PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Hyaluronic Acid Binding Sperm Selection for assisted reproduction treatment (HABSelect): study protocol for a multicentre randomised controlled trial
AUTHORS	Witt, Karolina; Beresford, Lee; Bhattacharya, Siladitya; Brian, Kate; Coomarasamy, Arri; Hooper, Richard; Kirkman-Brown, Jackson; Khalaf, Yakoub; Lewis, Sheena; Pacey, Allan; Pavitt, Sue; West, Robert; Miller, David

VERSION 1 - REVIEW

REVIEWER	Kathryn C. Worrilow, Ph.D. LifeAire Systems USA
REVIEW RETURNED	30-May-2016

GENERAL COMMENTS	Please clearly describe the limitations of the protocol and study as designed. There are limitations described in various sections of the protocol but a summary would provide the reviewer and reader a more robust understanding of the strength and scope of the study.

REVIEWER	Lodovico Parmegiani GynePro Medical Centres Bologna Italy
REVIEW RETURNED	01-Jun-2016

GENERAL COMMENTS	The aim of this article by Witt and colleagues is to describe a multicenter study based on PICSI HA technology. The results that will arise from this study investigating the chromatin status of HA-selected sperm and clinical outcomes of PICSI treatments are potentially interesting. Major comments: Pag. 30 line 25-29: “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” I would suggest to add “in a selected patient population (Worrilow et al Hum Reprod 2013)”. Furthermore, also Sperm Slow which is the other commercially available HA-based selection systems can be introduced into the ART procedure with minimal training and it has been demonstrated to have the same efficiency of PICSI (Parmegiani et al, Fertil Steril 2012).

REVIEWER	Prof Dr Nikolaos P. Polyzos Center for Reproductive Medicine Universitair Ziekenhuis Brussel Vrije Universiteit Brussel BELGIUM
REVIEW RETURNED	07-Jun-2016

GENERAL COMMENTS	I have read with interest the HABSelect by Witt et al. The protocol is very robustly designed and following the GCP guidelines and should be published. I would like to highlight several issues which might help the authors to avoid criticism during the course or after the conduction of the study. 1. Why only those performing PICSI will be aware of the allocation? Would it be more correct to keep allocation concealed from all participants? Personally I believe the study would benefit more if it was blinded for all. 2. Randomisation will be performed 24h prior insemination and it is indeed stratified for several confounders by using the minimization technique. However although I saw stratification for multiple variables, I failed to find any notion of the confounder which is ovarian response (number of follicles or number of oocytes). Given that the primary outcome is live birth rates wouldn't the authors believe that the number of follicle oocytes and embryos could have an impact on the final outcome? Should such a variable be included in the stratification (I don't necessarily ask for it ,…but I would like to see their rationale) 3. Maternal and paternal age are consider 2 different variables (please clarify) 4. Ovarian reserve defined as FSH or AMH is not very clear to me. We do know that AMH and AFC are much better predictors for ovarian reserve and I would stick to these variables and not FSH. On the other hand, a serious issue is the AMH assay used (depending on the generation of the assay measurements can be 20% lower) 5. How many embryos are they allowed to transfer? Should this also be included as a stratification variable? 6. As I assume all stimulation protocols can be used. Could this also be a confounding factor needing to adjust for? A great advantage of the RCT the huge sample size of the study, able to detect an increase in LBR from 24 to 29% which is 20% relative increase Finally, possibly the authors could consider a cost effective analysis to be done based on their raw data. I understand that an increase in the LBR is the primary outcome …but for me I would like to know whether using PICSI instead of ICSI could be cost-effective. Keep in mind that all these patients will more likely have several embryos frozen and possibly they will get pregnant in the subsequent frozen embryo transfer even if they fail in the fresh cycle. Thus, to justify the use of such a method routinely, economic data would be of great valued for decision making. Overall nice design by a respectful group of investigators. Green light for publication of the protocol (from this reviewer) and i am looking forward to see the study up and running.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1. Please clearly describe the limitations of the protocol and study as designed. There are limitations described in various sections of the protocol but a summary would provide the reviewer and reader a more robust understanding of the strength and scope of the study.

We have modified Table 1 to (hopefully) provide a more inclusive and clearer view of the protocol's strengths and weaknesses.

Reviewer: 2

1. Pag. 30 line 25-29:[3]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[2]IwouldCPRsuggest to[3]inaddselected patient population (Worrilow et al Hum Reprod 2013)[2] .

This comment refers to the unedited version of the protocol (p30) as seen by the ethics review panel. This unexpurgated version was submitted at the journal's request but will not be published by the journal.

2. Furthermore, also Sperm Slow which is the other commercially available HA-based selection systems can be introduced into the ART procedure with minimal training and it has been demonstrated to have the same efficiency of PICSI (Parmegiani et al, Fertil Steril 2012).

We accept that the wording in this section of the original submission was somewhat overstated and we have modified it accordingly (see page 8, section 2.5).

Reviewer: 3

1. Why only those performing PICSI will be aware of the allocation? Would it be more correct to keep allocation concealed from all participants? Personally I believe the study would benefit more if it was blinded for all.

Although other clinical care providers and participating couples will be blinded, it is not practical to blind the embryologists who actually perform the ICSI or PICSI procedure at each site and so only they will be aware of the treatment allocation. Steps will be taken to militate against biases arising from embryologists being unblinded and we have reworded the relevant section in the Abstract and included more information in Table 1, accordingly.

