycle with first fresh embryo transfer post randomisation per arm 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. The proportion will be compared between arms using multivariable logistic regression adjusting for centre and for factors used in the minimisation. An odds ratio with 95% confidence interval will be calculated.

13.2.2 Secondary endpoint analyses

The secondary endpoints are the respective proportions of women who:

- experience a clinical pregnancy based on presence of fetal heartbeat or fetal sac at 6-9 weeks' gestation
- experience a clinical pregnancy and miscarry
- experience a clinical pregnancy and proceed to a live birth <37 weeks

These proportions have as their denominator the number of women who are followed up after their first ICSI cycle post randomisation, and as their numerator the number with the respective outcome. The proportions of each will be compared between arms using multivariable logistic regression adjusting for centre and for factors used in the minimisation. An odds ratio with 95% confidence interval will be calculated.

13.3 Handling 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. If baseline assessments of covariates are missing we will use mean imputation or missing value indicators to replace them [64]. If any outcome data are missing we will analyse only those with outcomes data, adjusting for baseline covariates (this approach is unbiased if reasons for the outcome being missing can be related to observed covariates – the so-called "missing at random" assumption). We will also perform sensitivity analyses as suggested by White et al. [65].

13.4 Sample Size – Clinical evaluations

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 and given our broad inclusion criteria, we conservatively expect 40% of the couples to meet eligibility and be willing to be consented to the study i.e. 3730 over 21 months. Trial recruitment is based on pro rata targets at each of the participating sites based on HFEA data and the need to recruit at least 3,266 couples into the trial to detect a 5% improvement in clinical efficacy**.**

For PICSI, average improvements in LBR per treatment cycle of 7.5% are likely based on maternal age and paternal semen profile ([42, 48, 51]). Older women (≥37) are of particular interest to us because their eggs may have a decreased capacity for the repair of sperm DNA damage

in their older partners [1]. Lower and higher improvement score[s] among, respectively, younger and older women are likely. Assuming 5% for the former and 15% for the latter, rates will rise from 32.7% to 37.7% LBR (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 LBR 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 score 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 comprise almost 30% of ART procedures, providing 1007 women for the study.

13.4.1 Projected recruitment and retention rates

We have estimated from site feasibility analysis that the sites' recruitment targets (couples) will be as follows:

- Leeds (6.7% or 250)
- Guys (18.5% or 690)
- Bart's & The London (12.1% or 450)
- Manchester (7.7% or 288)
- Birmingham (6% or 224)
- Sheffield (4.3% or 161)
- Dundee (3.8% or 143)
- Aberdeen (3% or 113)
- Coventry (5.6% or 209)
- Leicester (3.1% or 115)
- Homerton (7.5% or 279)
- IVF Hammersmith (5.4% or 200)

Other sites will be recruited as required.

A 10% loss to follow-up (the 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 Study Flow Diagram – Appendix 3).

13.5 Sample size - Mechanistic evaluation

The sample size for the clinical trial (3,730) is based on detecting a 5% improvement in live birth outcome at 90% power (3,266). In contrast, the mechanistic sample size is based on an estimated minimum of 180 required for logistic regression analysis of the relationship between HBS and clinical outcomes [62] and can therefore be far smaller. While this number accommodates the potentially limiting availability of samples with low (<50%) HB scores (estimated at ~10% or ~370), it is a minimum estimate that does not satisfy the need to more accurately specify the relationships between the mechanistic and clinical outputs of this study that are less dependent on HB scoring (such as sperm concentration). Furthermore, as indicated elsewhere, many of the samples will not be amenable to mechanistic tests. Hence, the chosen mechanistic sample size is a balance between minimum sample size requirements (180) required to link clinical and mechanistic outcomes, the need to access more samples in order to accommodate limitations in their use and the relative cost of analysis. Assuming that ~20% of samples can be examined by one or more tests based on cytology, only 36/180 will satisfy the criteria. Hence ~900 samples (180/36*180) are required overall. All mechanistic tests are needed to facilitate the identification of the form of damage that compromises sperm function in relation to clinical outcome (Fig 6).

All samples will be transferred to the Human Biomaterials Resource Centre, University of Birmingham as outlined in Section 11.9 for initial cytology. Cytology will be performed retrospectively on as many of the 3730 trial samples as required for mechanistic analysis. It will provide an important first step characterisation on the integrity of the semen samples. The rationale for banking semen samples on all trial participants is based on maximising the success of the exploratory mechanistic components of the study. The sampling has been described in Section 11.9.1. In summary, the mechanistic evaluations will be performed retrospectively following PCTU stratification by sample characteristics based primarily on the HBS to assay across the three, <50%, 50-65%, >65% HBA scoring strata, with the cytology secondarily determining if there is sufficient sample available. It is anticipated that a significant proportion of semen samples will be identified as having insufficient sperm for further mechanistic evaluation. The retrospective stratification will generate a balanced sampling

frame from each trial arm to obtain high quality information for each test in each stratum even if only a fraction of the samples is amenable to global examination by *all* tests (refer to Figure 5; Table 3).

