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Advanced sperm selection techniques for assisted reproduction — Source link

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[Intervention Review]

Advanced sperm selection techniques for assisted reproduction

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ABSTRACT

Background

Assisted reproductive technologies (ART) such as in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) bring together gametes outside of the body to enhance the probability of fertilisation and pregnancy. Advanced sperm selection techniques are increasingly being employed in ART, most commonly in cycles utilising ICSI. Advanced sperm selection techniques are thought to improve the chance that structurally intact and mature sperm with high DNA integrity are selected for fertilisation. Advanced sperm selection strategies include selection according to surface charge; sperm apoptosis; sperm birefringence; ability to bind to hyaluronic acid; and sperm morphology under ultra-high magnification. These techniques theoretically improve ART outcomes.

Objectives

To evaluate the impact of advanced sperm selection techniques on ART outcomes.

Search methods

Systematic search of electronic databases (Cochrane Menstrual Disorders and Subfertility Group Specialised Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, PsycINFO, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Latin American and Caribbean Health Science Information Database (LILACS)), trials registers (ClinicalTrials.gov, Current Controlled Trials, World Health Organization International Clinical Trials Registry Platform), conference abstracts (Web of Knowledge) and grey literature (OpenGrey) for relevant randomised controlled trials. We handsearched the reference lists of included studies and similar reviews. The search was conducted in May 2014.

Selection criteria

We included randomised controlled trials (RCTs) comparing an advanced sperm selection technique versus standard IVF or ICSI or versus another advanced sperm selection technique. We excluded studies of sperm selection using ultra-high magnification (intracytoplasmic morphologically selected sperm injection, or IMSI), as they are the subject of a separate Cochrane review. Quasi-randomised and pseudo-randomised trials were excluded. Our primary outcome measure was live birth rate per woman randomly assigned. Secondary outcome measures included clinical pregnancy per woman randomly assigned, miscarriage per clinical pregnancy and fetal abnormality per clinical pregnancy.

Data collection and analysis

Two review authors independently assessed eligibility of studies and risk of bias, and performed data extraction. Disagreements were resolved by consultation with a third review author. Study investigators were consulted to resolve other queries that arose. Risk ratios (RRs) were calculated with 95% confidence intervals (CIs). We planned to combine studies using a fixed-effect model, if sufficient data were available. The quality of the evidence was evaluated using Grades of Recommendation, Assessment, Development and Evaluation (GRADE) methods.

Main results

Two RCTs were included in the review. Both evaluated sperm selection by hyaluronanic acid binding for ICSI, but only one reported live births. No studies were identified that were related to surface charge selection, sperm apoptosis or sperm birefringence.

One RCT compared hyaluronanic acid binding versus conventional ICSI. Live birth was not reported. Evidence was insufficient to show whether there was a difference between groups in clinical pregnancy rates (RR 1.01, 95% CI 0.84 to 1.22, one RCT, 482 women). This evidence was deemed to be of low quality, mainly as the result of poor reporting of methods and findings. Miscarriage data were unclear, and fetal abnormality rates were not reported.

The other RCT compared two different hyaluronanic acid binding techniques, SpermSlow and physiological intracytoplasmic sperm injection (PISCI). Evidence was insufficient to indicate whether there was a difference between groups in rates of live birth (RR 1.16, 95% CI 0.65 to 2.05, one RCT, 99 women), clinical pregnancy (RR 1.07, 95% CI 0.67 to 1.71, one RCT, 99 women) or miscarriage (RR 0.76, 95% CI 0.24 to 2.44, one RCT, 41 women). The evidence for these comparisons was deemed to be of low quality, as it was limited by imprecision and poor reporting of study methods. Fetal abnormality rates were not reported.

Authors' conclusions

Evidence was insufficient to allow review authors to determine whether sperm selected by hyaluronanic acid binding improve live birth or pregnancy outcomes in ART, and no clear data on adverse effects were available. Evidence was also insufficient to show whether there is a difference in efficacy between the hyaluronic acid binding methods SpermSlow and PICSI. No randomised evidence evaluating sperm selection by sperm apoptosis, sperm birefringence or surface charge was found.

Further studies of suitable quality are required to evaluate whether any of these advanced sperm selection techniques can be recommended for use in clinical practice.

PLAIN LANGUAGE SUMMARY

Advanced sperm selection techniques for assisted reproduction

Review question

Cochrane review authors reviewed the evidence on advanced sperm selection techniques for assisted reproduction. We sought studies of all advanced sperm techniques apart from ultra-high magnification, which is the subject of a separate Cochrane review. Our outcomes of interest included live birth, clinical pregnancy, miscarriage and fetal abnormalities.

Background

In vitro fertilisation (IVF) with or without intracytoplasmic sperm injection (ICSI) is a commonly used treatment for subfertile couples. It is thought that selection of high-quality sperm may improve live birth rates for these couples. Advanced sperm selection techniques use complex methods to select healthy, mature and structurally sound sperm for fertilisation. Despite use of these techniques in many centres worldwide, their effectiveness is unclear.

Study characteristics

Two randomised controlled trials (with a total of 581 women) were included in the review. Both studies evaluated sperm selection by hyaluronanic acid binding for ICSI, but only one reported live birth. One study compared hyaluronanic acid binding versus conventional ICSI and reported clinical pregnancy. The other study compared two different hyaluronanic acid binding techniques, SpermSlow and physiological intracytoplasmic sperm injection (PICSI), and reported live birth, clinical pregnancy and miscarriage rates. No studies were found on other techniques. Evidence is current to May 2014.

Key results

Current evidence was insufficient to permit evaluation of the effectiveness of advanced sperm selection strategies in assisted reproductive technologist (ART). No evidence showed a difference between the groups in terms of any of the reported outcomes. Further studies of suitable quality are required before any of these advanced sperm selection techniques can be recommended for use in clinical practice.

Evidence quality

The evidence gathered was of low quality. The main limitations were imprecision, discrepancies in the data and poor reporting of study methods. Data on important clinical outcomes such as live births and adverse effects were scant.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Conventional sperm selection (ICSI) versus hyaluronan selected sperm (HA-ICSI) for assisted reproduction

Population: women with infertility requiring assisted reproductive technology
Settings: ART
Intervention: hyaluronan sperm selection (HA-ICSI)
Comparison: conventional sperm selection (ICSI)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Conventional sperm selection (ICSI)	Hyaluronan sperm selection (HA-ICSI)				
Clinical pregnancy per woman randomly assigned	**47 per 100**	**48 per 100** (39-57)	**RR 0.99** (0.82-1.20)	482 (1 study)	⊕⊕◯◯ **low**[a,b]	

*The basis for the **assumed risk** is the median control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
ART: assisted reproductive technologies; **CI:** confidence interval; **OR:** odds ratio.

GRADE Working Group grades of evidence.
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[a] Serious risk of bias: discrepancy in reporting of pregnancy losses.
[b] Serious imprecision: confidence intervals compatible with substantial benefit or harm from the intervention, or with no effect.

BACKGROUND

Description of the condition

In vitro fertilisation (IVF) is a form of assisted reproductive technology (ART) that is used for treating infertility-a condition affecting an estimated 15% of the population. IVF usually involves controlled ovarian hyperstimulation, surgical oocyte retrieval, in vitro fertilisation and embryo transfer. Intracytoplasmic sperm injection (ICSI) involves injecting a single sperm into the cytoplasm of each oocyte to achieve fertilisation. ICSI is commonly used as treatment for male factor infertility when semen parameters are poor, when sperm have been surgically retrieved or when repeated fertilisation with standard IVF has failed (Palermo 1992).

Successful embryo development and subsequent pregnancy outcome are likely to be impacted by the quality of the sperm that fertilise an oocyte (Sakkas 2000). Ideally only sperm with a high chance of successful fertilisation and subsequent embryo growth would be used for ART. These sperm would be viable and mature, would have high DNA integrity and would be structurally sound. Sperm preparation and selection in IVF are limited to semen washing, density gradient centrifugation and use of swim-up techniques (Boomsma 2007). In ICSI, routine sperm selection is based on motility and gross morphology (sperm are examined under a microscope at 200× to 400× magnification) after one or more of the above methods of semen preparation has been applied. Advanced sperm selection techniques based on alternative characteristics might enable further selection of the most appropriate sperm for use in ART.

Description of the intervention

Advanced sperm selection techniques have developed as a means of improving ART outcomes in certain clinical scenarios. Techniques can be categorised as follows.

Surface charge selection

Electrophorectic sperm selection and sperm zeta potential are surface charge selection protocols utilised in both IVF and ICSI. The zeta potential of the sperm is the electrical potential between the sperm membrane and its surroundings. The zeta potential decreases with capacitation, and normally differentiated sperm are charged electronegatively. Semen is placed into an electrophoretic device and a current applied. Normally differentiated negatively charged sperm are rapidly separated and collected from an adjacent chamber (Ainsworth 2005).

