

Sperm selection for assisted reproduction by prior hyaluronan binding: the HABSelect RCT

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Scientific summary

The HABSelect RCT

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Scientific summary

Introduction and background

The male contribution to human infertility is not fully understood and estimates of its prevalence vary. However, concern over the recent decline in sperm counts at least in the developed world, alongside the increasing age at which couples come forward for treatment, has led to calls for improvements in the care of the male partner. Such improvements include a better understanding of the causes of male infertility and how best to ameliorate the condition sufficiently to boost treatment success rates (hitherto focused mainly on boosting female fertility). With the advent, rapid uptake and expansion of interventional intracytoplasmic sperm injection (ICSI) treatment, the practitioner has only one chance per egg to pick the right sperm with the greatest potential for live birth for injection and methods aimed at increasing the likelihood of doing so are in development. One such method involves the selection of sperm based on their innate ability to bind hyaluronan (HA), which occurs naturally in the cumulus–oophorous complex. Such sperm appear to have better indicators of genomic integrity, including lower levels of deoxyribonucleic acid (DNA) fragmentation, chromosomal aneuploidy and cytoplasmic retention and, hence, increased maturity relating to these measures. A number of clinical trials have tested the claim that ICSI with HA-selected sperm improves clinical outcomes, but with the exception of a reduction in miscarriage following the use of selected sperm, results for other outcomes, including live births, have remained equivocal at best. Hyaluronic Acid Binding sperm selection (HABSelect) was designed to detect a minimum 5% difference (per cycle started) in full-term live birth outcomes, which was also sufficient to detect significant changes in other (secondary) outcomes including miscarriage rates.

Hypotheses

1. By selecting sperm able to bind to HA [physiological intracytoplasmic sperm injection (PICSI)] live birth rates (LBRs) would be increased.
2. Any observed improvement in outcomes would be attributable to sperm DNA integrity and chromatin structure.

Objectives

The main clinical objectives of HABSelect were to determine if sperm selected for ICSI by HA binding could increase full-term LBRs per fresh treatment cycle. Secondary objectives were to detect a reduction in miscarriage rates and associated improvements in clinical pregnancy (CP) and preterm LBRs. The main mechanistic objective was to relate clinical outcomes to aspects of sperm DNA integrity, including DNA fragmentation and compaction.

Methods

HABSelect was a parallel-arm, randomised clinical trial with associated laboratory-based studies investigating sperm DNA integrity (fragmentation and compaction). The intervention was based on sperm binding to the HA substrate in the Conformité Européenne (CE) and UK Medicines and Healthcare products Regulatory Agency-approved PICSI™ dish (Origio, Måløv, Denmark). This substrate binds and immobilises sperm for ICSI. The study was as inclusive as possible with regard to both partners' eligibility to participate. Approximately 6700 couples were assessed for eligibility and 2772 were randomised into either the selection (PICSI, $n = 1387$) or the control (standard ICSI, $n = 1385$) arm of the trial, although six couples were excluded post randomisation

as they were subsequently found not to have met eligibility criteria. Following post-randomisation withdrawals, the number of couples included in the primary analysis was 2752. Following treatment, residual sperm samples were frozen and stored for retrospective analysis of DNA integrity using a number of complementary assays measuring variables for DNA fragmentation [acridine orange, comet and terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL)] and compaction [aniline blue (AB)] with halo assays linking both variables. Participants, clinical care providers in in vitro fertilisation (IVF) licensed units, maternity and neonatal wards, and research nurses responsible for participants' follow-up were blinded to treatment allocation. The only unblinded group at study sites were the embryologists who performed the PICSI/standard ICSI procedure, HA binding scoring and randomisation. The study data manager and independent statistician, both residing within the trials' unit, were also unblinded and helped prepare reports for the Data Monitoring and Ethics Committee. When it became clear part-way through that the laboratory effort would be unable to process all samples, the mechanistic statistician was unblinded to provide a sample set enriched for miscarriage.

Results

Approximately 6700 couples were assessed for eligibility and 2772 were finally randomised into either the selection (PICSI) or control (standard ICSI) arms of the trial. Outcome data were available for 2752 couples. For the primary outcome, 379 out of 1381 (PICSI 27.4%) and 346 out of 1371 (ICSI 25.2%) eligible couples randomised achieved a full-term live birth (≥ 37 weeks). This corresponds to an odds ratio for all treatment cycles of 1.12 [95% confidence interval (CI) 0.95 to 1.34], which was not statistically significant ($p = 0.18$). Of the secondary outcomes, miscarriage rates per couple treated were significantly reduced in the PICSI arm, with 60 out of 1381 (4.3%) clinical pregnancies lost per couple treated, compared with ICSI at 96 out of 1371 (7.0%), corresponding to an odds ratio for all treatment cycles of 0.61 (95% CI 0.43 to 0.84; $p = 0.003$). Clinical pregnancy rates (CPRs) per couple treated were not significantly different and subgroup analyses of both primary and miscarriage outcomes across hyaluronan binding score (HBS), female age, anti-Müllerian hormone or follicle stimulating hormone subgroups did not find a difference in treatment effect. DNA fragmentation in the sperm prepared for ICSI/PICSI was not discriminatory of clinical outcomes, although DNA compaction may have influenced establishment of CP. The mechanistic analysis, which explored the relationship between mechanistic and clinical data for the purposes of hypothesis generation, found statistically significant relationships between HBS, sperm motility, sperm concentration and sperm DNA integrity. However, with the exception of establishment of CP, which was related to sperm DNA compaction (AB staining), no other measure of sperm DNA integrity predicted or was associated with a clinical outcome, including miscarriage. Assays of DNA integrity also correlated poorly with each other. Classification tree and linear regression highlighted female age and male HBS as most predictive of clinical outcome, with PICSI showing some benefit for older women. The PICSI intervention led to a drop in fertilisation rates, although this did not affect subsequent CPRs.

Limitations

Use of processed sperm samples rather than original semen for DNA integrity assays was unavoidable but also uninformative, and mechanistic analysis depended on the randomness of missing data.

Conclusions

The PICSI-based sperm selection showed no advantage for raising CP or LBRs in couples undergoing ICSI. The intervention, however, afforded some protection against miscarriage. The mechanistic analysis suggested that this effect was more related to female age than to sperm DNA integrity, although the processing and quality of the sperm sampled for the mechanistic work might have reduced the sensitivity of our analysis, which is still ongoing. Data from existing and future trials of PICSI should be combined with HABSelect to confirm and

provide a more precise assessment of the efficacy of PICS1 at reducing miscarriage risk and determine whether or not reductions in the number of miscarriages can lead to a corresponding increase in LBRs.

Trial registration

This trial is registered as ISRCTN99214271.

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