Of note the mechanistic tests do not inconvenience participants, or their management or treatment as they use residual semen samples from the ISCI/PICSI IVF procedure.

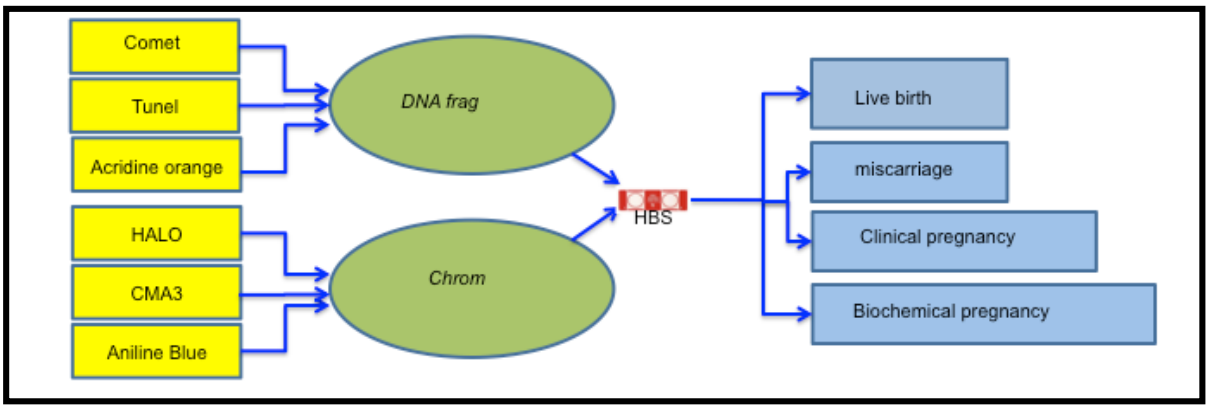

Figure 6. Schematic representation of structural equation modelling showing relationship between measured quantities (boxes) and latent variables (ellipses). *DNA frag* and *Chrom* are treated as covariates in the regression model for HBS score.

13.6 Statistical Analysis

13.6.1 Clinical Evaluation

Our unit of analysis will be couples randomised to alternative interventions. In addressing the research question in this grant application, we have elected to focus on the outcome of the ICSI cycle with first fresh embryo transfer in each randomised couple and powered the trial accordingly. We believe that, if effective, the impact of the intervention will be evident in the first fresh study cycle. Attempting to evaluate cumulative live birth rates after an ICSI treatment culminating in transfer of fresh embryos followed by the subsequent transfer of frozen embryos in those not achieving a pregnancy would require an extended duration of follow-up. This would significantly increase the complexity and costs of the proposed trial, without generating outcomes of sufficient additional value.

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 tabulated. Outcomes in intervention and non-intervention arms will be compared using multivariable regression with effects summarised as odds ratios with 95% confidence intervals.

If there is evidence that the clinical pregnancy rate differs between the trial arms, then secondary analyses will be carried out taking only women with a clinical pregnancy as the denominator.

Sensitivity analyses will be carried out which adjust for other potentially prognostic factors in addition to the minimisation factors. Factors to be included will be selected blind to their distribution between recruitment arms or the effect of treatment on outcome, and will be those that are associated with the outcome or believed a priori to be prognostic.

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

Subgroup analyses. For binary outcomes, results will be expressed as odds ratio with 95% confidence intervals of pregnancy success in either arm. The exploratory subgroup analyses

- Hyaluronan Binding Score; HBS (High (>65%) versus low (≤65%)),
- maternal age (≤35 years versus >35),
- Previous miscarriages (none versus any),
- FSH hormone level (<6.0miU/ml versus ≥6.0miU/ml) or AMH hormone level (<17pmol/L versus ≥17pmol/L) where FSH testing is not available.
- sperm concentration (<15mml versus ≥15mml).
- We may also analyse a very low (≤25%) vs. low (>25%, ≤65%) HBS sub-group.

In each case, an interaction test will first be used to determine whether there is a basis for investigating treatment efficacy within subgroups. Sub-group analyses results will be treated with caution, and will be undertaken in relation to the advanced analytical assays summarised in section 13.7 solely for the purposes of hypothesis generation.

As the two arms of this study are compatible with the equivalent arms of the US NIH trial (NCT00741494) they can be included in any future meta analysis of the data.