Sperm apoptosis

Selection of non-apoptotic sperm for use in ART is based on the presence of phosphatidylserine on the external surface of the sperm membrane in the early stages of apoptosis. Magnetic activated cell sorting (MACS) and glass wool separation columns utilise the magnetic properties of phosphatidylserine to separate apoptotic sperm from non-apoptotic sperm (Grunewald 2001).

Hyaluronic acid binding

Hyaluronic acid (HA) is the main component of the extracellular matrix of the cumulus oophorus. Hyaluronic acid binding sites on the sperm plasma membrane indicate sperm maturity. Mature sperm bind to and digest HA and thus have a better chance of reaching the oocyte for fertilisation. In vitro, HA is utilised as a 'physiological selector' of mature intact sperm.

Two systems for HA sperm selection are currently available. Physiological intracytoplasmic sperm injection (PICSI; Origio, Måløv, Denmark) is a plastic culture dish with spots of HA attached to its base. Sperm are bound by the head to HA and are selected for microinjection (Huszar 2007). SpermSlow is a viscous medium containing HA. Appropriate sperm appear 'slowed' and are selected.

Sperm birefringence

The mature sperm nucleus has high intrinsic birefringence due to longitudinally orientated subacrosomal protein filaments. With the use of polarised light microscopy, sperm birefringence can be evaluated and a mature sperm selected (Gianaroli 2008).

Sperm morphology (intracytoplasmic morphologically selected sperm injection, IMSI)

Subtle defects in sperm morphology (acrosome, nucleus, mitochondria, tail, postacrosoma lamina and neck) can be observed using ultra-high magnification (6000×) microscopy (motile sperm organelle morphology examination, MSOME) (Bartoov 2002). Intracytoplasmic morphologically selected sperm injection (IMSI) is a modification of ICSI that uses this technique (Bartoov 2003). This review does not evaluate IMSI, as it is the subject of another Cochrane review (Teixeira 2013).

How the intervention might work

Each sperm selection modality utilises different characteristics of sperm structure, physiology or function to promote selection of the most normal sperm. Selection of the most appropriate sperm for fertilisation in vitro may help improve fertilisation and the quality of embryos created. Advanced sperm selection protocols aim to improve ART outcomes and may limit possible deleterious effects on offspring of using sperm with defective DNA (Aitken 2007).

Why it is important to do this review

Advanced sperm selection techniques are hypothesised to improve ART outcome through the selection of sperm with a variety of 'beneficial characteristics.' Although individual small studies have suggested that these techniques have clinical benefit, there remains no comprehensive review of randomised controlled trials (RCTs) in this area. The current review includes only RCTs, so the results can better guide clinical practice and further research efforts.

OBJECTIVES

To evaluate the impact of advanced sperm selection techniques on ART outcomes.

METHODS

Criteria for considering studies for this review

Types of studies

Published and unpublished RCTs investigating the impact of advanced sperm selection techniques in ART were eligible for inclusion. Non-randomised studies were excluded because of high risk of bias. Cross-over studies are inappropriate in this context and were excluded.

Types of participants

Women or couples undergoing ART.

Types of interventions

Trials comparing an advanced sperm selection technique with either another advanced sperm selection technique or an advanced sperm selection technique with standard sperm preparation techniques (e.g. semen washing, density gradient centrifugation, swim-up techniques).
Advanced sperm selection techniques include the following.

- Surface charge selection.
- Sperm apoptosis.
- Hyaluronic acid binding.
- Sperm birefringence.

Sperm selection by sperm morphology using ultra-high magnification (IMSI) was excluded, as this is the subject of another Cochrane review (Teixeira 2013).

Types of outcome measures

Primary outcomes

Effectiveness

- Live birth per woman randomly assigned.

(Live birth is defined as the delivery of a live fetus beyond 20 completed weeks' gestation.)

Secondary outcomes

Effectiveness

- Clinical pregnancy per woman randomly assigned.

(Clinical pregnancy is defined as identification of a gestational sac on ultrasound at ≥ seven weeks' gestation.)

Adverse events

- Miscarriage, fetal abnormalities per clinical pregnancy.

(Miscarriage is defined as pregnancy loss at < 20 completed weeks' gestation, or when the fetus weighs < 500 grams. Miscarriage must be confirmed by ultrasound and pregnancy test or histology and includes partial loss of multiple pregnancies.)
Fertilisation rates, implantation rates and outcomes related to embryo development and quality are of importance to this review and are described under Characteristics of included studies. These outcomes were not included in the meta-analysis because standardised grading systems for morphology are lacking, and denominators for fertilisation and implantation rates differ.

Search methods for identification of studies

We searched for all published and unpublished RCTs using the search terms 'IVF,' 'ICSI,' 'ART,' 'sperm selection,' 'sperm preparation,' 'sperm parameter,' 'Hyaluronic acid,' 'PICSI,' 'apoptosis,' 'DNA,' 'membrane maturity,' 'magnetic cell sorting,' 'morphology,' 'intracytoplasmic morphologically selected sperm injection,' 'polarization microscopy,' 'polscope' and 'sperm birefringence.'
The search included no language restriction and was designed and conducted by SM and BK, in consultation with the Menstrual Disorders and Subfertility Group (MDSG) Trials Search Co-ordinator. The search was conducted in May 2014.

Electronic searches

We searched the following electronic databases, trial registers and websites.

- Menstrual Disorders and Subfertility Group (MDSG) Specialised Register
- Cochrane Central Register of Controlled Trials (CENTRAL)
- MEDLINE
- EMBASE
- PsycINFO
- Cumulative Index to Nursing and Allied Health Literature (CINAHL)

Other electronic sources of trials included the following.

- Trial registers for ongoing and registered trials: http://www.controlled-trials.com, http://clinicaltrials.gov/ct2/home, http://www.who.int/trialsearch/Default.aspx.
- Citation indexes: http://scientific.thomson.com/products/sci/.
- Conference abstracts in the Web of Knowledge: http://wokinfo.com/
- Latin American and Caribbean Health Science Information Database (LILACS), for trials from the Portuguese and Spanish-speaking world: http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&base=LILACS&lang=i&form=F.
- PubMed: http://www.ncbi.nlm.nih.gov/pubmed/.
- Open System for Information on Grey Literature in Europe (OpenSIGLE) database (http://opensigle.inist.fr/) and Google Scholar for grey literature.

Searching other resources

We searched the reference lists of articles retrieved by the search.

Data collection and analysis

Selection of studies

After an initial screen of titles and abstracts retrieved by the search, we retrieved the full texts of all potentially eligible studies. Two review authors (SM and BK) independently examined these full-text articles for compliance with the inclusion criteria and selected studies eligible for inclusion in the review. We corresponded with study investigators as required to clarify study eligibility. Disagreements as to study eligibility were resolved by discussion or by discussion with a third review author (AY). We documented the selection process by using a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart.

Data extraction and management

Two review authors independently extracted data from eligible studies. Disagreements were resolved by discussion or by consultation with a third review author. Data extracted included study characteristics and outcome data and details of methods, participants, setting, context, interventions (sperm selection protocols), outcomes, results and publications. We attempted to contact study investigators via email to obtain additional information. No replies were received from any of the study authors.

Assessment of risk of bias in included studies

Two review authors (SM and BK) independently assessed the included studies for risk of bias using the Risk of bias assessment tool of The Cochrane Collaboration (Higgins 2011). This instrument assessed random sequence generation and allocation concealment (selection bias); blinding of participants and personnel; blinding of outcome assessors; incomplete outcome data; selective reporting; and other bias. Disagreements were resolved by discussion or by consultation with a third review author (AY). We have fully described all judgements and have presented our conclusions in the Risk of bias table, which we planned to incorporate into the interpretation of review findings by means of sensitivity analyses. We took care to search for within-trial selective reporting, such as trials failing to report obvious outcomes, or trials reporting outcomes in insufficient detail to allow inclusion. We sought published protocols and compared outcomes between the protocol and the final published study.

Measures of treatment effect

The data extracted were dichotomous (e.g. live birth rate, miscarriage rate). Using RevMan software (RevMan 2011), we entered the numbers of events in control and intervention groups of each study to calculate Mantel-Haenszel risk ratios (RRs). We presented 95% confidence intervals (CIs) for all outcomes.

Unit of analysis issues

The primary analysis was performed per woman randomly assigned. Per-pregnancy data were included for some miscarriage outcomes. We briefly summarised data that did not allow valid analysis (e.g. 'per cycle' data), but did not meta-analyse these data. If studies reported only 'per-cycle' data, we attempted to contact the study authors to obtain 'per-woman randomised' data.
We counted multiple live births (e.g. twins, triplets) as a single live birth event.

Dealing with missing data

We analysed the data on an intention-to-treat basis as far as possible and made attempts to obtain missing data from the original trialists. When the data could not be obtained, we assumed that

the outcome measure (e.g. live birth, clinical pregnancy) did not occur. For other outcomes, we analysed available data. We planned to subject any imputation undertaken to sensitivity analysis (see below).