2. Randomisation will be performed 24h prior insemination and it is indeed stratified for several confounders by using the minimization technique. However, although I saw stratification for multiple variables, I failed to find any notion of the confounder which is ovarian response (number of follicles or number of oocytes). Given that the primary outcome[1] istheliveauthorsbirthratesbelievewouldnthat the number of follicle oocytes and embryos could have an impact on the final outcome? Should such a variable be included in the stratification (I don[1]tecessarily ask for it, but I would like to see their rationale)

The key prognostic factors for the number of oocytes and pregnancy outcomes are female age and ovarian reserve. We are implementing a minimisation strategy to balance female age and ovarian reserve (FSH or AMH). We therefore believe a satisfactory balance can be expected between the two arms for outcomes with regard to the number of follicles and number of oocytes.

3. Maternal and paternal age are consider 2 different variables (please clarify)

These are two separate minimisation factors (though obviously they are correlated) and relate mainly to the deterioration in gamete quality (particularly in the female) with age. The wording in the relevant section has been changed to make this clearer.

4. Ovarian reserve defined as FSH or AMH is not very clear to me. We do know that AMH and AFC are much better predictors for ovarian reserve and I would stick to these variables and not FSH. On the other hand, a serious issue is the AMH assay used (depending on the generation of the assay measurements can be 20% lower).

We accept that AMH and AFC are better tests of ovarian reserve than FSH. However, HABselect is a multi-centre trial conducted across multiple sites with varying care pathways, and insisting that AMH or AFC is always used in every centre was not practical. The purpose of using AMH or FSH levels for minimisation was to try to achieve a degree of balance across the trial arms for ovarian reserve. For this purpose, we believe AMH or FSH is adequate. The wording in the relevant section has been modified to indicate that these markers are currently the most commonly used across the UK.

5. How many embryos are they allowed to transfer? Should this also be included as a stratification variable?

The decision on the number of embryos for transfer is generally made after randomisation and trial intervention. It is therefore not possible to stratify or minimise for this variable. UK regulations allow for a maximum of 3 embryo transfers (rare) and case report forms record how many embryos were transferred. However, we can include the number of embryos transferred as an adjustment factor.

6. As I assume all stimulation protocols can be used. Could this also be a confounding factor needing to adjust for?

There are many variations in the regimens for pituitary suppression and controlled ovarian stimulation, but a variation in them is not known to make a material difference in the outcomes (compared with other key prognostic factors such as a woman's age). Furthermore, given the randomised nature of the study, we would expect a balance in the pituitary suppression/ COS regimens. We do not therefore believe that there is a clear need to minimise or adjust for stimulation protocols although provisions have been made for this in the clinical statistical analysis plan (accompanying).

7. Finally, possibly the authors could consider a cost effective analysis to be done based on their raw data. I understand that an increase in the LBR is the Šbutprimary outcome for me I would like to know whether using PICSI instead of ICSI could be cost-effective. Keep in mind that all these patients will more likely have several embryos frozen and possibly they will get pregnant in the subsequent frozen embryo transfer even if they fail in the fresh cycle. Thus, to justify the use of such a method routinely, economic data would be of great valued for decision making.

As no economic data (either from an NHS or societal perspective) are being collected, a cost effectiveness analysis may be difficult. However, cost effectiveness modelling alongside an IPD meta-analysis including the Worrilow study may be possible, but the cost data would need to be generated and the time horizon of the model may need to be longer than that used for this trial i.e. beyond live birth, in order to assess neonatal and post neonatal health of offspring.

VERSION 2 – REVIEW

REVIEWER	Prof Dr Nikolaos Polyzos Universitair Ziekenhuis Brussel Vrije Universiteit Brussel BELGIUM
REVIEW RETURNED	07-Sep-2016

GENERAL COMMENTS	The authors successfully addressed all the queries. Good luck with this very interesting and clinically relevant RCT.

Correction: Hyaluronic Acid Binding Sperm Selection for assisted reproduction treatment (HABSelect): study protocol for a multicentre randomised controlled trial

Witt KD, Beresford L, Bhattacharya S, *et al*. Hyaluronic Acid Binding Sperm Selection for assisted reproduction treatment (HABSelect): study protocol for a multicentre randomised controlled trial. *BMJ Open* 2016;**6:**e012609. doi: 10.1136/bmjopen-2016-012609

An additional author should appear in the article: Rachel Cutting. Her correspondence details are "Assisted Conception Unit, Jessop Wing, Royal Hallamshire Hospital, Tree Root Walk, Sheffield, S10 2SF, UK; rachel.cutting@sth.nhs.uk".

The updated Contributors statement should read:

Contributors Witt and Miller designed and wrote the protocol. Pavitt provided expert assistance on trial design and management. Kirkman-Brown, Lewis and Pacey provided expert assistance on the application of laboratory methods. Hooper and West provided essential statistical support on clinical and mechanistic aspects of the study, respectively. Cutting provided essential support with the clinical embryology embedded in the protocol. Khalaf, Coomarasamy and Bhattacharaya provided expert clinical support and checked the protocol for accuracy. Beresford designed the clinical Statistical Analysis Plan. Kate Brian is our Patient & Public Involvement Contributor.

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