13.7 Mechanistic Evaluation

The mechanistic evaluation will be conducted through a structural equation modelling approach that is particularly well suited to estimating causal relationships using a combination of quantitative data and qualitative causal assumptions [62, 63]. DNA fragmentation will be measured though the comet assay and through slide-based acridine orange assay and summarised through the latent variable DNAfrag (Fig 6). Similarly, chromatin compaction measured by aniline blue and CMA3 assays will be summarised through the latent variable Chrom. HALO provides a separate covariate of sperm nuclear integrity. These two latent variables (DNAfrag, Chrom), and HALO are covariates in a regression model for Hyaluronan Binding Score (HBS) which in turn is a covariate for the logistic regressions for each of the clinical outcomes

(biochemical pregnancy at two weeks, clinical pregnancy at 6–8 weeks, miscarriage rate, and live birth at ≥37 weeks. The structural equation modelling can be undertaken with the software MPLUS version 6.12.

14. CLINICAL GOVERNANCE ISSUES

14.1 Ethical and Trust Management Approval

Ethical approval (IRAS) and R&D approvals will be obtained before recruitment starts. The primary ethical issue is the substitution of PVP with PICSI in 50% of the trial's participants. However, the existing data from earlier trials suggests a significant benefit and absence of serious safety concerns with PICSI use in pregnancy [42, 51]. Information will be provided verbally and through an information sheet, which will be developed in consultation with patients and representatives of the patient involvement group. The information sheet will clearly explain the participation in the trial is voluntary with the option of withdrawing from the trial at any stage, and that participation or non-participation will not affect their usual care. Only individuals who are NHS employees (substantive or honorary) and who have access permissions will examine hospital databases for potentially eligible participants.

The Local Comprehensive Research Network will conduct governance checks and assess the facilities and resources needed to run the trial, in order to give host site permission. The PCTU trial coordinator will assist the local Principal Investigator in the process of the Trust research governance approval by co-ordinating the Site Specific Information section of the standard IRAS form. The PCTU trial coordinator will liaise with the local Principal Investigator who will be responsible for their local site and communication with the Trust management with respect to locality issues and obtaining the necessary approvals at their Trust.

As soon as Trust approval has been obtained, the PCTU coordinator will send a site file containing all trial materials to the local Principal Investigator and assist the CI to conduct site initiation and training. Only once all site approvals and trial training is complete can trial start recruiting participants into the study.

14.2 Funding and Cost implications

The research costs of the trial are funded by a grant from the NIHR Efficacy and Mechanistic Evaluation programme awarded to the University of Leeds.

The trial has been designed to minimise extra 'service support' costs for participating hospitals as far as possible. Additional costs service support costs associated with the trial, e.g. gaining consent, pre-pregnancy clinic visits etc, are estimated in the Site Specific Information section

of the standard IRAS form. These costs should be met by accessing the Trust's Support for Science budget via the Local Comprehensive Research Network.

14.2.1 Indemnity

There are no special arrangements for compensation for non-negligent harm suffered by patients as a result of participating in the study. The study is not an industry-sponsored trial and so ABPI/ABHI guidelines on indemnity do not apply. The normal NHS indemnity liability arrangements for research detailed in HSG96 (48) will operate in this case. However, it should be stressed that in terms of negligent liability, NHS Trust hospitals have a duty of care to a patient being treated within their hospital, whether or not that patient is participating in a clinical trial. Apart from defective products, legal liability does not arise where there is non-negligent harm. NHS Trusts may not offer advance indemnities or take out commercial insurance for non-negligent harm.

14.2.2 Ethical Considerations

This protocol and any subsequent amendments, along with any accompanying material provided to the patient in addition to any advertising material will be submitted by the Investigator to an Independent Research Ethics Committee. Written Approval from the Committee must be obtained and subsequently submitted to the local R&D departments to obtain Final R&D approval.

14.3 Participant Record Retention and Archiving

During the course of research, all records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the Research Governance Framework and Trust Policy that the records are kept for a further 15 years. The Sponsor or sponsor's representative will hold responsibility for record retention and archiving to relevant procedures. A clear distinction will be made between storage period of study records and records performed on human male gametes as required by HFEA. The latter ones will be kept for a further 50 years after trial completion. Archiving of site files and participants' records at each participating centres will be the responsibility of the local R&D department.

14.4 Quality Control and Quality Assurance

14.4.1 Summary Monitoring Plan

The PCTU QA manager had conducted risk assessment of the study and determined the potential risk as Moderate level. The nature, frequency and intensity of trial monitoring will be outlined in the trial monitoring plan as determined by the PCTU risk assessment. The monitoring plan will explain what will be monitored, which/what proportion of data fields and who will be responsible for conducting the monitoring visits, and who will be responsible for ensuring that monitoring findings are addressed. Investigators and their host Trusts will be required to permit trial-related monitoring and audits, providing direct access to source data and documents as requested. Trial participants will be made aware of the possibility of external audit of data they provide in the participant information

14.4.2 Audit and Inspection

Auditing: Definition "A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s)."