Assessment of heterogeneity

We considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. We planned to assess statistical heterogeneity using the I^2 statistic, with an I^2 measurement greater than 50% indicating moderate heterogeneity (Higgins 2003; Higgins 2008). Substantial heterogeneity was deemed to be greater than 60%. If substantial heterogeneity was apparent, we planned to explore possible explanations using a sensitivity analysis (see below) and to consider subgroup analyses. We planned to take any statistical heterogeneity into account when interpreting the results, especially if any variation in the direction of effect was noted.

Assessment of reporting biases

Reporting bias was minimised by ensuring a comprehensive search for eligible studies. If 10 or more studies were included in an analysis, we planned to use a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies).

Data synthesis

If the studies were sufficiently similar, we planned to combine data using a fixed-effect model for the following comparisons.

- IVF versus advanced sperm selection technique, stratified by individual sperm selection technique (refer to Description of the intervention above for details).
- ICSI versus advanced sperm selection technique, stratified by individual sperm selection technique.
- Advanced sperm selection technique versus another advanced sperm selection technique.

We graphically displayed an increase in the risk of a particular outcome, which may be beneficial (e.g. live birth) or detrimental (e.g. adverse effects), in the meta-analysis to the right of the centre line and a decrease in the risk of a particular outcome to the left of the centre line.

We planned to calculate number needed to treat for an additional beneficial outcome (NNTB), if significant findings were identified.

Subgroup analysis and investigation of heterogeneity

If sufficient data were available, we planned to conduct subgroup analyses to identify separate evidence within the following subgroups.

- Sperm morphology: when the Kruger score is equal to or less than 4%.
- Increased DNA fragmentation index (according to the study cut-off).
- Surgically retrieved sperm.
- Female participants over 38 years of age.

Sensitivity analysis

We planned to conduct sensitivity analyses for primary outcome measures to determine whether the conclusions were robust to arbitrary decisions made regarding eligibility and analysis. These analyses would include consideration of whether the review conclusions would have differed if:

- eligibility were restricted to studies without high risk of bias;
- a random-effects model had been adopted; or
- alternative imputation strategies had been implemented.

Overall quality of the body of evidence: Summary of findings table

We generated a Summary of findings table using GRADEpro software. This table evaluated the overall quality of the body of evidence for the main review outcomes, using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness and publication bias). We justified, documented and incorporated into the reporting of results for each outcome our judgements about the quality of evidence (high, moderate or low).

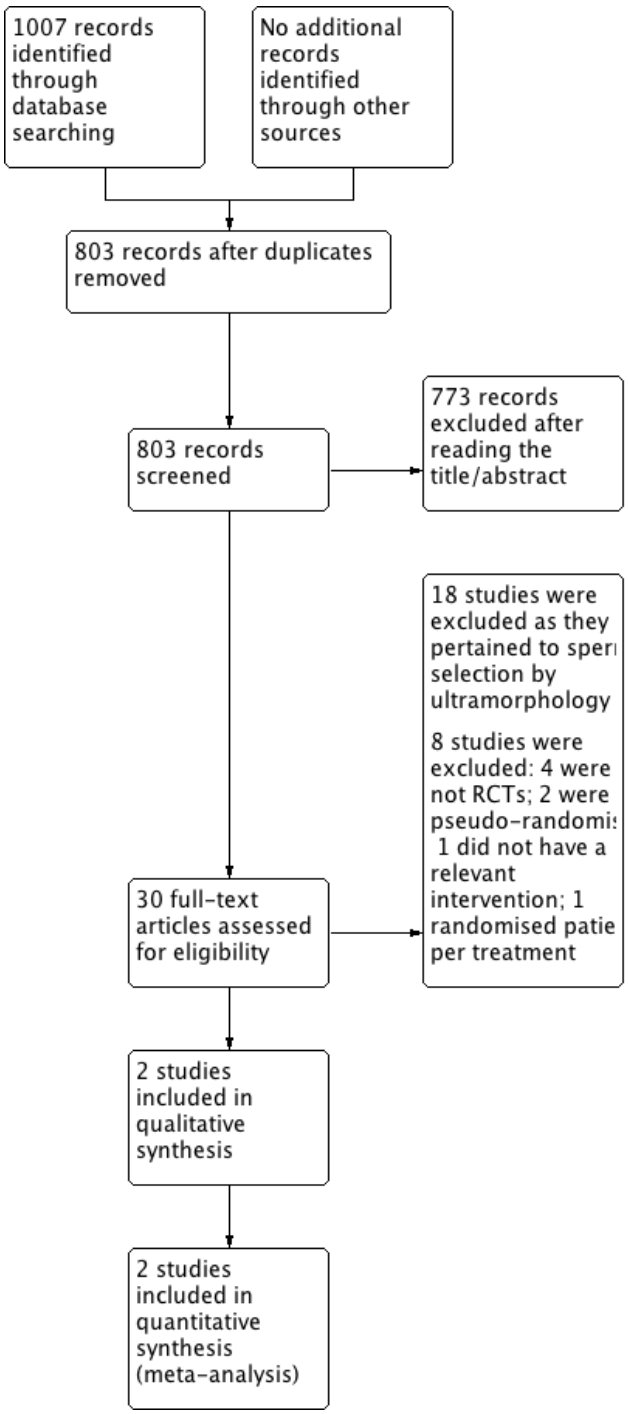
RESULTS

Description of studies

Results of the search

The search strategy identified 1007 studies. Thirty studies were potentially eligible and were retrieved in full text. Following publication of our protocol, a Cochrane review was published regarding sperm selection by sperm morphology under ultra-high magnification (Teixeira 2013). After discussion with the Cochrane Menstrual Disorders and Subfertility Group, the scope of our review was amended to exclude the use of ultra-high magnification for sperm selection. Nine more studies were therefore excluded. Two studies met our inclusion criteria (Parmegiani 2012; Worrilow 2013). Eight studies were excluded (see PRISMA flow chart; Figure 1). We identified no suitable studies regarding sperm selection by apoptosis, birefringence or surface charge. The remainder of the meta-analysis therefore pertains to hyaluronic acid binding only. See study tables Characteristics of included studies and Characteristics of excluded studies.

Figure 1. Study flow diagram.



Included studies

Study design and setting

Two parallel-design RCTs were included in the review (Parmegiani 2012; Worrilow 2013). One was a single-centre study conducted in Italy, and the other was a multi-centre study, performed at 10 IVF programs in the USA. Two further conference abstracts were identified that contained data from the above two trials; these have been listed as secondary references.

Participants

- The first study (Parmegiani 2012) included 49 women in the PICSI group and 50 in the SpermSlow group. No study arm received standard ICSI only. Couples were included if the woman was ≤ 41 years of age, ICSI treatment was to be utilised, total sperm number was ≥ 1 million and sperm motility was ≥ 5%. Couples using sperm collected surgically or with severe oligoasthenoteratozoospermia were excluded.
- The second study (Worrilow 2013) included 240 women in the intervention group (PICSI) and 242 in the control group (standard ICSI). Couples were included if they were receiving ICSI as part of their ART treatment. Participants were excluded if the woman was > 40 years old, or if testicular sperm was used. Participants were divided into cohorts on the basis of the proportion of sperm bound to hyaluronan in the unprocessed sample. Participants were further excluded if HB binding was < 2%. Participants were divided into those with hyaluronan-bound sperm between 2% and 65% or > 65%, and then were further divided into study groups (intervention or control).

Interventions

- 1/2 studies compared PICSI versus SpermSlow.
- 1/2 studies compared PICSI versus standard ICSI.

Outcomes

- 1/2 studies reported live birth.
- 1/2 studies reported clinical pregnancy rate.
- 1/2 studies reported miscarriage rate.

Excluded studies

Eight studies were excluded from the review for the following reasons.

- 4/8 were not RCTs (Berkovitz 2006; Fleming 2007; Gnosh 2007; Parmegiani 2010b).
- 2/8 were pseudo-randomised (Gianaroli 2008; Gianaroli 2010).
- 1/8 did not analyse a relevant intervention (Blanchard 2010).
- 1/8 analysed participants per treatment randomly assigned (Parmegiani 2010a), and despite attempts to contact the study investigators, we were unable to obtain "per-woman" data.

Nine studies pertaining to IMSI were excluded, as this intervention is the subject of a separate Cochrane review (Teixeira 2013).