A study may be identified for audit by any method listed below:

1. A project may be identified via the risk assessment process.
2. An individual investigator or department may request an audit.
3. A project may be identified via an allegation of research misconduct or fraud or a suspected breach of regulations.
4. Projects may be selected at random. The Department of Health states that Trusts should be auditing a minimum of 10% of all research projects.
5. Projects may be randomly selected for audit by an external organisation.

Triggered audit would be conducted by PCTU QA manager if any concern arises with the study, protocol deviation or if any of the findings from monitoring remains unresolved. The documents to be verified will be randomly selected. Any major discrepancies found at a site visit would trigger a more extensive audit of trial data at the site involved. In addition, the sponsor may also carry out an audit throughout the duration of the trial

14.5 Compliance

The CI will ensure that the trial is conducted in compliance with the protocol, principles of the Declaration of Helsinki (1996), and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework (2005), Good Clinical Practice Guidelines (1996) and Trust and Research Office policies and procedures and any subsequent amendments.

14.5.1 Non-Compliance

Definition-: “A noted systematic lack of both the CI and the study staff adhering to Declaration of Helsinki (1996), applicable regulatory requirements including but not limited to the Research Governance Framework, GCP, Sponsor’s and Sponsor’s delegated representatives’ policies and procedures and any subsequent amendments, which leads to prolonged collection of deviations, breaches or suspected fraud.”

These non-compliances may be captured from a variety of different sources including monitoring visits, CRFs, communications and updates. The sponsor will maintain a log of the non-compliances to ascertain if there are any trends developing which to be escalated. The sponsor will assess the non-compliances and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the JRO will agree an appropriate action, including an on-site audit.

15. TRIAL ORGANISATION AND RESPONSIBILITIES

To ensure the smooth running of the trial and to minimise the overall procedural workload, it is proposed that each participating centre should designate individuals who would be chiefly responsible for local co-ordination of clinical and administrative aspects of the trial (see Communication Chart in Appendix 5).

All investigators are responsible for ensuring that any research they undertake follows the agreed protocol, for helping care professionals to ensure that participants receive appropriate care while involved in research, for protecting the integrity and confidentiality of clinical and other records and data generated by the research, and for reporting any failures in these respects, adverse events or suspected misconduct through the appropriate systems.

15.1 Local Coordinator at each centre

Each Centre will have a local Principal Investigator who will be responsible for the conduct of research at their centre and must sign a declaration to acknowledge these responsibilities. Close collaboration between all clinical teams is particularly important in HABSelect in order that patients for whom HABSelect is an option can be identified sufficiently early for entry. The responsibilities of the local Principal Investigator will be to ensure that all medical, nursing and midwifery staff involved in the care of miscarriages and infertility services are well informed about the study and trained in trial procedures, including obtaining informed consent and conduct of the trial according to good clinical practice. The local Principal Investigator will liaise with the Trial Coordinator on logistic and administrative matters connected with the trial.

15.2 Nursing or Midwifery Coordinator at each centre

Each participating centre should also designate one nurse or midwife as local Nursing/Midwifery Coordinator. This person would be responsible for ensuring that all eligible patients are considered for the study, that patients are provided with study information sheets, and have an opportunity to discuss the study if required. The nurse may be responsible for collecting the baseline and randomisation data and for coordinating the follow-up evaluations. Again, this person would be sent updates and newsletters, and would be invited to training and progress meetings.

15.3 The HABSelect Trial Office

The Pragmatic Clinical Trials Unit (PCTU) at Queen Mary, University of London will provide set-up and monitoring of trial conduct to PCTU SOPs and the GCP Conditions and Principles

as detailed in the UK Research Governance Framework 2005 including, randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, monitoring schedule and statistical analysis for the trial. In addition the PCTU will support main REC, Site Specific Assessment and R&D submissions and clinical set-up, ongoing management including training, monitoring reports and promotion of the trial.

The PCTU trial coordinator will be responsible for supplying investigator site files to each collaborating centre after relevant ethics committee approval and local R&D approval has been obtained.

The PCTU will be responsible for the day-to-day running of the trial including trial administration, database administrative functions, data management, safety reporting and all statistical analyses.

The PCTU develop trial monitoring plan for the trial and will assist the CI to resolve any local problems that may be encountered during the trial, and resolve any issues of non-compliance.

15.4 Trial Committees

15.4.1 Trial Steering Committee

The TSC, with an independent Chair, will provide overall supervision of the trial, in particular trial progress, adherence to protocol, participant safety and consideration of new information. It will include an Independent Chair, not less than two other independent members and a consumer representative. The Chief Investigator and other members of the TMG may attend the TSC meetings and present and report progress. The Committee will meet annually as a minimum.

The TSC provides independent supervision for the trial, providing advice to the Chief and Co-Investigators and the Sponsor on all aspects of the trial and affording protection for patients by ensuring the trial is conducted according to the MRC Guidelines for Good Clinical Practice in Clinical Trials. If the Chief and Co-Investigators are unable to resolve any concern satisfactorily, Principal Investigators, and all others associated with the study, may write through the Trial Office to the chairman of the TSC, drawing attention to any concerns they may have about the possibility of particular side-effects, or of particular categories of patient requiring special study, or about any other matters thought relevant.

Members of HABSelect TSC are listed in Section 1.8.

15.4.2 Data Monitoring and Ethics Committee

The DMEC will adopt a DAMOCLES charter to define its terms of reference and operation in relation to oversight of the HABSelect trial. They will not be asked to perform any formal interim analyses of effectiveness. They will, however, have access to unblinded treatment allocations obtained from the independent statistician at the PCTU, and for their meetings they may wish to see copies of data accrued to date, or summaries of that data by treatment group. They will also consider emerging evidence from other trials or research on PICSI compared with PVP-ICSI. They may advise the chair of the Trial Steering Committee at any time if, in their view, the trial should be stopped for ethical reasons, including concerns about patient safety. DMEC meetings will be held every 6 months, the first after recruitment has commenced.
Members of HABSelect DMEC are listed in Section 1.7.

16. PUBLICATION POLICY

The trial is registered with an authorised registry (ISRCTN99214271) according to the ICMJE Guidelines**.**

16.1 Authorship and Acknowledgement

A meeting will be held after the end of the study to allow discussion of the main results among the collaborators prior to publication. The success of the study depends entirely on the wholehearted collaboration of a large number of doctors, nurses and others. For this reason, chief credit for the main results will be given to all those who have collaborated in the study, through authorship and by contribution. Uniform requirements for authorship for manuscripts submitted to medical journals will guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- conception and design, or acquisition of data, or analysis and interpretation of data drafting the article or revising it critically for important intellectual content
- final approval of the version to be published

and that all these conditions must be met as per ICMJE guidelines (www.icmje.org).

In light of this, the Chief Investigator, Co-Applicants and senior PCTU staff will be named as authors in any publication, and an appropriate first and senior author agreed through discussion amongst the Trial Management Group (TMG) members. In addition, all collaborators will be listed as contributors for the main study publication, giving details of their roles in planning, conducting and reporting the study. The HABSelect team should be acknowledged in all publications, as should NIHR EME (as detailed in Section 16.5 below). Other key individuals will be included as authors or contributors as appropriate and at the discretion of the TMG. Any disputes relating to authorship will be resolved by the Trial Steering Committee (TSC).

The Chairs and Independent members of the TSC and Data Monitoring and Ethics Committee (DMEC) will be acknowledged, but will not qualify for full authorship, in order to maintain their independence.

Relevant NIHR Clinical Research Networks (e.g. CCRN) support should be acknowledged appropriately in trial publications.

Centres may seek permission to publish data obtained from participants in the HABSelect Trial that use trial outcome measures but do not relate to the trial randomised evaluation and hypothesis, provided they inform the TMG of their intentions prior to submission. Individual

collaborators must not publish data concerning their participants which is directly relevant to the questions posed in the study until the main results of the study have been published.

16.2 Data Sharing

Anonymous data will be made available to other researchers, for example for individual patient data meta-analysis, if the aim is to answer further resolved questions in a scientifically rigorous study design.

The TSC will agree a publication plan and must be consulted prior to release or publication of any study data.

16.3 Ancillary studies

It is requested that any proposals for formal additional studies of the effects of the trial treatments on some participants (e.g. special investigations in selected hospitals) be referred to the TMG for consideration. In general, it would be preferable for the trial to be kept as simple as possible, and add-on studies will need to be fully justified.

16.4 Processes for the Drafting, Review and Submission of Abstracts and Manuscripts

The agreed first author of abstracts is responsible for circulating these to the other members of the TMG and the Sponsor for review at least 15 days prior to the deadline for submission.

The agreed first author of manuscripts is responsible for ensuring:

- timely circulation of all drafts to all co-authors during manuscript development and prior to submission
- timely (and appropriate) circulation of reviewers' comments to all co-authors incorporation of comments into subsequent drafts
- communication with the TSC (i.e. ensuring submission is in line with TSC publication plan, and ensuring TSC receive the final draft prior to submission)

The first author is responsible for submission of the publication and must keep the TMG and all authors informed of the abstracts or manuscripts status. The TSC will be kept informed of rejections and publications as these occur. On publication, the first author should send copies of the abstract or manuscript to the TSC, the TMG, the Sponsor and to all co-authors, and ensure communication with NIHR EME programme as outlined below.

16.5 Funders' Requirements

16.5.1 NIHR-Efficacy and Mechanism Evaluation (EME) programme requirements

In accordance with the NIHR EME programme requirements, all materials to be submitted for publication (written, audio/visual and electronic) should be sent to the NIHR Coordinating Centre for EME (NCCEMEM) at the time of submission or at least 28 days before the publication date, whichever is earlier. This applies to all publications regardless of whether or not the primary results have been published.