Risk of bias in included studies

See Characteristics of included studies; Figure 2; and Figure 3 for detailed information.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

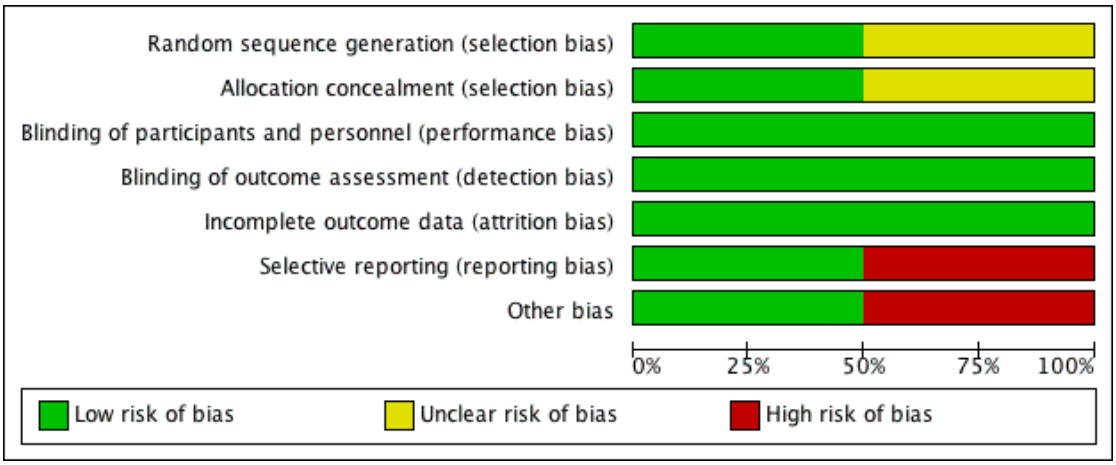


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Allocation

Random sequence allocation

One study was at low risk of bias related to sequence generation: it used computer-generated randomisation (Worrilow 2013). The other study was at unclear risk of bias, as the method of random sequence generation was not reported (Parmegiani 2012).

Allocation concealment

One study was at low risk of bias, as the investigator performing randomisation had no involvement in the trial (Worrilow 2013). Information was insufficient to permit judgement in the other trial (Parmegiani 2012).

Blinding

One study was double-blinded (Worrilow 2013), and the other was unblinded (Parmegiani 2012). Blinding however is unlikely to affect any of the outcome measures. Both studies were judged to be at low risk of bias for this domain.

Incomplete outcome data

In one study the risk of attrition bias was unclear, as it could not be accurately determined which study group participants with incomplete data belonged to (Worrilow 2013). Data were incomplete in 4/482; therefore the risk in this domain was not considered high. The other study was deemed to be at low risk of bias in this domain (Parmegiani 2012).

Selective reporting

One study was considered to be at high risk of selective reporting bias, as data regarding all outcome measures were not available (Worrilow 2013). The remaining study was deemed to be at low risk of selective reporting bias (Parmegiani 2012). No evidence was found to suggest that specific outcomes were reported on the basis of statistical significance.

Other potential sources of bias

One study was potentially biased, as it was stopped prematurely because of financial constraints and a slower than expected time to recruit (Worrilow 2013).

Effects of interventions

See: **Summary of findings for the main comparison** Conventional sperm selection (ICSI) versus hyaluronan sperm selection (HA-ICSI) for assisted reproduction; **Summary of findings 2** HA culture dish (PICSI) compared with viscous medium containing HA (SpermSlow) for infertility requiring intracytoplasmic sperm injection

See Summary of findings for the main comparison. Conventional sperm selection (ICSI) versus hyaluronan selected sperm (HA-ICSI); Summary of findings 2. HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow).

HA culture dish (PICSI) versus ICSI

Primary outcome

Live birth (effectiveness)

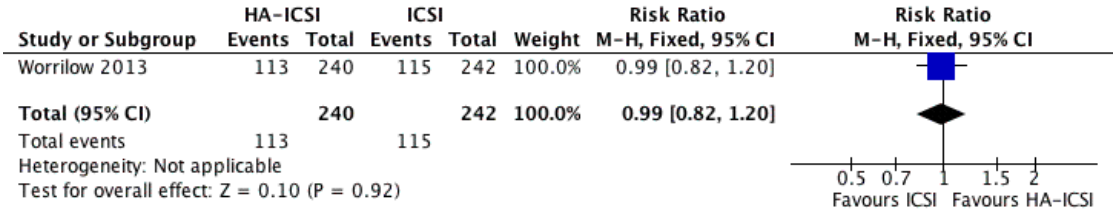
Live birth was not reported in the included study.

Secondary outcomes

Clinical pregnancy (effectiveness)

Evidence was insufficient to show whether there is a difference between the interventions (RR 0.99, 95% CI 0.82 to 1.20, one RCT, 482 women, low-quality evidence; Analysis 1.1; Figure 4).

Figure 4. Forest plot of comparison. Hyaluronan sperm selection (HA-ICSI) versus ICSI, outcome: 1.1 Clinical pregnancy per woman randomly assigned.



Miscarriage (adverse event)

Pregnancy loss rate was included in the reported study; however the data were not suitable for meta-analysis. From the data provided, we were unable to accurately determine to which treatment group a miscarriage pertained.

Fetal abnormality (adverse event)

The included study did not report fetal abnormality.

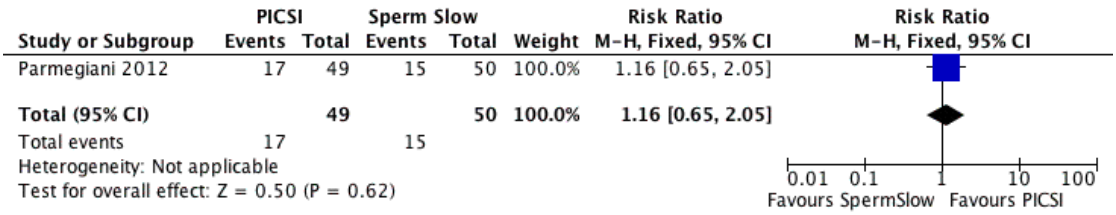
HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow)

Primary outcome

Live birth (effectiveness)

Evidence was insufficient to show whether there is a difference between the interventions in live birth rates (RR 1.16, 95% CI 0.65 to 2.05, one RCT, 99 women, low-quality evidence; Analysis 2.1; Figure 5).

Figure 5. Forest plot of comparison: 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow), outcome: 2.1 Live birth per woman randomly assigned.

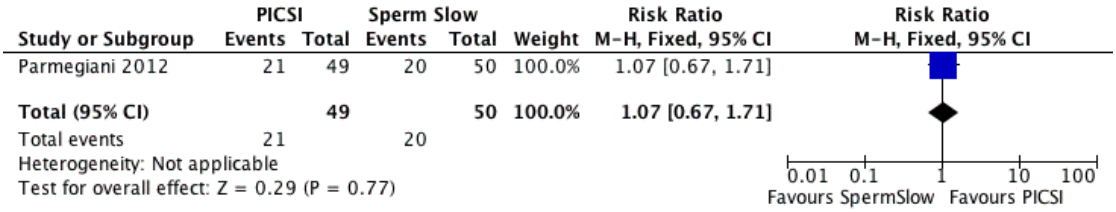


Secondary outcomes

Clinical pregnancy (effectiveness)

Evidence was insufficient to show whether there is a difference between the interventions in clinical pregnancy rates (RR 1.07, 95% CI 0.67 to 1.71, one RCT, 99 women, low-quality evidence; Analysis 2.2; Figure 6).

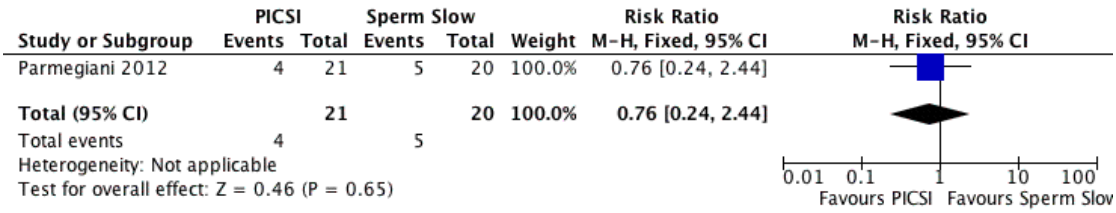
Figure 6. Forest plot of comparison: 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow), outcome: 2.2 Clinical pregnancy per woman randomly assigned.



Miscarriage (adverse event)

Evidence was insufficient to indicate whether there is a difference between the interventions in miscarriage rates (RR 0.76, 95% CI 0.24 to 2.44, one RCT, 41 women, low-quality evidence; Analysis 2.3; Figure 7).

Figure 7. Forest plot of comparison: 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow), outcome: 2.3 Miscarriage per woman randomly assigned.



Fetal abnormality (adverse event)

The included study did not report fetal abnormality.

Secondary analyses

Data were insufficient for review authors to conduct any subgroup or sensitivity analyses or to construct a funnel plot to assess reporting bias.

ADDITIONAL SUMMARY OF FINDINGS *[Explanation]*

HA culture dish (PICSI) compared with viscous medium containing HA (SpermSlow) for infertility requiring intracytoplasmic sperm injection

Population: women with infertility requiring intracytoplasmic sperm injection
Settings: ART
Intervention: HA culture dish (PICSI)
Comparison: viscous medium containing HA (SpermSlow)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Viscous medium containing HA (SpermSlow)	HA culture dish (PICSI)				
Live birth per woman randomly assigned	**30 per 100**	**35 per 100** (19-55)	**RR 1.16** (0.65-2.05)	99 (1 study)	⊕⊕◯◯[a,b] low	
Clinical pregnancy per woman randomly assigned	**40 per 100**	**43 per 100** (25-62)	**RR 1.07** (0.67-1.71)	99 (1 study)	⊕⊕◯◯[a,b] low	
Miscarriage per clinical pregnancy	**25 per 100**	**19 per 100** (5-51)	**RR 0.76** (0.24-2.44)	41 (1 study)	⊕⊕◯◯[a,b] low	

*The basis for the **assumed risk** is the median control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
ART: assisted reproductive technologies; **CI:** confidence interval; **HA:** hyaluronic acid; **OR:** odds ratio.

GRADE Working Group grades of evidence.
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[a] Serious risk of bias: study methods not reported in adequate detail.

[b]Serious imprecision: confidence intervals compatible with substantial benefit or harm from the intervention, or with no effect.

DISCUSSION

Summary of main results

Overall, this review does not allow any conclusions to be drawn regarding the use of advanced sperm selection techniques for assisted reproduction.

Live birth rate was reported in only one included trial, and evidence was insufficient to indicate whether there is a difference in effectiveness between PICSI and SpermSlow.

Evidence was insufficient to show whether there is a difference in clinical pregnancy rates between PICSI and standard ICSI or between PICSI and SpermSlow. Congenital abnormality outcomes were not reported.

Data were insufficient to permit any conclusions with regard to miscarriage.

No suitable studies were identified that would have allowed determination of the effect of sperm selected by surface charge, sperm apoptosis or sperm birefringence. None of the included studies reported a subgroup suitable for analysis. See Summary of findings for the main comparison and Summary of findings 2 for further details.

Overall completeness and applicability of evidence

The objectives of this review were incompletely addressed by the studies included. No suitably controlled data were available to address the primary outcome measure (live birth per allocated couple) for any of the advanced sperm selection techniques described, and data on other important clinical outcomes such as miscarriage and fetal abnormalities were lacking.

Only one study (Worrilow 2013) provided data pertaining to clinical pregnancy per allocated couple for hyaluronanic acid binding compared with standard ICSI. The other included study (Parmegiani 2012) compared two different hyaluronanic acid binding techniques-PICSI and SpermSlow-but did not include a control population receiving standard ICSI. No study allowed a suitable subgroup analysis.

Quality of the evidence

See Figure 2 and Figure 3.

The quality of the evidence for reported outcomes was low. The main limitations were poor reporting of study methods, discrepancies in the data and serious imprecision. The 95% confidence intervals were compatible with substantial benefit or harm from the intervention, or with no effect. Available RCT data were very sparse, and the two included studies did not address the same clinical comparison. We were unable to assess the risk of reporting bias.

The included study for the main comparison (Worrilow 2013) had several limitations. Overall the methodology was of a very poor standard. Study findings were difficult to interpret, and absolute values for outcome measures were not easily identifiable. The trial was stopped early for financial reasons and slow recruitment; this may have hindered the investigators' ability to adequately complete the stated objectives. It was unclear to which study group dropouts belonged. Attempts were made to contact the lead author to clarify several findings; however no reply was forthcoming (see Summary of findings for the main comparison).

Evidence on the comparison of two different hyaluronic acid binding methods for sperm selection (Parmegiani 2012) was similarly limited by imprecision and by poor reporting of study methods (see Summary of findings 2).

Potential biases in the review process

No potential biases were identified in the review process.

Agreements and disagreements with other studies or reviews

Another systematic review (Said 2007) has addressed effects of advanced sperm selection on sperm quality and ART outcomes. Sperm selection techniques similar to those described in this review were investigated. A total of 44 studies were identified, but few were strictly randomised, and nearly all were deemed unsuitable for inclusion in this review. However, the authors' conclusions were in concordance with our findings. Further clinical trials are required before advanced sperm selection techniques can be recommended in routine practice.

AUTHORS' CONCLUSIONS

Implications for practice

Evidence is insufficient to show whether sperm selected by hyaluronanic acid binding improves live birth or pregnancy outcomes in ART, and no clear data on adverse effects are available. Evidence is also insufficient to show whether there is a difference in efficacy between hyaluronic acid binding methods-SpermSlow and PICSI.

No randomised evidence describes evaluation of sperm selection by sperm apoptosis, sperm birefringence or surface charge.

Implications for research

Suitable RCTs are needed to evaluate the effects of sperm selection based on hyaluronanic acid binding, surface charge selection, sperm apoptosis and sperm birefringence on live birth, clinical

pregnancy, miscarriage and congenital abnormality, and to investigate whether certain patient subgroups such as those with high sperm DNA fragmentation might benefit from these advanced sperm selection techniques. Trials should use intention-to-treat analysis and should report outcomes per woman randomly assigned.

ACKNOWLEDGEMENTS

We acknowledge the valuable help and support provided by the Cochrane Menstrual Disorders and Subfertility Group. Attempts were made to contact the authors of both of the included studies, but neither author replied.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Parmegiani 2012

Methods	Randomised controlled trial conducted in a single private assisted reproduction centre (Italy) between September 2010 and March 2011
Participants	**Inclusion criteria:** all infertile women aged ≤ 41 years; undergoing ICSI treatment; total sperm number ≥ 1 million; sperm motility ≥ 5% **Exclusion criteria:** testicular spermatozoa; severe oligoasthenoteratozoospermia; total sperm number < 1 million; sperm motility < 5% **Randomly assigned:** 99 participants
Interventions	Couples were randomly assigned to 2 groups: **Intervention 1 (PICSI):** A 2-μL droplet suspension of treated spermatozoa is placed near each 5-μL culture (3 microdots in total) medium droplet and is subsequently connected to the droplet using the tip of a Gilson pipette. The PICSI dish is incubated at 37°C under oil (FertiCult Mineral Oil; FertiPro, Beernem, Belgium); within 5 minutes, the bound spermatozoa attach by their head to the surface of the HA microdots and spin. An ICSI injecting pipette (ICSI Micropipets; Humagen Fertility Diagnostics-Origio, Jyllinge, Denmark) are used to pick HA-bound sperm and inject them 1 by 1 into each oocyte. Spermatozoa spinning faster are preferred. The ICSI injecting pipette was previously loaded with SpermSlow to facilitate sperm micromanipulation. 49 women **Intervention 2 (SpermSlow):** On a plastic culture dish (IVF Petri dishes; Nunc, catalog no. 150255), a 2-μL droplet suspension of treated spermatozoa is connected with a pipette tip to a 5-μL droplet of fresh culture medium (FertiCult Flushing Medium). Simultaneously, a 5-μL droplet of SpermSlow is connected with a pipette tip to a 5-μL droplet of fresh culture medium. The spermatozoa on this culture dish are then incubated for 5 minutes at 37°C under oil (FertiCult Mineral Oil; FertiPro). Spermatozoa bound to HA are slowed in the junction zone of the 2 droplets; these spermatozoa are selected and collected with an injecting pipette (ICSI Micropipets) and then are injected into oocytes. 50 women **Both PICSI and SpermSlow:** PICSI and SpermSlow procedures are performed at 400× magnification. The spermatozoa are selected according to their morphology (World Health Organization guidelines 2010)
Outcomes	Clinical pregnancy rate; miscarriage rate; live birth rate
Notes	Study authors were emailed to clarify aspects of methodology, but no reply was received

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method of sequence generation not described

Parmegiani 2012 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement: states that randomisation was "by sealed envelopes"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All participants were informed of their allocated treatment. Given lack of blinding of participants, it can be assumed that other study personnel were unblinded. Lack of blinding is unlikely to affect any of the outcome measures
Blinding of outcome assessment (detection bias) All outcomes	Low risk	It is unclear whether blinding was performed. Lack of blinding of outcome assessment is unlikely to affect the outcomes measured
Incomplete outcome data (attrition bias) All outcomes	Low risk	Omission of a single case is unlikely to have a significant impact. Participant was excluded because of high-risk ovarian hyperstimulation syndrome
Selective reporting (reporting bias)	Low risk	All prespecified outcomes have been reported
Other bias	Low risk	None