All publications must acknowledge NIHR EME as the trial's funding source and include an appropriate disclaimer regarding expressed views and opinions (example text is provided on the EME website).

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18. APPENDICES

Appendix 1 – Information with regards to Safety Reporting in Non-CTIMP Research

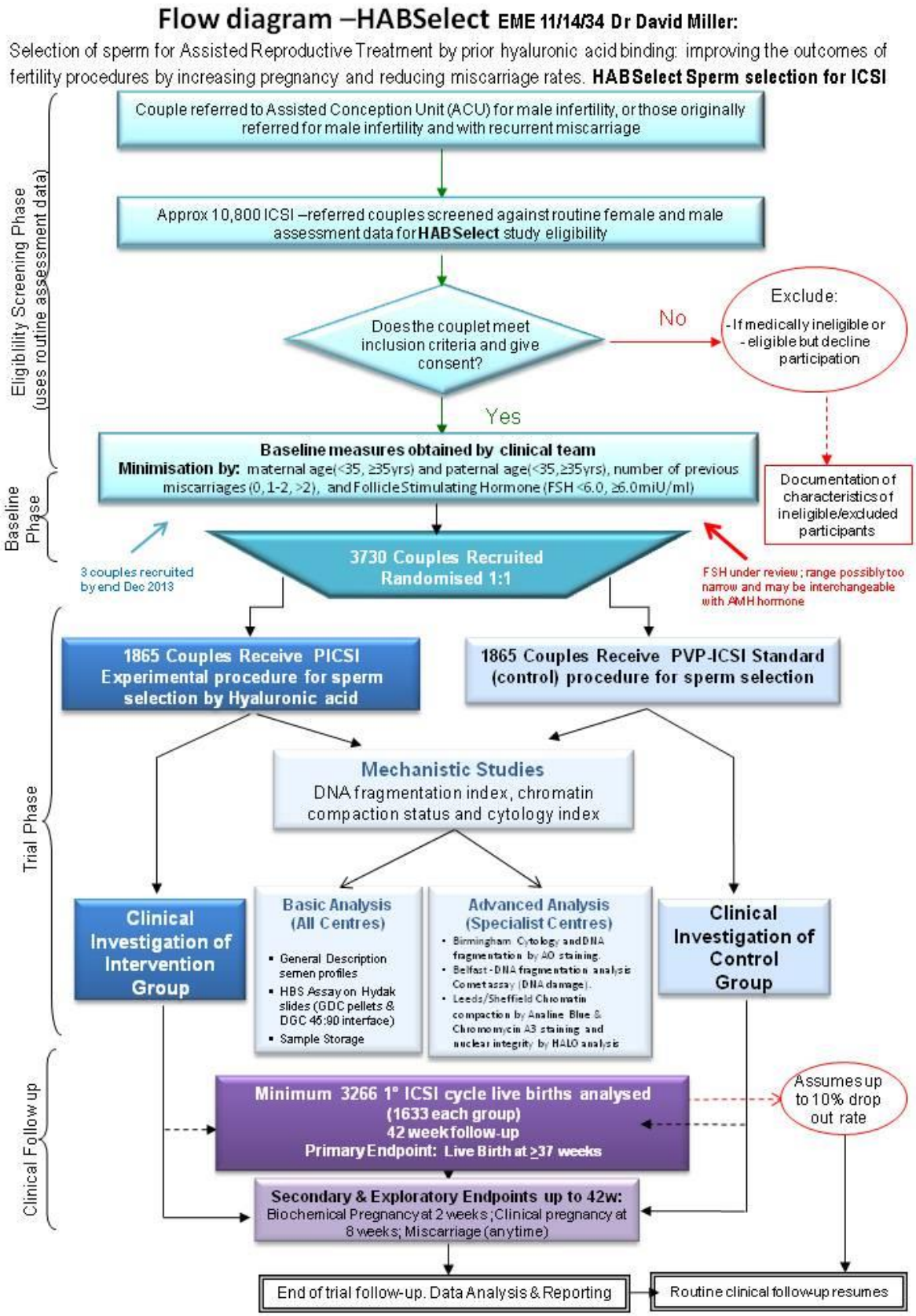
	Who	When	How	To Whom
SAE	Chief Investigator	-Report to Sponsor within 24 hours of learning of the event -Report to the MREC within 15 days of learning of the event	SAE Report form for Non-CTIMPs, available from NRES website.	Sponsor and MREC
Urgent Safety Measures	Chief Investigator	Contact the Sponsor and MREC Immediately Within 3 days	By phone Substantial amendment form giving notice in writing setting out the reasons for the urgent safety measures and the plan for future action.	Main REC and Sponsor Main REC with a copy also sent to the sponsor, and PCTU QA manager. The MREC will acknowledge this within 30 days of receipt.
Progress Reports	Chief Investigator	Annually (starting 12 months after the date of	Annual Progress Report Form (non-CTIMPs) available from	Main REC