Worrilow 2013

Methods	Prospective, double-blind, randomised controlled trial conducted in 10 IVF programs (USA). Period of enrolment not reported
Participants	**Inclusion criteria:** IVF patients who received ICSI as part of their ART treatment **Exclusion criteria:** use of testicular sperm; use of donor or cryopreserved gametes; patients receiving PIGD; use of sperm sorting procedures; patients for whom only a proportion of oocytes receive ICSI; maternal age > 40 years; < 4 metaphase 2 oocytes at time of oocyte retrieval; initial hyaluronan binding score < 2%; sperm count < 10,000 motile sperm/mL **Randomly assigned:** 482 participants
Interventions	Participants were divided into 2 cohorts based on the proportion of HB sperm in their unprocessed or initial semen (I-HB). The 2 cohorts were divided based on an I-HB ≤ 65% or > 65%. Patients with an I-HB score ≤ 65% were randomly assigned to routine ICSI (control) or sperm selection based on hyaluronan binding. Patients with an I-HB score > 65% were randomly assigned to 3 groups: control, hyaluronan binding or non-participation. The non-participating group was present to balance the numbers of participants with high and low I-HB scores The initial hyaluronan binding score of sperm was evaluated using the HBA Sperm Hyaluronan Binding Assay, a dual-chambered slide containing an attached layer of

	hyaluronan located beneath 2 individual coverslips (Biocoat, Inc., Horsham, PA, USA) . In accordance with the manufacturer's instructions, the HB score was determined by calculating the number of motile HB sperm divided by the number of total motile sperm. Following assessment of I-HB score, the sperm were subjected to centrifugation on a discontinuous gradient and were washed with sperm processing media, according to the specific protocol for the participating site **Intervention:** The final sperm suspension was placed upon microdots of hyaluronan in the PICSI Sperm Selection Device (Biocoat, Inc.) and was overlaid with oil. Following a 5- to 10-minute incubation, HB sperm were selected for microinjection. 240 women **Control:** The final sperm suspension was placed into standard ICSI dishes for selection. 242 women
Outcomes	Clinical pregnancy rate; pregnancy loss rate
Notes	Longer time than expected was taken to recruit participants; therefore the trial was prematurely closed because of cost implications Results for the study groups were combined to yield outcome measures regardless of percentage of HB sperm Study authors were contacted to clarify numerous areas of methodology and results. No reply was received

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Permuted block design with a computer random number generator
Allocation concealment (selection bias)	Low risk	Investigator performing randomisation has no clinical involvement in the trial
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Apart from embryologists, all participants and personnel were blinded. Non-blinding of embryologists is unlikely to affect the outcome
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Information was insufficient to permit judgement. Lack of blinding of outcome assessment is unlikely to affect the outcomes measured
Incomplete outcome data (attrition bias) All outcomes	Low risk	Results of 4 participants were not reported. Overall, this was not a substantial departure from the intervention received. It is not clear to which study group the incomplete data belong

Worrilow 2013 (*Continued*)

Selective reporting (reporting bias)	High risk	The outcome 'pregnancy loss rate' was incompletely reported. Absolute numbers were not given and could not be determined from the information provided. The absolute number of pregnancies given for pregnancy loss was higher than that given for clinical pregnancy. No explanation is given
Other bias	High risk	Participant recruitment took longer than expected; therefore the study was closed prematurely because of higher than expected costs

Abbreviations:
ART: assisted reproductive technologies.
HB: hyaluronan binding.
HA: hyaluronic acid.
ICSI: intracytoplasmic sperm injection.
I-HB: initial hyaluronan binding.
IVF: in vitro fertilization.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Antinori 2008	This study did not meet our inclusion criteria
Balaban 2011	This study did not meet our inclusion criteria
Berkovitz 2006	Not a randomised controlled trial (RCT) (couples were matched)
Blanchard 2010	The study did not analyse a relevant intervention
Figueira 2011	This study did not meet our inclusion criteria
Fleming 2007	Not a randomised controlled trial (RCT) (observational study)
Gianaroli 2008	The study was pseudo-randomised
Gianaroli 2010	The study was pseudo-randomised
Gnosh 2007	Not a randomised controlled trial (RCT) (observational study)

(*Continued*)

Knez 2011	This study did not meet our inclusion criteria
Knez 2012	This study did not meet our inclusion criteria
Mahmoud 2011	This study did not meet our inclusion criteria
Parmegiani 2010a	Results were 'per-treatment randomised' rather than 'per-woman or couple randomised.' Study authors were contacted to adjust results, but no reply was received
Parmegiani 2010b	This study did not meet our inclusion criteria
Setti 2011	This study did not meet our inclusion criteria
Setti 2012a	This study did not meet our inclusion criteria
Setti 2012b	This study did not meet our inclusion criteria

DATA AND ANALYSES

Comparison 1. Hyaluronan sperm selection (HA-ICSI) versus ICSI

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy per woman randomly assigned	1	482	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.82, 1.20]

Comparison 2. HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth per woman randomly assigned	1	99	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [0.65, 2.05]
2 Clinical pregnancy per woman randomly assigned	1	99	Risk Ratio (M-H, Fixed, 95% CI)	1.07 [0.67, 1.71]
3 Miscarriage per woman randomly assigned	1	41	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.24, 2.44]

Analysis 1.1. Comparison 1 Hyaluronan sperm selection (HA-ICSI) versus ICSI, Outcome 1 Clinical pregnancy per woman randomly assigned.

Review: Advanced sperm selection techniques for assisted reproduction

Comparison: 1 Hyaluronan sperm selection (HA-ICSI) versus ICSI

Outcome: 1 Clinical pregnancy per woman randomly assigned

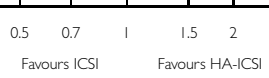
Study or subgroup	HA-ICSI n/N	ICSI n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Worrilow 2013	113/240	115/242		100.0 %	0.99 [0.82, 1.20]
Total (95% CI)	**240**	**242**		**100.0 %**	**0.99 [0.82, 1.20]**

Total events: 113 (HA-ICSI), 115 (ICSI)

Heterogeneity: not applicable

Test for overall effect: Z = 0.10 (P = 0.92)

Test for subgroup differences: Not applicable

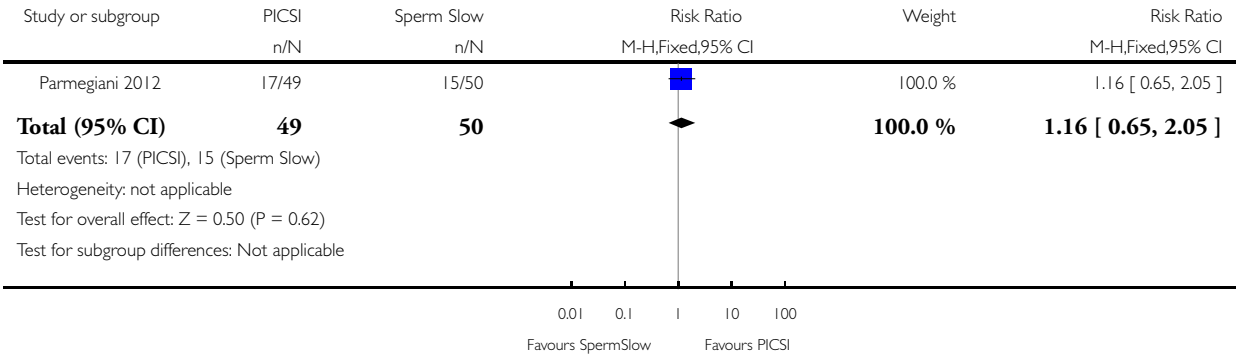


Analysis 2.1. Comparison 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow), Outcome 1 Live birth per woman randomly assigned.

Review: Advanced sperm selection techniques for assisted reproduction

Comparison: 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow)

Outcome: 1 Live birth per woman randomly assigned



Analysis 2.2. Comparison 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow), Outcome 2 Clinical pregnancy per woman randomly assigned.

Review: Advanced sperm selection techniques for assisted reproduction

Comparison: 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow)

Outcome: 2 Clinical pregnancy per woman randomly assigned

Study or subgroup	PICSI n/N	Sperm Slow n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Parmegiani 2012	21/49	20/50		100.0 %	1.07 [0.67, 1.71]
Total (95% CI)	**49**	**50**		**100.0 %**	**1.07 [0.67, 1.71]**
Total events: 21 (PICSI), 20 (Sperm Slow)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.29 (P = 0.77)					
Test for subgroup differences: Not applicable					

Analysis 2.3. Comparison 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow), Outcome 3 Miscarriage per woman randomly assigned.

Review: Advanced sperm selection techniques for assisted reproduction

Comparison: 2 HA culture dish (PICSI) versus viscous medium containing HA (SpermSlow)

Outcome: 3 Miscarriage per woman randomly assigned

Study or subgroup	PICSI n/N	Sperm Slow n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Parmegiani 2012	4/21	5/20		100.0 %	0.76 [0.24, 2.44]
Total (95% CI)	**21**	**20**		**100.0 %**	**0.76 [0.24, 2.44]**
Total events: 4 (PICSI), 5 (Sperm Slow)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.46 (P = 0.65)					
Test for subgroup differences: Not applicable					

APPENDICES

Appendix 1. Menstrual Disorders and Subfertility Group keyword search

26.05.2014
Keywords CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or"intracytoplasmic sperm injection" or "in-vitro fertilization" or "assisted reproduction" or "subfertility"or "infertility" or "male factor" or"male fertility" or "male infertility" or "male subfertility" or "subfertility-male" or Title CONTAINS"IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or"intracytoplasmic sperm injection" or "in-vitro fertilization" or "assisted reproduction"or "subfertility"or "infertility" or "male factor" or"male fertility" or "male infertility" or "male subfertility" or "subfertility-male"
AND
Keywords CONTAINS "sperm morphology"or "sperm motility"or "sperm preparation"or"sperm preparation techniques"or "sperm select"or "sperm selection"or "sperm selection techniques"or"sperm separation"or "sperm sorting"or "birefringent sperm"or "Magnetic Activated Sorting Selection"or"magnetic sperm selection"or "hyaluronan-bound (HB) sperm"or "hyaluronan bound sperm"or "hyaluronic acid sperm selection"or "hyaluronic acid intracytoplasmic sperm injection"or "IMSI"or "intracytoplasmic morphologically selected sperm injection" or "apoptosis"or "semen preparation"or "membrane properties" or Title CONTAINS "sperm morphology"or "sperm motility"or "sperm preparation"or"sperm preparation techniques"or "sperm select"or "sperm selection"or "sperm selection techniques"or"sperm separation"or "sperm sorting"or "birefringent sperm"or "Magnetic Activated Sorting Selection"or"magnetic sperm selection"or "hyaluronan-bound (HB) sperm"or "hyaluronan bound sperm"

Appendix 2. CENTRAL search strategy

26.05.2014
1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (1743)
2 embryo transfer$.tw. (1017)
3 vitro fertili?ation.tw. (1475)
4 ivf-et.tw. (288)
5 ivf.tw. (2170)
6 icsi.tw. (801)
7 intracytoplasmic sperm injection$.tw. (490)
8 (blastocyst adj2 transfer$).tw. (97)
9 assisted reproduct$.tw. (461)
10 ovulation induc$.tw. (517)
11 (ovari$ adj2 stimulat$).tw. (861)
12 superovulat$.tw. (148)
13 ovarian hyperstimulation.tw. (604)
14 COH.tw. (133)
15 infertil$.tw. (2085)
16 subfertil$.tw. (158)
17 (ovari$ adj2 induction).tw. (30)
18 exp Reproductive Techniques, Assisted/ (2440)
19 ART.tw. (1279)
20 or/1-19 (7011)
21 (sperm$ adj7 selection$).tw. (61)
22 (sperm$ adj7 separat$).tw. (48)
23 surface charge.tw. (8)
24 electrophore$.tw. (541)
25 (zeta adj2 potential).tw. (6)
26 magnetic cell sorting.tw. (1)
27 glass wool.tw. (4)
28 membrane matur$.tw. (1)
29 magnetic activated cell sort$.tw. (7)
30 ultramorpholog$.tw. (8)

31 (hyaluronic acid adj2 binding).tw. (7)
32 (sperm$ adj5 birefringence).tw. (2)
33 (sperm$ adj3 morphology).tw. (147)
34 ultra high magnification.tw. (0)
35 motile sperm$ organelle.tw. (0)
36 MSOME.tw. (1)
37 IMSI.tw. (20)
38 Intracytoplasmic morphologically selected sperm injection$.tw. (17)
39 Raman spectroscopy.tw. (23)
40 confocal light absorption.tw. (0)
41 (scattering adj3 microscopy).tw. (2)
42 polarization microscopy.tw. (5)
43 polarisation microscopy.tw. (0)
44 polscope.tw. (3)
45 (sperm$ adj3 apopto$).tw. (10)
46 zeta method.tw. (0)
47 (nonapoptotic$ adj3 sperm$).tw. (1)
48 sperm$ preparation.tw. (58)
49 (sperm$ adj3 prepar$).tw. (97)
50 (semen adj2 prepar$).tw. (27)
51 (sperm$ adj5 chemotaxis).tw. (0)
52 hyaluronan bound.tw. (4)
53 (hyaluronic acid adj2 bound).tw. (1)
54 or/21-53 (964)
55 20 and 54 (230)
56 limit 55 to yr="2013 -Current" (14)

Appendix 3. EMBASE search strategy

26.05.2014
1 (sperm$ adj7 selection$).tw. (1241)
2 (sperm$ adj7 separat$).tw. (1543)
3 surface charge.tw. (7586)
4 electrophore$.tw. (200594)
5 (zeta adj2 potential).tw. (8166)
6 magnetic cell sorting.tw. (676)
7 glass wool.tw. (441)
8 membrane matur$.tw. (64)
9 magnetic activated cell sort$.tw. (620)
10 ultramorpholog$.tw. (163)
11 (hyaluronic acid adj2 binding).tw. (395)
12 (sperm$ adj5 birefringence).tw. (27)
13 (sperm$ adj3 morphology).tw. (3995)
14 ultra high magnification.tw. (32)
15 motile sperm$ organelle.tw. (64)
16 MSOME.tw. (83)
17 IMSI.tw. (159)
18 Intracytoplasmic morphologically selected sperm injection$.tw. (111)
19 Raman spectroscopy.tw. (8853)
20 confocal light absorption.tw. (5)
21 (scattering adj3 microscopy).tw. (1180)
22 polarization microscopy.tw. (509)

23 polarisation microscopy.tw. (42)
24 polscope.tw. (95)
25 (sperm$ adj3 apopto$).tw. (962)
26 zeta method.tw. (8)
27 (nonapoptotic$ adj3 sperm$).tw. (13)
28 sperm$ preparation.tw. (465)
29 (sperm$ adj3 prepar$).tw. (1489)
30 (semen adj2 prepar$).tw. (273)
31 (sperm$ adj5 chemotaxis).tw. (178)
32 hyaluronan bound.tw. (21)
33 (hyaluronic acid adj2 bound).tw. (40)
34 or/1-33 (235101)
35 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (52093)
36 embryo$ transfer$.tw. (12557)
37 in vitro fertili?ation.tw. (20392)
38 icsi.tw. (9487)
39 intracytoplasmic sperm injection$.tw. (6241)
40 (blastocyst adj2 transfer$).tw. (1069)
41 ivf.tw. (24351)
42 assisted reproduct$.tw. (12983)
43 ovulation induc$.tw. (4280)
44 (ovari$ adj2 stimulat$).tw. (6899)
45 superovulat$.tw. (2970)
46 ovarian hyperstimulation.tw. (5159)
47 COH.tw. (1457)
48 infertil$.tw. (53568)
49 subfertil$.tw. (4288)
50 (ovari$ adj2 induction).tw. (265)
51 exp infertility therapy/ (76551)
52 or/35-51 (127317)
53 Clinical Trial/ (831083)
54 Randomized Controlled Trial/ (342096)
55 exp randomization/ (62087)
56 Single Blind Procedure/ (18281)
57 Double Blind Procedure/ (113240)
58 Crossover Procedure/ (38945)
59 Placebo/ (239466)
60 Randomi?ed controlled trial$.tw. (98059)
61 Rct.tw. (13774)
62 random allocation.tw. (1301)
63 randomly allocated.tw. (20081)
64 allocated randomly.tw. (1916)
65 (allocated adj2 random).tw. (710)
66 Single blind$.tw. (14169)
67 Double blind$.tw. (139778)
68 ((treble or triple) adj blind$).tw. (362)
69 placebo$.tw. (196253)
70 prospective study/ (250502)
71 or/53-70 (1353706)
72 case study/ (25890)
73 case report.tw. (256873)
74 abstract report/ or letter/ (889492)
75 or/72-74 (1166690)

76 71 not 75 (1316227)
77 34 and 52 and 76 (434)
78 (2013$ or 2014$).em. (2235119)
79 (2013$ or 2014$).dp. (262788)
80 78 or 79 (2235838)
81 77 and 80 (75)

Appendix 4. MEDLINE search strategy

26.05.2014
1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (32488)
2 embryo transfer$.tw. (8205)
3 vitro fertili?ation.tw. (16893)
4 ivf-et.tw. (1837)
5 ivf.tw. (16329)
6 icsi.tw. (5489)
7 intracytoplasmic sperm injection$.tw. (4956)
8 (blastocyst adj2 transfer$).tw. (549)
9 assisted reproduct$.tw. (9089)
10 ovulation induc$.tw. (3397)
11 (ovari$ adj2 stimulat$).tw. (4899)
12 superovulat$.tw. (2913)
13 ovarian hyperstimulation.tw. (3799)
14 COH.tw. (1141)
15 infertil$.tw. (42532)
16 subfertil$.tw. (3513)
17 (ovari$ adj2 induction).tw. (224)
18 exp Reproductive Techniques, Assisted/ (53012)
19 ART.tw. (49558)
20 or/1-19 (145434)
21 (sperm$ adj7 selection$).tw. (1002)
22 (sperm$ adj7 separat$).tw. (1487)
23 surface charge.tw. (7193)
24 electrophore$.tw. (204269)
25 (zeta adj2 potential).tw. (6747)
26 magnetic cell sorting.tw. (449)
27 glass wool.tw. (418)
28 membrane matur$.tw. (65)
29 magnetic activated cell sort$.tw. (398)
30 ultramorpholog$.tw. (172)
31 (hyaluronic acid adj2 binding).tw. (383)
32 (sperm$ adj5 birefringence).tw. (17)
33 (sperm$ adj3 morphology).tw. (3368)
34 ultra high magnification.tw. (13)
35 motile sperm$ organelle.tw. (31)
36 MSOME.tw. (38)
37 IMSI.tw. (57)
38 Intracytoplasmic morphologically selected sperm injection$.tw. (52)
39 Raman spectroscopy.tw. (10662)
40 confocal light absorption.tw. (5)
41 (scattering adj3 microscopy).tw. (1238)
42 polarization microscopy.tw. (514)