		favourable opinion)	the NRES website	
Declaration of the conclusion or early termination of the study	Chief Investigator	Within 90 days (conclusion) Within 15 days (early termination) *The end of study should be defined in the protocol*	End of Study Declaration form available from the NRES website	Main REC with a copy to be sent to the sponsor
Summary of final Report	Chief Investigator	Within one year of conclusion of the Research	No Standard Format However, the following Information should be included:- Where the study has met its objectives, the main findings and arrangements for publication or dissemination including feedback to participants	Main REC with a copy to be sent to the sponsor

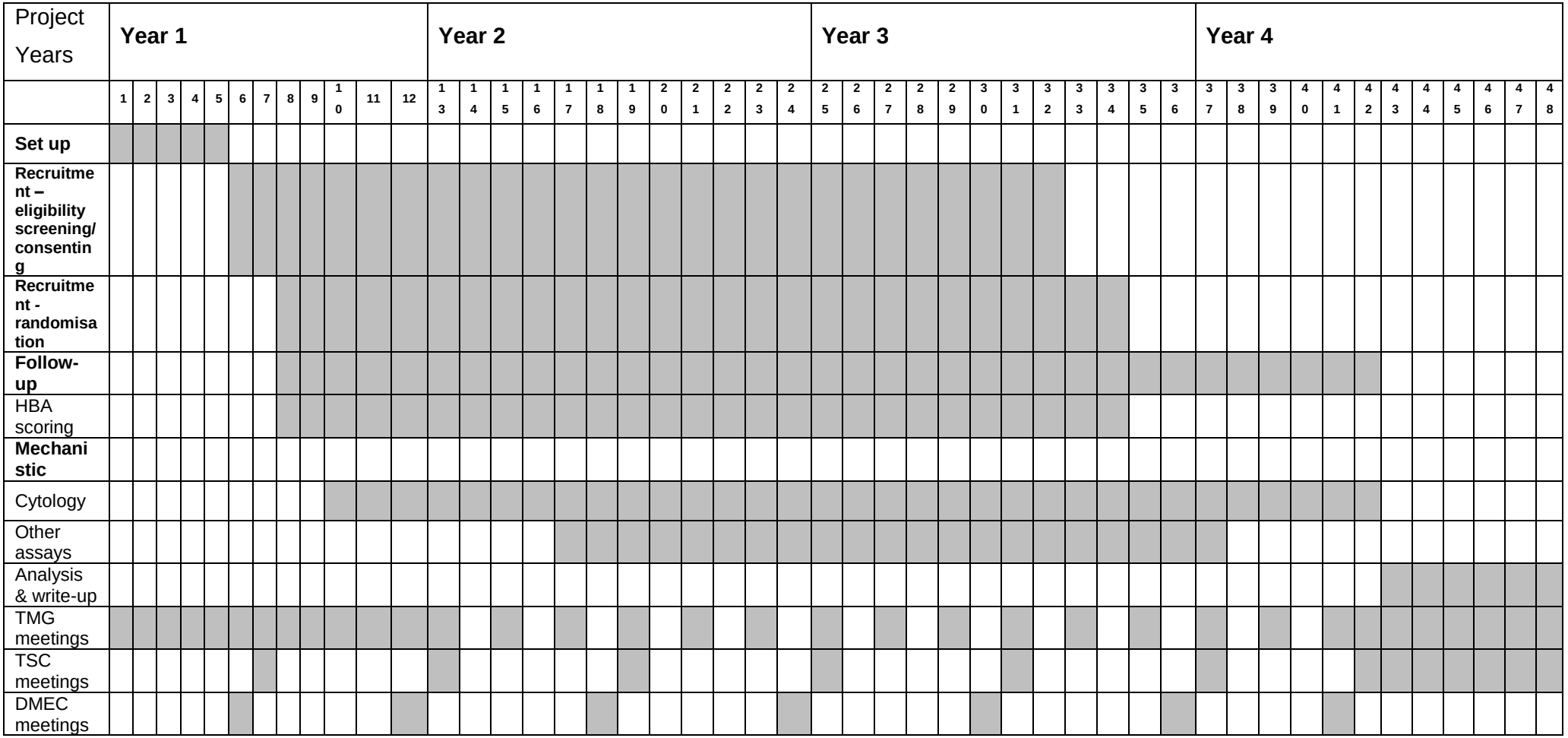
Appendix 2. Site codes

SITE NAME	SITE CODE
The Leeds Centre for Reproductive Medicine	0314
The Assisted Conception Unit, Guy's and St Thomas' NHS Foundation Trust	0102
Centre for Reproductive Medicine; Barts and The London NHS Trust	0094
The Department of Reproductive Medicine, Manchester	0067
Birmingham Women Fertility Centre	0119
Centre for Reproductive Medicine and Fertility, Sheffield	0196
Assisted Conception Unit; Ward 35; Ninewells Hospital; Dundee	0004
Aberdeen Fertility Centre	0019
Coventry Centre for Reproductive Medicine	0013
Leicester Fertility Centre	0068
Homerton Fertility Centre	0153
IVF Hammersmith	0078