43 polarisation microscopy.tw. (38)
44 polscope.tw. (64)
45 (sperm$ adj3 apopto$).tw. (781)
46 zeta method.tw. (6)
47 (nonapoptotic$ adj3 sperm$).tw. (10)
48 sperm$ preparation.tw. (360)
49 (sperm$ adj3 prepar$).tw. (1339)
50 (semen adj2 prepar$).tw. (203)
51 (sperm$ adj5 chemotaxis).tw. (166)
52 hyaluronan bound.tw. (18)
53 (hyaluronic acid adj2 bound).tw. (42)
54 or/21-53 (237463)
55 randomized controlled trial.pt. (374162)
56 controlled clinical trial.pt. (88396)
57 randomized.ab. (294053)
58 randomised.ab. (58894)
59 placebo.tw. (158322)
60 clinical trials as topic.sh. (169995)
61 randomly.ab. (212907)
62 trial.ti. (126568)
63 (crossover or cross-over or cross over).tw. (60716)
64 or/55-63 (944515)
65 exp animals/ not humans.sh. (3940682)
66 64 not 65 (871027)
67 20 and 54 and 66 (246)
68 (2013$ or 2014$).ed. (1349970)
69 (2013$ or 2014$).dp. (1396112)
70 68 or 69 (1838941)
71 67 and 70 (39)

Appendix 5. PsycINFO search strategy

13.3.2013; no new results at 26.05.2014
1 (sperm$ adj7 selection$).tw. (86)
2 (sperm$ adj7 separat$).tw. (23)
3 surface charge.tw. (13)
4 electrophore$.tw. (842)
5 (zeta adj2 potential).tw. (5)
6 magnetic cell sorting.tw. (2)
7 glass wool.tw. (3)
8 membrane matur$.tw. (1)
9 magnetic activated cell sort$.tw. (2)
10 ultramorpholog$.tw. (2)
11 (hyaluronic acid adj2 binding).tw. (0)
12 (sperm$ adj5 birefringence).tw. (0)
13 (sperm$ adj3 morphology).tw. (35)
14 ultra high magnification.tw. (1)
15 motile sperm$ organelle.tw. (0)
16 MSOME.tw. (0)
17 IMSI.tw. (2)
18 Intracytoplasmic morphologically selected sperm injection$.tw. (0)
19 Raman spectroscopy.tw. (5)

20 confocal light absorption.tw. (0)
21 (scattering adj3 microscopy).tw. (3)
22 polarization microscopy.tw. (1)
23 polarisation microscopy.tw. (0)
24 polscope.tw. (0)
25 (sperm$ adj3 apopto$).tw. (2)
26 zeta method.tw. (0)
27 (nonapoptotic$ adj3 sperm$).tw. (0)
28 sperm$ preparation.tw. (0)
29 (sperm$ adj3 prepar$).tw. (7)
30 (semen adj2 prepar$).tw. (1)
31 (sperm$ adj5 chemotaxis).tw. (6)
32 hyaluronan bound.tw. (1)
33 (hyaluronic acid adj2 bound).tw. (0)
34 or/1-33 (1032)
35 exp reproductive technology/ (1218)
36 in vitro fertili?ation.tw. (496)
37 ivf-et.tw. (17)
38 (ivf or et).tw. (89138)
39 icsi.tw. (39)
40 intracytoplasmic sperm injection$.tw. (33)
41 (blastocyst adj2 transfer$).tw. (2)
42 assisted reproduct$.tw. (469)
43 ovulation induc$.tw. (17)
44 (ovari$ adj2 stimulat$).tw. (47)
45 ovarian hyperstimulation.tw. (8)
46 COH.tw. (58)
47 superovulat$.tw. (5)
48 infertil$.tw. (2371)
49 subfertil$.tw. (56)
50 (ovari$ adj2 induction).tw. (4)
51 or/35-50 (92225)
52 random.tw. (37660)
53 control.tw. (293008)
54 double-blind.tw. (16852)
55 clinical trials/ (6705)
56 placebo/ (3440)
57 exp Treatment/ (544615)
58 or/52-57 (828902)
59 34 and 51 and 58 (4)

Appendix 6. CINAHL search strategy

26.05.2014

#	Query	Results
S56	S41 AND S55	8
S55	S42 OR S43 or S44 or S45 OR S46 OR S47 OR S48 OR S49 OR S50 OR S51 OR S52 OR S53 OR S54	891,112
S54	TX allocat* random*	3,905
S53	(MH "Quantitative Studies")	12,016
S52	(MH "Placebos")	8,741
S51	TX placebo*	31,574
S50	TX random* allocat*	3,905
S49	(MH "Random Assignment")	37,244
S48	TX randomi* control* trial*	72,875
S47	TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))	715,853
S46	TX ((trebl* n1 blind*) or (trebl* n1 mask*))	105
S45	TX ((trebl* n1 blind*) or (trebl* n1 mask*))	0
S44	TX clinic* n1 trial*	163,316
S43	PT Clinical trial	75,963
S42	(MH "Clinical Trials+")	174,859
S41	S8 AND S40	30
S40	S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39	5,541
S39	TX (hyaluronic acid N2 bound)	1
S38	TX (hyaluronan bound)	1
S37	TX (sperm* N5 chemotaxis)	43

(*Continued*)

S36	TX (sperm* N5 chemotaxis)	0
S35	TX (nonapoptotic* N3 sperm*)	0
S34	TX (nonapoptotic* N3 sperm*)	0
S33	TX zeta method	1
S32	TX (sperm* N3 apopto*)	17
S31	TX polscope	2
S30	TX (polarisation microscopy)	2
S29	TX (polarization microscopy)	9
S28	TX (scattering N3 microscopy)	10
S27	TX confocal light absorption	1
S26	TX Raman spectroscopy	77
S25	TX MSOME	2
S24	TX (motile sperm* organelle)	2
S23	TX (ultra high magnification)	1
S22	TX (sperm* N3 morphology)	90
S21	TX (sperm* N5 birefringence)	4
S20	TX (sperm* N5 birefringence)	0
S19	TX (hyaluronic acid N2 binding)	8
S18	TX ultramorpholog*	6
S17	TX (magnetic activated cell sort*)	12
S16	TX (membrane matur*)	16
S15	TX (glass wool)	8
S14	TX (magnetic cell sorting)	25
S13	TX(zeta N2 potential)	22

(*Continued*)

S12	TX electrophore*	5,173
S11	TX (surface charge)	31
S10	TX(sperm* N3 separat*)	7
S9	TX(sperm* N3 selection*)	17
S8	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7	3,443
S7	TX embryo* N3 transfer*	699
S6	TX ovar* N3 hyperstimulat*	301
S5	TX ovari* N3 stimulat*	223
S4	TX IVF or TX ICSI	1,134
S3	(MM "Fertilization in Vitro")	1,348
S2	TX vitro fertilization	2,672
S1	TX vitro fertilisation	259

CONTRIBUTIONS OF AUTHORS

Protocol

SM wrote the first draft of the protocol. BK wrote the revised draft of the protocol. EF contributed methodological and statistical expertise to the protocol. AY commented on all drafts of the protocol, as well as methods and statistics. DG assisted with revision of the protocol. YH provided technical expertise and will contribute in the analysis phase of the review.

Full review

SM wrote the draft. BK provided clinical input. EF and AY supplied methodological and statistical expertise. DG commented on the revised draft. YH provided technical input.

DECLARATIONS OF INTEREST

No conflicts of interest have been reported.

SOURCES OF SUPPORT

Internal sources

- None, Not specified.

External sources

- None, Not specified.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Following completion of our protocol, a Cochrane review titled 'Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction' was published (Teixeira 2013). This publication considerably overlapped that of our protocol, and after consultation with the Cochrane Menstrual Disorders and Subfertility Group in Auckland, the scope of our review was amended to exclude the use of ultra-high magnification (IMSI) for sperm selection. The title of our review was amended accordingly.

INDEX TERMS

Medical Subject Headings (MeSH)

*Sperm Retrieval; Apoptosis [physiology]; Birefringence; Hyaluronic Acid [metabolism]; Randomized Controlled Trials as Topic; Sperm Injections, Intracytoplasmic [*methods]; Spermatozoa [*physiology]

MeSH check words

Humans; Male