Appendix 3. Study Flow Diagram

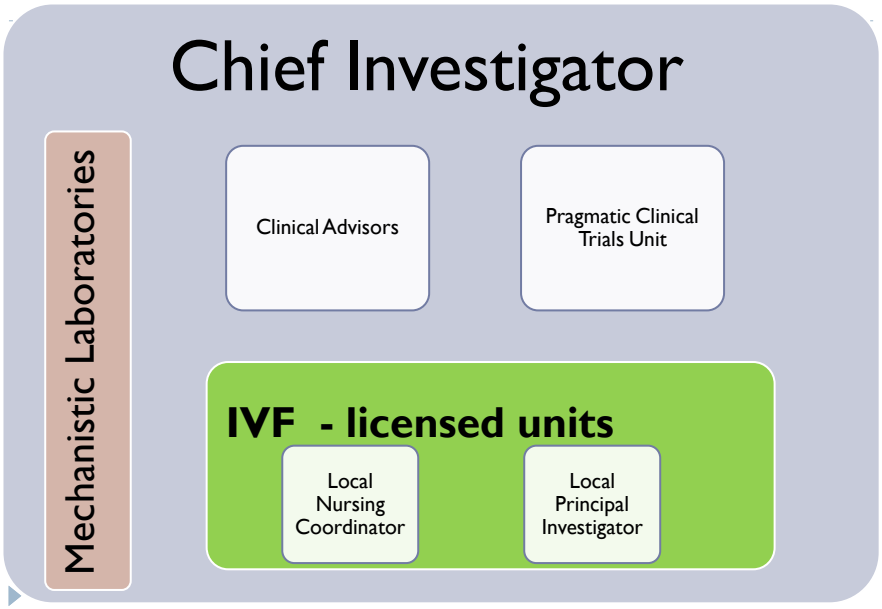


Appendix 4. GANTT chart of the project milestones



Appendix 5. HABSelect Communication Chart

HABSelect Communication Chart



Chief Investigator Agreement Page

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Chief Investigator Name: Dr David Miller

Chief Investigator Site: Leeds Institute of Genetics,
Health and Therapeutics,
University of Leeds
West Yorkshire
Leeds
LS 2 9JT

Signature and Date:

Statistician Agreement Page

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Statistician Name:

Professor Robert West[a]

Dr Richard Hooper[b]

Site:

[a] Leeds Institute of Health Sciences
University of Leeds
Room 1.2
Charles Thackrah Building
101 Clarendon Road
Leeds, LS2 9LJ

[b]Pragmatic Clinical Trials Unit
Centre for Primary Care and
Public Health, QMUL
Yvonne Carter Building
58 Turner Street
Whitechapel, E1 2AB

Signature and Date:

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Dr Yacoub Khalaf
Consultant/Senior Lecturer in Reproductive Medicine and Surgery Director

Principal Investigator Sites:
The Assisted Conception Unit & Centre for Pre-implantation Genetic Diagnosis

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Dr Vinay Sharma

Principal Investigator Site:
The Leeds Centre for Reproductive Medicine

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Dr Allan Pacey

Principal Investigator Site:
Centre for Reproductive Medicine and Fertility, Sheffield

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Dr Jackson Kirkman-Brown

Principal Investigator Site:
Birmingham Women Fertility Centre

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Professor Geraldine Hartshorne

Principal Investigator Site:
Coventry Centre for Reproductive Medicine, CV2 2DX

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Katherine Whalley

Principal Investigator Site:
Assisted Conception Unit, Ninewells Hospital, Dundee

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Bonnie Collins

Principal Investigator Site:
Centre for Reproductive Medicine, Bart's and the London

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Jane Blower

Principal Investigator Site:
Leicester Fertility Centre, LE1 5WW

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Prof Siladitya Bhattacharya

Principal Investigator Site:
Aberdeen Fertility Centre

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Prof Daniel Brison

Principal Investigator Site:
Department of Reproductive Medicine, Manchester

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Dr Srikantharajah Arasaratnam

Principal Investigator Site:
Homerton Fertility Centre

Signature and Date:

Principal Investigator Agreement Page

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Principal Investigator Name:
Dr Marta Jansa - Perez

Principal Investigator Site:
IVF Hammersmith

Signature and Date: