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Hyaluronic acid in embryo transfer media for assisted reproductive technologies (Review)

Heymann D, Vidal L, Or Y, Shoham Z

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[Intervention Review]

Hyaluronic acid in embryo transfer media for assisted reproductive technologies

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ABSTRACT

Background

This is an update of a Cochrane Review first published in the Cochrane Library (2010, Issue 7).

To increase the success rate of assisted reproductive technologies (ARTs), adherence compounds such as hyaluronic acid (HA) have been introduced into subfertility management. Adherence compounds are added to the embryo transfer medium to increase the likelihood of embryo implantation, with the potential for higher clinical pregnancy and live birth rates.

Objectives

To determine whether adding adherence compounds to embryo transfer media could improve pregnancy outcomes, including improving live birth and decreasing miscarriage, in women undergoing assisted reproduction.

Search methods

We searched the Cochrane Gynaecology and Fertility Group Trials Register, CENTRAL, MEDLINE, Embase, and PsycINFO electronic databases on 7 January 2020 for randomised controlled trials that examined the effects of adherence compounds in embryo transfer media on pregnancy outcomes. Furthermore, we communicated with experts in the field, searched trials registries, checked reference lists of relevant studies, and conference abstracts were handsearched.

Selection criteria

Only truly randomised controlled trials comparing embryo transfer media containing functional concentrations of adherence compounds to media with no or low adherence compound concentrations were included.

Data collection and analysis

Two review authors selected trials for inclusion according to the above criteria, after which the same two review authors independently extracted data for subsequent analysis. Statistical analysis was performed according to the guidelines developed by Cochrane. We combined data to calculate pooled risk ratios (RRs) and 95% confidence intervals (CIs). We assessed statistical heterogeneity using the I^2 statistic. We used GRADE methods to assess the overall quality of evidence for the main comparisons.

Main results

We analysed 26 studies with a total of 6704 participants. Overall, the certainty of evidence was low to moderate: the main limitations were imprecision and/or heterogeneity. Compared to embryos transferred in media containing no or low (0.125 mg/mL) HA, the addition of functional (0.5 mg/mL) HA concentrations to the transfer media probably increases the live birth rate (RR 1.21, 95% CI 1.1 to 1.31; 10 RCTs,

N = 4066; I^2 = 33%; moderate-quality evidence). This suggests that if the chance of live birth following no HA addition in media is assumed to be 33%, the chance following HA addition would be between 37% and 44%. The addition of HA may slightly decrease miscarriage rates (RR 0.82, 95% CI 0.67 to 1.00; 7 RCTs, N = 3091; I^2 = 66%; low-quality evidence). Nevertheless, when only studies with low risk of bias were included in the analysis, there was no conclusive evidence of a difference in miscarriage rates (RR 0.96, 95% CI 0.75 to 1.23; N = 2219; I^2 = 36%).

Adding HA to transfer media probably results in an increase in both clinical pregnancy (RR 1.16, 95% CI 1.09 to 1.23; 17 studies, N = 5247; I^2 = 40%; moderate-quality evidence) and multiple pregnancy rates (RR 1.45, 95% CI 1.24 to 1.70; 7 studies, N = 3337; I^2 = 36%; moderate-quality evidence). We are uncertain of the effect of HA added to transfer media on the rate of total adverse events (RR 0.86, 95% CI 0.40 to 1.84; 3 studies, N = 1487; I^2 = 0%; low-quality evidence).

Authors' conclusions

Moderate-quality evidence shows improved clinical pregnancy and live birth rates with the addition of HA as an adherence compound in embryo transfer media in ART. Low-quality evidence suggests that adding HA may slightly decrease miscarriage rates, but when only studies at low risk of bias were included in the analysis, the results were inconclusive. HA had no clear effect on the rate of total adverse events. The increase in multiple pregnancy rates may be due to combining an adherence compound and transferring more than one embryo. Further studies of adherence compounds with single embryo transfer need to be undertaken.

PLAIN LANGUAGE SUMMARY

In IVF, does transferring the embryo in media containing high concentrations of hyaluronic acid result in more live births?

What is IVF?

In vitro fertilisation (IVF) is a fertility treatment that helps people with fertility problems to have a baby. During IVF an egg from a woman's ovaries is fertilised with sperm in a laboratory. The egg can be placed in a dish with multiple sperm to fertilise it, or a single sperm can be injected directly into it (intracytoplasmic sperm injection; ICSI). The fertilised egg (an embryo) is then placed (implanted) into the woman's womb to grow and develop.

The embryo is transferred to the womb in a special transfer media, a solution containing compounds that help the embryo stick (adhere) successfully to the inside of the womb (implantation). Hyaluronic acid is a natural compound found in the body that acts as a binding and protective agent in tissues. It is often added to embryo transfer media to help implant the embryo.

Why we did this Cochrane Review

We wanted to find out whether using transfer media with high concentrations of adherence compounds for embryo transfer such as hyaluronic acid improves success in implanting embryos, resulting in more live births.

What did we do?

We searched for studies that investigated the use of embryo transfer media containing different concentrations of hyaluronic acid in IVF/ICSI.

We looked for randomised controlled studies in which the treatments received are decided at random, because these studies usually give the most reliable evidence about the effects of a treatment. We assessed the evidence by looking at how the studies were conducted, study sizes, and whether study findings were consistent.

Search date: we included evidence published up to January 2020.

What we found

We found 26 studies including 6704 women aged 27 to 35 years who underwent IVF/ICSI. These studies compared embryo transfer using media containing high concentrations of hyaluronic acid versus solutions containing no or low concentrations of hyaluronic acid.

We were interested in learning how the concentration of hyaluronic acid in the transfer solution affected the numbers of:

· live births;

· miscarriages (loss of pregnancy before 20 weeks' gestation);

· clinical pregnancies;

· multiple pregnancies; and

· adverse (unwanted) events.

What are the results of our review?

Embryo transfer using media with high concentrations of hyaluronic acid probably increases the number of live births compared with using solutions with low concentrations or no hyaluronic acid (10 studies). If transfer media with low concentrations or no hyaluronic acid have a 33% chance of resulting in a live birth, solutions with high concentrations increase the chance of a live birth to between 37% and 44%. There would probably be 1 additional live birth for every 14 embryos transferred in a high-concentration hyaluronic acid solution.

High concentrations of hyaluronic acid in the embryo transfer solution probably also increase the number of clinical pregnancies (17 studies) and the number of multiple pregnancies (7 studies).

Using transfer solutions containing high concentrations of hyaluronic acid may result in slightly fewer miscarriages (7 studies). But our analysis did not show a clear difference if we left out studies whose results varied widely.

Reported adverse events included ectopic pregnancies (when an embryo becomes implanted outside the womb) and abnormalities affecting the embryo or the foetus. Similar numbers of adverse events were reported for both types of transfer solution (high and low concentrations of hyaluronic acid): we found no evidence that the concentration of hyaluronic acid in the transfer solution affected the number of adverse events reported.

How reliable are these results?

We are moderately confident about our results for the numbers of live births, clinical pregnancies, and multiple pregnancies. Our results may change if further evidence becomes available.

We are less confident about the rate of miscarriage and the number of adverse events, because results for these varied widely. Our results are likely to change if further evidence becomes available.

Conclusions

Embryo transfer using solutions containing high concentrations of hyaluronic acid probably increases the number of live births in IVF/ICSI. Transfer solutions containing high concentrations of hyaluronic acid may slightly decrease the rate of miscarriage.

SUMMARY OF FINDINGS

Summary of findings 1. High versus low or no hyaluronic acid for assisted reproductive technologies

High versus low or no hyaluronic acid for assisted reproductive technologies

Population: couples undergoing embryo transfer
Settings: assisted reproduction
Intervention: high hyaluronic acid
Comparison: low or no hyaluronic acid

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of evidence (GRADE)	Number needed to treat/ harm (NNTB/ NNTH)
	Assumed risk	Corresponding risk				
	Low or no hyaluronic acid	High hyaluronic acid				
Live birth rate - high vs low or no hyaluronic acid	**333 per 1000**	**403 per 1000 (370 to 436)**	**RR 1.21** (1.11 to 1.31)	4066 (10 studies)	⊕⊕⊕⊖ **moderate** [a]	14
Miscarriage rate	**118 per 1000**	**97 per 1000 (79 to 118)**	**RR 0.82** (0.67 to 1.00)	3091 (7 studies)	⊕⊕⊖⊖ **low** [b,c]	48
Clinical pregnancy rate - high vs low or no hyaluronic acid	**402 per 1000**	**466 per 1000 (438 to 494)**	**RR 1.16** (1.09 to 1.23)	5247 (17 studies)	⊕⊕⊕⊖ **moderate** [d]	16
Multiple pregnancy rate	**126 per 1000**	**183 per 1000 (156 to 214)**	**RR 1.45** (1.24 to 1.70)	3337 (7 studies)	⊕⊕⊕⊖ **moderate** [b]	18
Adverse event rate	**19 per 1000**	**16 per 1000 (8 to 35)**	**RR 0.86** (0.40 to 1.84)	1487 (3 studies)	⊕⊕⊖⊖ **low** [e]	N/A

*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).
CI: confidence interval; N/A: not applicable; RR: risk 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]Downgraded once for high risk of bias - all studies except three at high risk of bias in one or more domains.
[b]Downgraded once for imprecision - small number of events and wide confidence interval.
[c]Downgraded once for substantial heterogeneity: $I^2 = 66\%$.
[d]Downgraded once for moderate heterogeneity: $I^2 = 40\%$.
[e]Downgraded twice for imprecision - small number of events and wide confidence interval.

BACKGROUND

Description of the condition

The first in vitro fertilisation (IVF) baby was born in 1978. Much progress has been made in the intervening years in assisted reproductive technology (ART) to improve live birth outcomes for subfertile couples. Embryo implantation into the lining of the endometrium is one of the major determining factors in successful human IVF (Gardner 2003). Much research has therefore focused on the interaction between the embryo and the endometrium at the time of implantation. The composition of the medium surrounding the embryo at the time of IVF transfer is considered to be important at this crucial stage of development. For these reasons, studies have been conducted on adding specific adherence compounds to the embryo transfer medium and effects of these compounds on implantation and pregnancy rates.

Description of the intervention

The literature describes multiple adhesion molecules that have been examined, including albumin, fibrin, and hyaluronic acid (HA) (Ben-Rafael 1995; Bungum 2002; Fancsovits 2015). The mechanisms for how these work are discussed in the next section. Studies included in this review examined how the addition of functional concentrations of adherence compounds, such as HA, to embryo transfer media affects pregnancy outcomes. Embryo transfer can occur anytime between day 2 and day 6 after in vitro culturing. Multiple factors may affect the influence of HA; therefore these factors will be analysed as subgroups. These include length of exposure to HA, day of transfer, frozen or fresh embryos, single or multiple embryo transfer, and participant groups with a poor versus good prognosis.

How the intervention might work

Albumin traditionally has been used as the main macromolecule in most embryo culture media. It serves as a source of hormones, energy, and vitamins and enables easier handling by increasing embryo culture viscosity (Fancsovits 2015; Simon 2003). Albumin in embryo culture media is associated with better pregnancy rates (Bungum 2002). However, serum albumin, which is derived from blood, is not a pure substance and carries a risk of viral contamination. Although the risks associated with a biologically derived product have been overcome in part by recombinant human serum albumin (Lane 2003), HA has largely replaced albumin as the sole macromolecule in an embryo transfer medium, resulting in high pregnancy rates (Simon 2003).

Another implantation-enhancing molecule that has been introduced into transfer media is fibrin in the form of a two-component fibrin sealant, which consists of fibrinogen and thrombin, together with a fibrinolysis inhibitor (aprotinin). Fibrin sealant is a viscous solution that quickly and firmly adheres to tissue and therefore was added to decrease the possibility of embryo expulsion and ectopic pregnancies (Ben-Rafael 1995; Feichtinger 1990). Fibrin sealant seemed to have an effect on the pregnancy rate only in older women (39 to 42 years of age) (Bar-Hava 1999; Ben-Rafael 1995). It was suggested that fibrinolysis provoked by the presence of fibrin in utero may cause chemical absorption of the zona pellucida's membrane, which is thickened in older women, resulting in hatching of the embryo. Other possible explanations for the beneficial effect of fibrin sealant is that the enhanced adhesive quality of the embryo surface facilitates the initial implantation process.

The main adhesion compound studied in randomised controlled trials is hyaluronic acid (Bontekoe 2014). Hyaluronic acid is a naturally existing molecule and is one of the major macromolecules present in the female reproductive tract. It is present in the human endometrium (Salamonsen 2001), and its levels have been shown to increase dramatically on the day of implantation in mice (Carson 1987). Both human and animal studies show that adding HA to the transfer medium significantly increases implantation rates and enhances foetal development when compared with no HA in the transfer medium (Gardner 1999; Valojerdi 2006). HA has several properties that make it a candidate for an implantation-enhancing molecule. Hyaluronic acid increases cell-to-cell adhesion and cell-to-matrix adhesion (Turley 1984). It is secreted by cumulus granulosa cells and is found in uterine, oviductal, and follicular fluids (Fancsovits 2015). It produces a viscous solution that can enhance the embryo transfer process and prohibit expulsion (Stojkovic 2002), and it may facilitate diffusion and integration of the embryo in intrauterine secreted fluid (Simon 2003). The viscosity alone, however, does not explain involvement of HA in implantation, as not all highly viscous solutions (such as human placental collagen) can improve implantation (Menezo 1989). HA also has autocrine and paracrine functions that act on CD44 receptors, which could explain its effect on implantation. The primary receptor for HA is CD44, which is expressed both on the pre-implantation embryo and in the stroma of the human endometrium (Behzad 1994; Campbell 1995), where peak concentrations of both HA and its CD44 receptor occur when the endometrium is most receptive to embryo implantation (Afify 2006). HA is known to have a role in regulating proliferation, differentiation, migration, and gene expression, and it may even have important roles in natural endometrial decidualisation and implantation and in normal embryo development (Fancsovits 2015). HA may have an effect on development of the embryo itself. Bovine studies showed that HA improved the developmental capacity of embryos by increasing the number of trophectoderm cells and the total number of cells of expanded blastocysts (Stojkovic 2002). The increase in embryo quality attained by HA was shown to be dependent on CD44 activity and mitogen-activated protein kinase (MAPK) signalling (Marei 2013). Furthermore, HA maintains viability on frozen embryos after thaw, and this was associated with an increased implantation rate (Gardner DK 2003; Lane 2003).

Why it is important to do this review

Because the rate of human implantation (and consequent pregnancy and delivery) is innately low, at between 10% and 30% (Gardner 2004), it is often difficult to establish small but significant improvements. Such improvements in pregnancy and birth rates are crucial in light of the high and increasing number of assisted reproductive technology (ART) cycles per year, especially if the intervention is relatively simple, such as the addition of HA. Any improvement in the implantation rate may lead to a reduction in the need to transfer multiple embryos, with a subsequent decrease in multiple pregnancy, maximising the chance of pregnancy while decreasing pregnancy complications. Systematic meta-analysis of all randomised controlled trials (RCTs) is therefore an important tool for assessing whether an innovation represents a true technological advancement. This is an update of a Cochrane Review first published in 2010, and previously updated in

2014, which showed beneficial effects of HA on clinical pregnancy and live birth and no change in the miscarriage rate. Since then, multiple studies have been published and need to be incorporated into the review.

OBJECTIVES

To determine whether adding adherence compounds to embryo transfer media could improve pregnancy outcomes, including improving live birth and decreasing miscarriage, in women undergoing assisted reproduction.

METHODS

Criteria for considering studies for this review

Types of studies

We included all randomised controlled trials (RCTs) comparing embryo transfer media containing high concentrations of adherence compounds versus embryo transfer media with no or low concentrations of adherence compounds. We did not include quasi-randomised trials. We included cross-over trials in the review only for completeness because the cross-over design is not valid in the context of subfertility trials (Vail 2003). Therefore, we included only data from the first phase.

Types of participants

Women undergoing embryo transfer after in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI), or an embryo thaw cycle for therapeutic reasons, or after oocyte donation.

Types of interventions

All known culture methods for IVF and/or ICSI comparing embryo transfer media containing functional concentrations of adherence compounds versus embryo transfer media with non-functional amounts of such adherence compounds. For clarification, HA groups are labelled as high (0.5 mg/mL), low (0.125 mg/mL), or no HA (0.0 mg/mL). Two- to six-day embryo transfers, as well as both fresh and frozen embryos, were included.

Types of outcome measures

Primary outcomes

- Live birth rate per randomly assigned woman
- Miscarriage rate per randomly assigned woman: defined as spontaneous loss of clinical pregnancy before 20 weeks' gestation

Secondary outcomes

- Clinical pregnancy rate per randomly assigned couple: defined as the number of clinical pregnancies (demonstrated by the presence of a gestational sac on ultrasound scan) per randomly assigned couple
- Multiple pregnancies per randomly assigned couple
- Total adverse events including ectopic pregnancies, foetal or congenital defects, and pelvic inflammation or other adverse events per randomly assigned couple

Additional outcome measures

- Implantation rate: defined as the number of gestational sacs divided by the number of embryos transferred
- Data on implantation rate cannot be pooled in a meta-analysis together with other outcome measures because of the difference in denominators (Vail 2003). “Implantation rate” is defined per number of embryos transferred, and other outcome measures are defined per randomly assigned couple. However, because of the frequency with which implantation rate is reported in the literature, and the fact that embryo transfer is a crucial step for investigating the effect of adherence compounds on implantation, it was decided to analyse these data separately for completeness

Search methods for identification of studies

All published and unpublished RCTs, on the addition of an adherence compound to the embryo transfer medium versus use of transfer medium devoid of an adherence compound, were sought using the following search strategies, with no language restrictions and in consultation with the Cochrane Gynaecology and Fertility Group Information Specialist.

Electronic searches

The following electronic databases, trial registers, and websites were searched, using the search terms provided in the appendices;

- Cochrane Gynaecology and Fertility Group Trials Register; Procite Platform, searched 7 January 2020 (Appendix 1)
- CENTRAL via the Cochrane Register of Studies Online (CRSO); Web platform, 7 January 2020 (Appendix 2)
- MEDLINE; OVID platform, searched from 1946 to 7 January 2020 (Appendix 3)
- Embase; OVID platform, searched from 1980 to 7 January 2020 (Appendix 4)
- PsycINFO; OVID platform, searched from 1806 to 7 January 2020 (Appendix 5).

Other electronic sources of trials that were searched were as follows;

- The Cochrane Library (www.cochrane.org/index.htm)
- Trial registers for ongoing and registered trials: ClinicalTrials.gov, a service of the US National Institutes of Health (http://clinicaltrials.gov/ct2/home); the World Health Organization International Trials Registry Platform search portal (www.who.int/trialsearch/Default.aspx)

Searching other resources

We handsearched appropriate journals and reference lists of trial reports retrieved by the search. Furthermore, we handsearched European Society of Human Reproduction & Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) supplements, and we contacted experts and manufacturers of transfer media, including adherence compounds, to obtain additional relevant data.

Data collection and analysis

Selection of studies

Two review authors (DH and LV) performed a selection of trials by scanning titles and abstracts retrieved from the search and removing those that were clearly irrelevant. The full text of all trials considered to be potentially eligible was retrieved. Two review

authors (DH and LV) independently examined the full-text articles for compliance with the inclusion criteria and selected eligible studies for inclusion in the review. When required, review authors corresponded with study investigators to clarify study eligibility. Disagreements on eligibility were resolved by consensus. Excluded articles are detailed in the Characteristics of excluded studies table. The included trials were assessed against risk of bias criteria and for methodological details. This information is presented in the Characteristics of included studies table and provides context for assessing the reliability of results.

Data extraction and management

Two review authors (DH and LV) independently extracted data by using a data extraction form designed and pilot-tested by the review authors (Appendix 5). If disagreements could not be resolved by consensus, a third review author (ZS) was available to resolve any discrepancies. Additional information on trial methods or on actual original trial data was requested from the authors of trials that appeared to meet eligibility criteria to clarify any aspects of methods or to obtain data in a suitable form. Reminder correspondence was sent when a reply was not received within several weeks. When studies had multiple publications, the main trial report was used as the reference and was supplemented by additional details from secondary papers. Authors ZS and YO reviewed the final draft.

Assessment of risk of bias in included studies

Two review authors (DH and LV) independently assessed the included studies for risk of bias using the Cochrane 'Risk of bias assessment tool' to assess selection (random sequence generation and allocation concealment); performance (blinding of participants and personnel); detection (blinding of outcome assessors); attrition (incomplete outcome data); reporting (selective reporting); and other bias (Higgins 2011). Judgements will be assigned as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions*, Section 8.5 (Higgins 2011). Disagreements were resolved by reaching consensus or by contacting a third review author (ZS). All judgements are fully described. Conclusions are presented in the risk of bias figures and are incorporated into the interpretation of review findings.

Similarity between treatment and control groups in culture and transfer media was assessed by checking with the media manufacturers and by ensuring that all parameters up to the moment of embryo transfer were comparable between groups.

With the addition of an adherence compound to the embryo transfer medium, it was important to report multiple pregnancies when the embryo transfer policy consisted of transferring multiple embryos per treatment cycle. It can be considered to be a bias risk when study authors failed to report the multiple pregnancy rates in these cases, because they had ignored a higher risk of the adverse event of a multiple pregnancy. Calculating the implantation rate can overcome this bias.

Measures of treatment effect

Dichotomous data (e.g. clinical pregnancy rate) outcomes from each study were expressed as risk ratios (RRs) with 95% confidence intervals (CIs) and, when possible, were combined for meta-analysis with Review Manager 2014 software using the Mantel-Haenszel method. All measured outcomes yielded dichotomous data, so analysis of continuous and ordinal data was not required.

Unit of analysis issues

The primary analysis of the review was expressed as per randomly assigned couple. Reported data that did not allow valid analysis (e.g. per embryo transfer) were presented in meta-view but were not pooled. Most included trials reported their results per randomly assigned woman or participant. When possible, reported multiple live births were counted as a single live birth event. Only first-phase data from cross-over trials were included. However, all included trials were parallel-group RCTs. When possible, data were analysed via intention-to-treat (ITT) analysis. The number of couples randomly assigned was used as the denominator.

Dealing with missing data

Data were analysed on an ITT basis as much as possible, and the original investigators were contacted regarding missing data. If unavailable, we undertook the imputation of individual values for the primary outcome only. Live births were regarded not to have occurred if not reported.

Only available data were analysed. Therefore, any imputation undertaken was subjected to sensitivity analysis.

Success rates of subfertility treatments can be affected by the number of treatment cycles and mostly by the woman's age (Schröder 2004). Study outcomes can be affected by participants enrolling in studies with multiple treatment cycles; this can create uncertainty about the number of cycles per participant. The number of cycles per participant generally was not stated in the articles. When not mentioned, original investigators were contacted for information on the number of cycles undertaken by participants in the trial in an attempt to resolve this matter.

Assessment of heterogeneity

Heterogeneity was considered by the review authors when clinical and methodological characteristics of the included studies were similar enough that a meta-analysis could provide a meaningful summary. Statistical analyses were performed in accordance with the guidelines for statistical analysis developed by Cochrane (Higgins 2011). Heterogeneity between results of different studies was assessed by using the I^2 statistic, which can be interpreted in the following broad terms.

- 0% to 40%: might not be important.
- 30% to 60%: represents moderate heterogeneity.
- 50% to 90%: represents substantial heterogeneity.
- 75% to 100%: represents considerable heterogeneity (Higgins 2011).

In cases of substantial or considerable heterogeneity, explanations were sought, including those involving the sensitivity analyses performed for the primary outcome measures. We planned to look at the possible contribution of differences in trials, for example, transfer of embryos on different days. When possible, the outcomes were pooled.

Assessment of reporting biases

Review authors aimed to minimise the potential impact of publication and reporting biases by performing comprehensive

searches for eligible studies and looking for data duplication. If 10 or more studies were included in an analysis, a funnel plot was used to investigate the possibility of small-study effects (the tendency for estimates of the intervention effect to have a bigger impact in smaller studies).

When included studies did not report the primary outcome measure of live births or interim outcomes such as clinical pregnancies, informal assessment was undertaken to check whether those studies reporting primary outcome measures reflected typical findings for the interim outcomes.

Assessment of reporting biases was addressed in the Included studies portion of the Main results section. See Other potential sources of bias.

Data synthesis

Data from primary studies were combined using a fixed-effect model for the following comparisons.

- Embryo transfer medium with inclusion of adherence compounds versus embryo transfer medium without such adherence compounds added, or with a lower concentration, stratified as follows.
    - High concentration versus low concentration or no hyaluronic acid.
    - Fibrin sealant versus no fibrin sealant.

As described in the Background section under How the intervention might work, the clinical trials included control groups that were completely devoid of HA or had low levels of HA (often also present in culture media). Based on the results of the previous Cochrane meta-analysis, consideration was given to combining these trials in the current review as a primary analysis for the overall treatment effect.

An increase in the risk of a particular outcome, either a beneficial effect or a detrimental effect, is displayed graphically in the meta-analyses to the right of the centre line, and a decrease in the risk of an outcome is displayed to the left of the centre line.

Subgroup analysis and investigation of heterogeneity

The following five subgroup analyses were performed.

- A: cleavage versus blastocyst stage.
- B: fresh versus frozen.
- C: time of exposure (up to and including 10 minutes versus longer than 10 minutes).
- D: number of embryos (single versus 2 or more).
- E: poor responders versus general population.

In humans, transferring the embryo back into the uterus can be performed after two, three, four, five, or six days of in vitro culturing. The day of transfer itself might be important, as it is not clear whether the small volume of adherence compound in media transferred on days 2 to 4 would still be present and would have a potential effect on the later days of implantation (days 5 to 6) (Simon 2003). However, adherence compounds in the media may play an important role at this early stage because of their physical properties and may prohibit expulsion. Therefore, in this review, the influence of the day of embryo transfer is analysed as a subgroup. It was not known whether inclusion of HA in the transfer media provides any added benefit in frozen embryos compared with fresh embryos, or vice versa. Therefore, fresh and frozen-thawed embryos are analysed as subgroups.

The effect of exposure time of the embryo to adherence compounds before embryo transfer is analysed in a third subgroup. It is possible that length of exposure to adherence compounds before the day of implantation (days 5 to 6) may have an impact on the outcome. Many included studies are expected to use EmbryoGlue, which contains HA, as the adherence compound. Therefore, it was decided that an exposure time of 10 minutes should be used as the cut-off point for this subgroup analysis. This is the time recommended by the manufacturer (Vitrolife; Gothenburg, Sweden). The outcomes of studies in which embryos were exposed to adherence compounds for up to 10 minutes are compared with the outcomes of studies in which embryos were exposed for longer periods.

It is very important to determine whether the combination of adherence compounds and an embryo transfer policy of transferring multiple embryos per treatment cycle affects outcome measures, especially multiple pregnancies and adverse event rates. Therefore, a fourth subgroup analysis compares different embryo transfer policies. Trials on single embryo transfer are also compared with trials in which a mean of two or more embryos were transferred.

The fifth and final subgroup analysis includes a comparison of participant groups with different prognoses. The outcomes of studies that actively selected poor prognosis participants on the basis of age, number of previous treatment failures, and, in some trials, embryo quality are compared with the outcomes of studies that selected good prognosis participants and studies with unselected participants.

Sensitivity analysis

Sensitivity analyses were performed for the two primary outcomes - live birth and miscarriage - to verify whether arbitrary decisions regarding study eligibility and data analysis could have impacted the results. The following parameters were examined.

- Eligibility was restricted to studies without high risk of bias. When a study was assessed as 'unclear risk' or 'high risk' in one of the following domains - adequate sequence generation, allocation concealment, or blinding - it no longer had low risk of bias.
- Studies with outlying results were excluded. Outlying results were those that caused heterogeneity because they differed too much from the other results included in the meta-analysis.
- Alternative imputation strategies were adopted.
- A random-effects model was adopted.
- Studies using a functional adherence compound concentration different from 0.5 mg/mL in the treatment group were excluded.

When sensitivity analyses identified particular data that greatly influenced the findings of the review, we tried to resolve uncertainties. This led the review authors to conclude that further research is mandated.

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

We generated summary of findings tables using GRADEpro GDT. These tables evaluated the overall quality of the body of evidence for main review outcomes (live birth, miscarriage, clinical pregnancy, multiple pregnancy, and total adverse events) using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness, and publication bias) (Higgins 2011). Judgements about evidence quality (high, moderate, or low) were justified, documented, and incorporated into the reporting of results for each outcome.

RESULTS

Description of studies

Results of the search

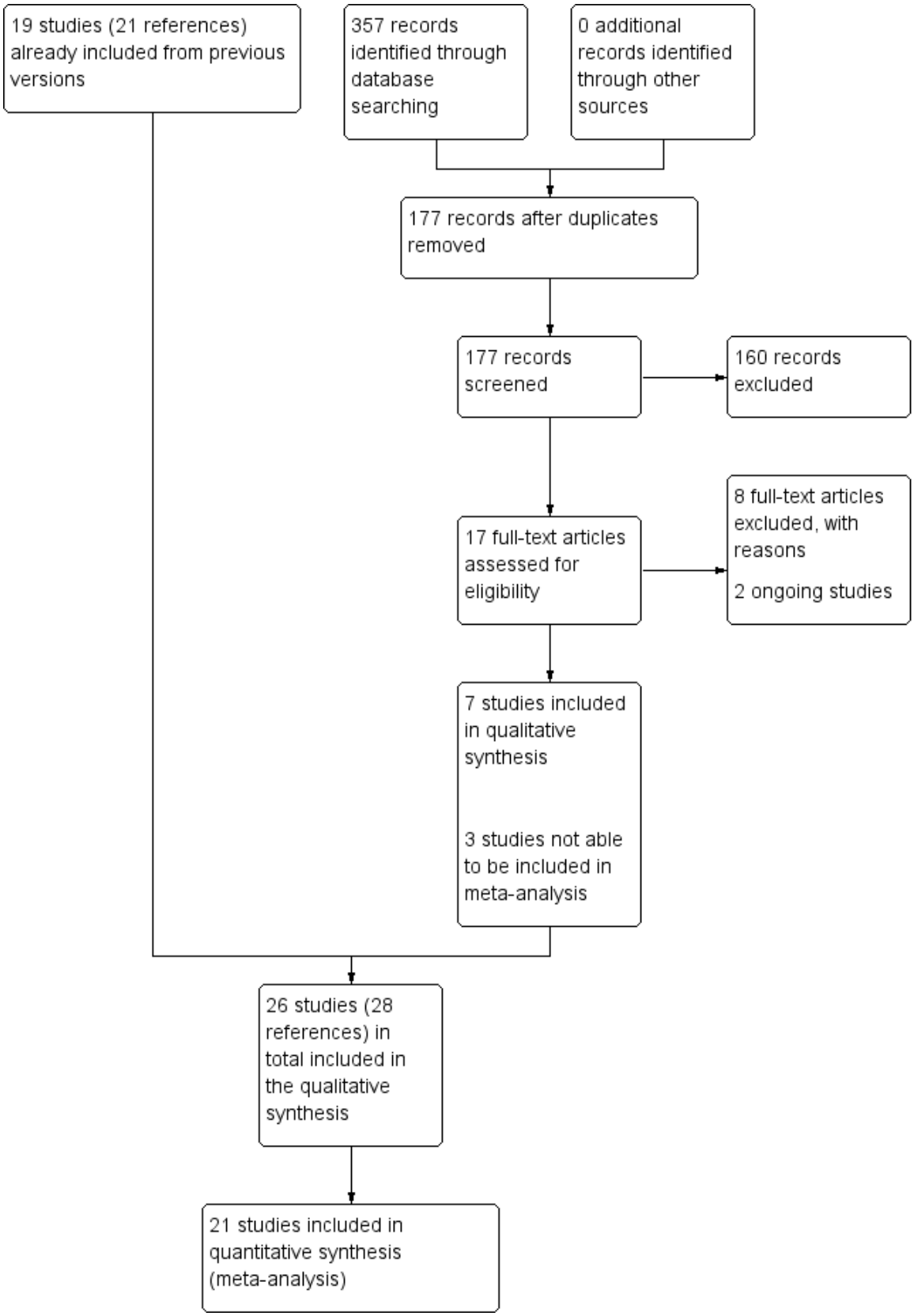
A total of 357 studies were located using the search strategies; 177 were screened after duplicates were removed in 2020 (Appendix 1; Appendix 2; Appendix 3; Appendix 4; Appendix 5). During the search update of January 2020, 17 potentially eligible trials that appeared to meet the basic inclusion criteria were identified. After further in-depth eligibility assessment, data examination, and contacting of principal investigators, eight of the potentially eligible studies were excluded and two were ongoing, resulting in seven additional included studies.

Nineteen studies (21 publications) were included in the last review (published in 2012). The search update from 2020 resulted in the inclusion of seven new studies (Drew 2014; Fancsovits 2015, Fasano 2016; Kandari 2019; Kleijkers 2016, Ten 2019; Yung 2019), which are incorporated within the current review.

Five new potentially eligible trials were found to be quasi-randomised upon further in-depth analysis (Nakagawa 2012; Nishihara 2017; Perez 2019; Schiewe 2013; Tomari 2014).

See Figure 1 and Characteristics of excluded studies for details of the screening and selection process.

Figure 1. Study flow diagram.



Two ongoing studies with no published outcomes were found (Mowafy 2016; Oxford Fertility 2017).

Only one study involving 211 participants compared the effect of fibrin sealant in the transfer medium versus the effect of a medium without fibrin sealant (Ben-Rafael 1995). Participants could enrol in the trial for one treatment cycle. A total of 759 embryos were transferred. No trials that compared fibrin sealant with a lower concentration of fibrin were found. Because of this paucity of data, fibrin was removed from the 'Summary of findings' table, and the title was changed from adhesion compounds to hyaluronic acid.

Included studies

Twenty-six studies with a total of 6704 participants were included (see Characteristics of included studies). Not all published data could be used for analysis (Appendix 6).

Four studies reported outcomes as percentages alone (Fasano 2016; Friedler 2005; Khan 2004; Walker 2005). See the Characteristics of included studies table for further information.

Morbeck 2007 did not publish actual data because the study was suspended prematurely. The data were retrieved by contacting the principal author. Chen 2001 reported only the biochemical pregnancy rate, which is not an outcome measure for this review.

In three studies, cycles - not women - were randomised (Drew 2014; Fasano 2016; Ten 2019); therefore these study data could not be used in the meta-analysis, except for data on implantation rate, which is defined per number of embryos transferred. Fancsovits 2015 randomised by cycle but first cycle data were retrieved by contacting the principal author; this study was included in the meta-analysis.

Twelve of the included studies reported implantation rates as well. However, the data on this outcome measure could not be used in a meta-analysis because the denominator in this analysis is the number of embryos transferred, rather than the number of randomly assigned couples.

Study characteristics

All included studies were RCTs that compared the results of an intervention group versus those of a control group. Methods of participant sampling varied between studies. Eleven studies recruited participants consecutively (Balaban 2004; Ben-Rafael 1995; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Kleijkers 2016; Korošec 2007; Morbeck 2007; Urman 2008), one study in non-consecutive order (Simon 2003), and the rest using an unclear method. Hazlett 2008 reported both consecutive and non-consecutive sampling in different publications of the same trial.

Fourteen were single-centre studies (Balaban 2004; Chen 2001; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Hazlett 2008; Khan 2004; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Urman 2008; Yakin 2004), and seven were multi-centre trials. Ten of the included studies were performed in part at academic medical centres (Ben-Rafael 1995; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Kleijkers 2016; Korošec 2007; Mahani 2007; Simon 2003). Five studies were performed in Israel (Ben-Rafael 1995; Friedler 2005; Friedler 2007; Ravhon 2005; Simon 2003), four in the United States (Hazlett 2008; Khan 2004; Morbeck 2007; Schoolcraft 2002), three in Turkey (Balaban 2004; Urman 2008; Yakin 2004), two in Hungary (Fancsovits 2011; Fancsovits 2015), one in China (Yung 2019), one in Germany and Switzerland (Dittmann-Műller 2009), one in India (Kandari 2019), one in Iran (Mahani 2007), one in the Netherlands (Kleijkers 2016), one in Slovenia and Austria (Korošec 2007), one in Spain (Ten 2019), and one in Taiwan (Chen 2001).

Ten studies used strict inclusion and exclusion criteria for participant selection (Ben-Rafael 1995; Friedler 2005; Friedler 2007; Hazlett 2008; Kandari 2019; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Simon 2003) (see Characteristics of included studies). These focused mainly on the woman's age and the number of previous treatment cycles. For example, Simon 2003 included only women up to 35 years of age with a maximum of three previous treatment failures. Six studies performed a power calculation to determine sample size (Friedler 2007; Hazlett 2008; Korošec 2007; Kleijkers 2016; Morbeck 2007; Urman 2008) (see Characteristics of included studies).

Participants

The age of participants was reported as a mean with a standard deviation or as a range. Mean age ranged from 27.5 to 35.8 years. Three studies did not report participants' ages (Dittmann-Műller 2009; Schoolcraft 2002; Ten 2019). Age analysis was performed in eight studies (Ben-Rafael 1995; Fancsovits 2011; Fancsovits 2015; Kandari 2019; Kleijkers 2016; Morbeck 2007; Urman 2008; Yung 2019). Ben-Rafael 1995 divided participants into subgroups of younger than 31 years of age, 31 to 38 years of age, and 39 to 42 years of age. Morbeck 2007 and Urman 2008 compared outcomes in women younger than 35 years versus those in women aged 35 or older, and Fancsovits 2011 and Fancsovits 2015 compared participants up to 40 years of age versus older participants (see Characteristics of included studies).

Regarding the number of treatment cycles per participant, after contact was made with the original authors, three studies were found to enrol patients in multiple treatment cycles (Balaban 2004; Hazlett 2008; Korošec 2007). Seven studies allowed only a single cycle per participant (Dittmann-Műller 2009; Friedler 2005; Friedler 2007; Morbeck 2007; Simon 2003; Urman 2008; Yakin 2004), and the policy of the other studies remains unclear. Information on the number of embryos transferred can be found under Characteristics of included studies.

Nine studies reported the primary cause of subfertility of study participants (Balaban 2004; Ben-Rafael 1995; Dittmann-Műller 2009; Fancsovits 2015; Friedler 2007; Kandari 2019; Kleijkers 2016; Korošec 2007; Urman 2008) (see Characteristics of included studies). Eight studies reported the mean duration of subfertility for participants before the start of the study (Balaban 2004; Ben-Rafael 1995; Dittmann-Műller 2009; Kandari 2019; Kleijkers 2016; Mahani 2007; Ravhon 2005; Urman 2008) (see Characteristics of included studies).

Thirteen studies reported the mean number of previous subfertility treatments that participants received as an inclusion criterion or as a study measure (Balaban 2004; Ben-Rafael 1995; Dittmann-Műller 2009; Friedler 2005; Friedler 2007; Kandari 2019; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Ravhon 2005; Simon 2003; Urman 2008) (see Characteristics of included studies).

Interventions

Embryo transfer in medium containing high versus low or no hyaluronic acid

Twenty-five studies comparing transfer medium containing HA versus transfer medium with low or no HA were included in this comparison (Balaban 2004; Balaban 2011; Chen 2001; Dittmann-Műller 2009; Drew 2014; Fancsovits 2011; Fancsovits 2015; Fasano 2016; Friedler 2005; Friedler 2007; Hazlett 2008 (days 3 and 5); and Kandari 2019; Khan 2004; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Ten 2019; Urman 2008; Walker 2005; Yakin 2004; Yung 2019). However, the results of seven studies could not be pooled (Chen 2001; Drew 2014; Fasano 2016; Friedler 2005; Khan 2004; Ten 2019; Walker 2005) (see Description of studies above and Characteristics of included studies), resulting in 20 studies with a total of 5568 participants analysed.

Of studies in the meta-analysis examining the effects of HA, 10 of the 20 studies, with a total of 2043 participants, compared transfer medium containing HA versus transfer medium without HA. In eight studies, the HA medium was specified to be 0.5 mg/mL HA (Friedler 2005; Friedler 2007; Hazlett 2008 (day three); and Kandari 2019; Khan 2004; Korošec 2007; Mahani 2007; Simon 2003). Kleijkers 2016 mentioned that one of the differences between the media used was the addition of HA but did not specify the concentration. Of the eight studies, four in this comparison used comparable embryo culture medium in both study arms up to the time of embryo transfer (Hazlett 2008 (day 3); and Khan 2004; Korošec 2007; Simon 2003). In five studies, it remains unclear whether the embryo culture media were comparable (Friedler 2005; Friedler 2007; Kandari 2019; Kleijkers 2016; Mahani 2007).

Twelve of the 20 studies with a total of 3525 participants compared high HA (0.5 mg/mL) versus low HA (0.125 mg/mL) (Balaban 2004; Balaban 2011; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Hazlett 2008 (day 5); and Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Urman 2008; Yakin 2004; Yung 2019). The data from Hazlett 2008 were divided into two subgroups for analysis: day 3 and day 5 embryo transfers. The day 3 subgroup compared HA in the transfer medium versus no HA in the medium. The day 5 subgroup compared high (0.5 mg/mL) versus low concentrations of HA (0.125 mg/mL). Transfer and culture media of the treatment and control groups in all these studies were comparable, except for Yung 2019, for which this information remained unclear.

Embryo transfer in medium containing fibrin sealant versus embryo transfer in medium with no fibrin sealant

One study examined the effect of transfer in medium with fibrin sealant versus transfer in medium without fibrin and included 211 participants (Ben-Rafael 1995). The transfer media used in treatment and control groups of this study were obtained from different manufacturers, and it is unclear whether the embryo culture medium was similar in the two groups (see Characteristics of included studies).

Further study design details

Timing of randomisation

Nine studies randomised participants to treatment or control arms on the day of embryo transfer (Balaban 2004; Friedler 2007; Kandari 2019; Korošec 2007; Mahani 2007; Ravhon 2005; Simon 2003; Urman 2008; Yakin 2004). Two studies performed randomisation before commencement of the treatment cycle (Morbeck 2007; Kleijkers 2016), and another between commencement of treatment and a fertilisation check (Dittmann-Műller 2009). Four studies randomly assigned participants between fertilisation check and the day of embryo transfer (Ben-Rafael 1995; Fancsovits 2011; Fancsovits 2015; Yung 2019). Timing of randomisation remains unclear in six studies (Chen 2001; Friedler 2005; Hazlett 2008; Khan 2004; Schoolcraft 2002; Ten 2019). Hazlett 2008 was inconsistent in describing the timing of randomisation in different publications of the same trial.

Duration of exposure to adherence compound

Seven studies exposed embryos in the treatment group to the adherence compound for up to 10 minutes before the transfer was made (Fancsovits 2011; Fancsovits 2015; Friedler 2007; Khan 2004; Mahani 2007; Schoolcraft 2002; Simon 2003). Seven studies exposed embryos in the treatment group to the adherence compounds for longer than 10 minutes (Balaban 2004; Dittmann-Műller 2009; Hazlett 2008 (days 3 and 5); and Kleijkers 2016; Korošec 2007; Morbeck 2007; Urman 2008). Exposure time remains unclear in the other eight studies (Ben-Rafael 1995; Chen 2001; Friedler 2005; Kandari 2019; Ravhon 2005; Ten 2019; Yakin 2004; Yung 2019).

Timing of embryo transfer: cleavage versus blastocyst stage

Thirteen studies performed the transfer at the cleavage stage of embryo development (days 2 to 3) (Ben-Rafael 1995; Chen 2001; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Khan 2004; Mahani 2007; Morbeck 2007; Schoolcraft 2002; Simon 2003; Yakin 2004). Three studies performed the transfer at the blastocyst stage of embryo development (day 5 and later) (Balaban 2004; Korošec 2007; Ten 2019). Five studies performed transfers at both cleavage and blastocyst stages (Hazlett 2008; Kandari 2019; Kleijkers 2016; Urman 2008; Yung 2019). Data from two of these trials could be analysed separately for the subgroup analysis on timing of the intervention (Hazlett 2008; Urman 2008). However, in Kleijkers 2016, one hospital transferred some of their embryos on day 5, but the rest of the embryos in this study were transferred between days 2 and 4. Data for the hospital with the blastocyst transfer protocol were not provided separately; therefore Kleijkers 2016 was excluded from this subgroup analysis. Similarly, Yung 2019 and Kandari 2019 were excluded from this subgroup analysis because only abstracts were available for these studies and separate results for cleavage and blastocyst stage embryos were not provided.

Fresh versus frozen-thaw protocol

Three studies transferred embryos only after following a frozen-thaw protocol (Morbeck 2007; Simon 2003; Yakin 2004). Two studies included both fresh and frozen-thawed embryos (Korošec 2007; Yung 2019). Data from Korošec 2007 were analysed separately for the subgroup analysis on frozen-thawed versus fresh embryos, but separate data from Yung 2019 were not available. Nine studies transferred only fresh embryos (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2011; Friedler 2007; Hazlett 2008 (days 3 and 5); and Mahani 2007; Kandari 2019; Ravhon 2005; Urman 2008). Procedures in the other studies remain unclear.

Number of embryos transferred per cycle

Korošec 2007 followed the procedure of transferring only singleton embryos per treatment cycle. All other studies transferred multiple

embryos per treatment cycle, with a mean range of 1.4 to 3.9 embryos per treatment cycle.

Method of pregnancy diagnosis

Pregnancy was determined by the presence of a foetal heartbeat on ultrasound scan in eight studies (Hazlett 2008 (days 3 and 5); and Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Schoolcraft 2002; Simon 2003; Yung 2019). Twelve studies used the presence of a gestational sac on ultrasound to determine pregnancy (Balaban 2004; Ben-Rafael 1995; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008 (days 3 and 5); and Korošec 2007; Mahani 2007, Morbeck 2007, Simon 2003, Urman 2008). Nine studies used biochemical pregnancy tests to determine pregnancy (Chen 2001; Fancsovits 2011; Friedler 2007; Hazlett 2008 (days 3 and 5); and Kleijkers 2016; Korošec 2007; Mahani 2007; Simon 2003; Urman 2008). The method of pregnancy determination used in the remaining studies is still unclear (Friedler 2005; Kandari 2019; Khan 2004; Ravhon 2005; Ten 2019; Yakin 2004).

Outcomes

Ten studies reported live birth rates (Hazlett 2008; Fancsovits 2011; Fancsovits 2015; Kandari 2019; Kleijkers 2016; Korošec 2007; Morbeck 2007; Simon 2003, Urman 2008; Yung 2019) (see Characteristics of included studies). All but one also reported clinical pregnancy rates (see Characteristics of included studies) (Kandari 2019).

Seven studies reported on miscarriages (Fancsovits 2015; Friedler 2005, Kandari 2019; Kleijkers 2016; Korošec 2007; Mahani 2007; Urman 2008). The data from Friedler 2005 could not be used, as this study reported miscarriages as a percentage without clarifying group size.

Seven studies reported the multiple pregnancy rate (Balaban 2004; Dittmann-Műller 2009; Friedler 2007; Kleijkers 2016; Simon 2003; Urman 2008; Yung 2019). All but two reported the multiple pregnancy rate as a percentage of the number of pregnancies (Kleijkers 2016; Yung 2019).

Four studies reported other adverse events (Ben-Rafael 1995; Friedler 2007; Kleijkers 2016; Yung 2019). Three studies reported ectopic pregnancies (Ben-Rafael 1995; Friedler 2007; Yung 2019); another reported on foetal malformations (Kleijkers 2016). These data were combined for analysis in the review.

Eighteen studies reported implantation rates (Balaban 2004; Ben-Rafael 1995; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Hazlett 2008; Kandari 2019; Khan 2004; Kleijkers 2016; Mahani 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Ten 2019; Urman 2008; Yakin 2004). Data from five studies could not be used (see Characteristics of included studies) (Friedler 2005; Khan 2004; Ravhon 2005; Schoolcraft 2002; Yakin 2004).

Ten studies reported outcome measures that were not included in this review (Balaban 2011; Chen 2001; Fancsovits 2011; Fancsovits 2015; Hazlett 2008; Kleijkers 2016; Korošec 2007; Simon 2003; Urman 2008; Yakin 2004). Chen 2001 reported pregnancy rate, as determined by a biochemical pregnancy test, which could not be used. Along with live birth and clinical pregnancy rates, Hazlett 2008 reported ongoing pregnancy rates as pregnancy demonstrated by foetal cardiac activity at seven weeks' gestation, as assessed as viable pregnancy. Kleijkers 2016 reported birth weights, including the numbers of small- and large-for-gestational-age infants. Korošec 2007 reported clinical pregnancy rates in cycles after a previous implantation failure. Simon 2003 reported deliveries, ongoing pregnancy rates per embryo transfer, singleton pregnancy rates, and clinical pregnancy rate per embryo transfer. Urman 2008 reported clinical pregnancy and implantation rates stratified by age, previous treatment failures, and quality of the embryos (see Characteristics of included studies). Balaban 2011, a follow-up study, reported the live birth rate resulting from the Urman 2008 trial per embryo transfer. Yakin 2004 reported on the cryosurvival rate. Fancsovits 2011 reported the fertilisation rate and the rate of positive human chorionic gonadotrophin (hCG) tests.

Studies that reported outcome measures in such a way that they could not be incorporated into this review are summarised in Appendix 6. The original investigators who responded to our additional data queries and the data they provided are summarised in Appendix 7.

Excluded studies

Twenty-six studies were excluded (and in this update, eight) (see Characteristics of excluded studies), 17 because they failed to use a truly randomised design (Balaban 2005; Chao 2008; Check 2012; Feichtinger 1990; Feichtinger 1992; Hambiliki 2010; Karimian 2004; Perez 2019; Nakagawa 2012; Nakagawa 2012-II; Nishihara 2017; Schiewe 2013; Singh 2015; Sun 2010; Thornton 2018; Tomari 2014; Valojerdi 2006). Two studies were reviews and meta-analyses and had no available data that could be incorporated into this systematic review (Loutradi 2008; Sallam 2010). Loutradi 2008 presented a review on the effect of HA on embryo implantation, but not all included studies were randomised controlled trials. Sallam 2010 provided a systematic review on the effects of assisted reproductive technologies, including EmbryoGlue (Vitrolife), but did not report actual data in the conference abstract in which the review was published. Six studies were excluded because they did not consider the comparison of interest (Bungum 2003; Chatziioannou 2010; de Moura 2017; Romano 2004; Sieren 2006; Venetis 2009). One study was excluded because oocytes instead of participants were randomly assigned (Sifer 2009).

Risk of bias in included studies

Based on descriptions provided within the original publications, the potential risks of bias seemed moderate. However, upon contact with the original authors, many concerns about sources of bias were resolved. See Appendix 7 for information on which ambiguities were resolved in this way.

Allocation

Twelve studies used a computerised random number generator for allocation of participants into different arms of the study (Balaban 2004; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008; Kandari 2019; Kleijkers 2016; Korošec 2007; Schoolcraft 2002; Ten 2019; Urman 2008; Yung 2019). Morbeck 2007 used a random number table for participant randomisation. Dittmann-Műller 2009 reported the use of a cube as a method of randomisation, allocating even numbers to the treatment arm and odd numbers to the control arm of the trial. The remaining seven studies did not report the specific method of randomisation used (Ben-Rafael 1995; Chen 2001; Friedler 2005; Khan 2004; Mahani 2007; Ravhon 2005; Yakin 2004).

Allocation concealment was reported in nine studies. Four of those studies used a third party or central computer randomisation for allocation concealment (Balaban 2004; Fancsovits 2015; Friedler 2007; Kleijkers 2016). The other five studies used serially numbered, sealed, opaque envelopes (Hazlett 2008; Morbeck 2007; Simon 2003; Urman 2008; Yung 2019). The remaining studies did not clearly report the method of allocation concealment used (see Characteristics of included studies; Figure 2 and Figure 3).

Figure 2. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

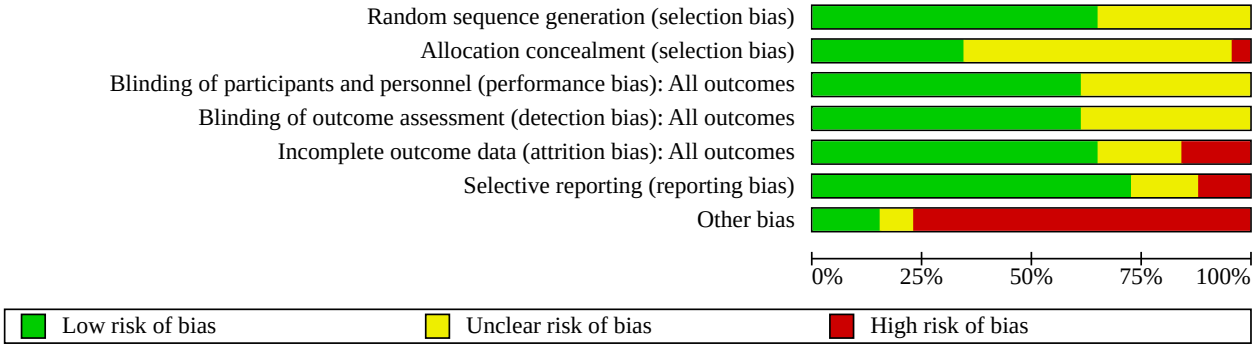


Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.

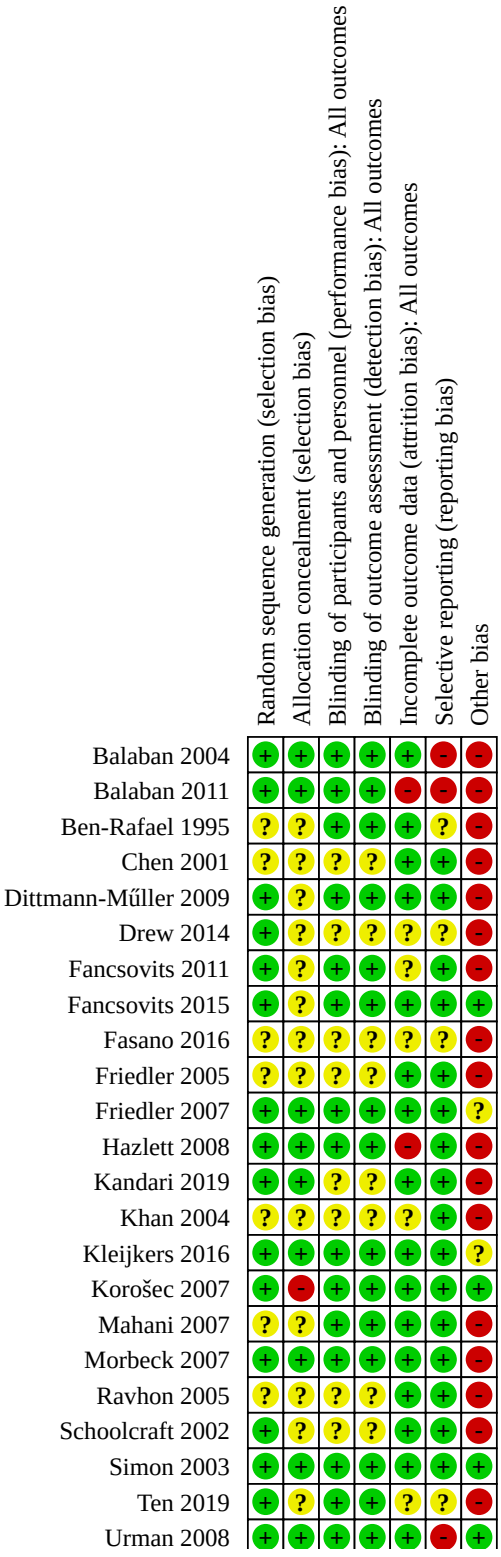
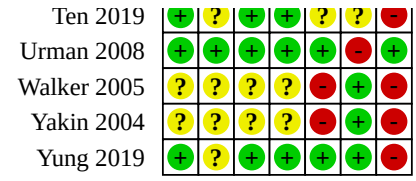


Figure 3. (Continued)



Blinding

Blinding was performed in 15 of the 21 studies (Balaban 2004; Ben-Rafael 1995; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Simon 2003; Ten 2019; Urman 2008; Yung 2019). Neither participants, treating physicians, nor nurses knew to which arm of the study participants had been allocated. None of the studies described the process of analysis used for blinded results, except for Kleijkers 2016, which specified that the allocation sequence was revealed at the end of the study, after data collection was complete.

Regarding detection bias, as mentioned above, clinicians were blinded in 15 of the 20 studies. This category is not applicable to the main outcome of this review - live births.

Incomplete outcome data

Sixteen studies reported length of follow-up per participant, or length of follow-up could be determined indirectly from the text (Balaban 2004; Ben-Rafael 1995; Chen 2001; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008; Kandari 2019; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Simon 2003; Urman 2008; Yung 2019).

Loss to follow-up was described in eight studies (Balaban 2004; Dittmann-Műller 2009 (no loss); and Hazlett 2008; Kleijkers 2016; Korošec 2007; Morbeck 2007, Urman 2008; Yung 2019). Fancsovits 2015 and Kandari 2019 provided this information after study authors were contacted for more information. Korošec 2007 accurately reported loss to follow-up but did not include the results of all participants in the results table (see Characteristics of included studies). Hazlett 2008 reported loss to follow-up, but there were discrepancies between the number of participants randomised, the number lost to follow-up, and the final number included in the analysis (see Characteristics of included studies).

An ITT analysis was performed in four studies (Balaban 2004; Kleijkers 2016; Urman 2008; Yung 2019).

Therefore, six studies have been classified as complete in reporting of outcome data (Fancsovits 2015; Kleijkers 2016; Korošec 2007; Morbeck 2007; Simon 2003; Urman 2008), and one study remains classified as unclear (Hazlett 2008). All six studies reported live births, length of follow-up, and loss to follow-up. However, not all studies performed an ITT analysis. In terms of risk of bias, Hazlett 2008 was assessed as unclear because of loss of participants. Simon 2003 reported no loss of participants, and Morbeck 2007 excluded 38 participants before randomisation.

Selective reporting

Twenty studies reported outcome measures in a pre-specified manner (Balaban 2004; Chen 2001; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Hazlett 2008; Kandari 2019; Khan 2004; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Ten 2019; Yakin 2004; Yung 2019). Some studies reported more outcome measures than planned. This was not considered to be a source of bias. However, when fewer outcome measures than planned were reported, this was considered to be a source of bias (see Characteristics of included studies). Urman 2008 reported fewer outcomes than planned, but a follow-up study from the same trial reported the live birth rate (Balaban 2011). This group of studies is considered to have high risk of bias because a pre-specified protocol before initiation of the study was not found and the outcome of live birth was added after completion of the trial. Ben-Rafael 1995 did not specify the outcome measures beforehand and therefore was assessed as unclear.

Ten studies reported live births (Fancsovits 2011; Fancsovits 2015; Hazlett 2008; Kandari 2019; Kleijkers 2016; Korošec 2007; Morbeck 2007; Simon 2003; Urman 2008; Yung 2019). As mentioned above, the live birth rate was not reported in Urman 2008 but rather in the follow-up study (Balaban 2011). Korošec 2007 recorded live births only in the subgroup for fresh embryo transfers.

Chen 2001 did not report the primary outcome measure of live births nor interim outcomes such as clinical pregnancies. Instead, Chen 2001 reported on the biochemical pregnancy rate alone. This study showed a trend in favour of adding HA to the embryo transfer medium over the control medium. These findings are plausible when compared with findings of the other included studies.

Other potential sources of bias

See Assessment of risk of bias in included studies for information on how the risk of other sources of bias was assessed.

Twelve studies reported that the study was free of commercial funding (Balaban 2004; Ben-Rafael 1995; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008; Kleijkers 2016; Korošec 2007; Morbeck 2007; Ravhon 2005; Simon 2003; Urman 2008). Two studies received commercial funding (Dittmann-Műller 2009; Schoolcraft 2002). The other studies did not report on funding.

Thirteen studies used similar embryo culture media and media brands for the treatment and control groups, so all parameters could be considered similar until the moment of embryo transfer (Balaban 2004; Chen 2001; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Khan 2004; Korošec 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Urman 2008; Yakin 2004).

Eight studies did not report on multiple pregnancy rates despite transferring multiple embryos per treatment cycle (Fancsovits 2011; Friedler 2005; Hazlett 2008; Mahani 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Yakin 2004).

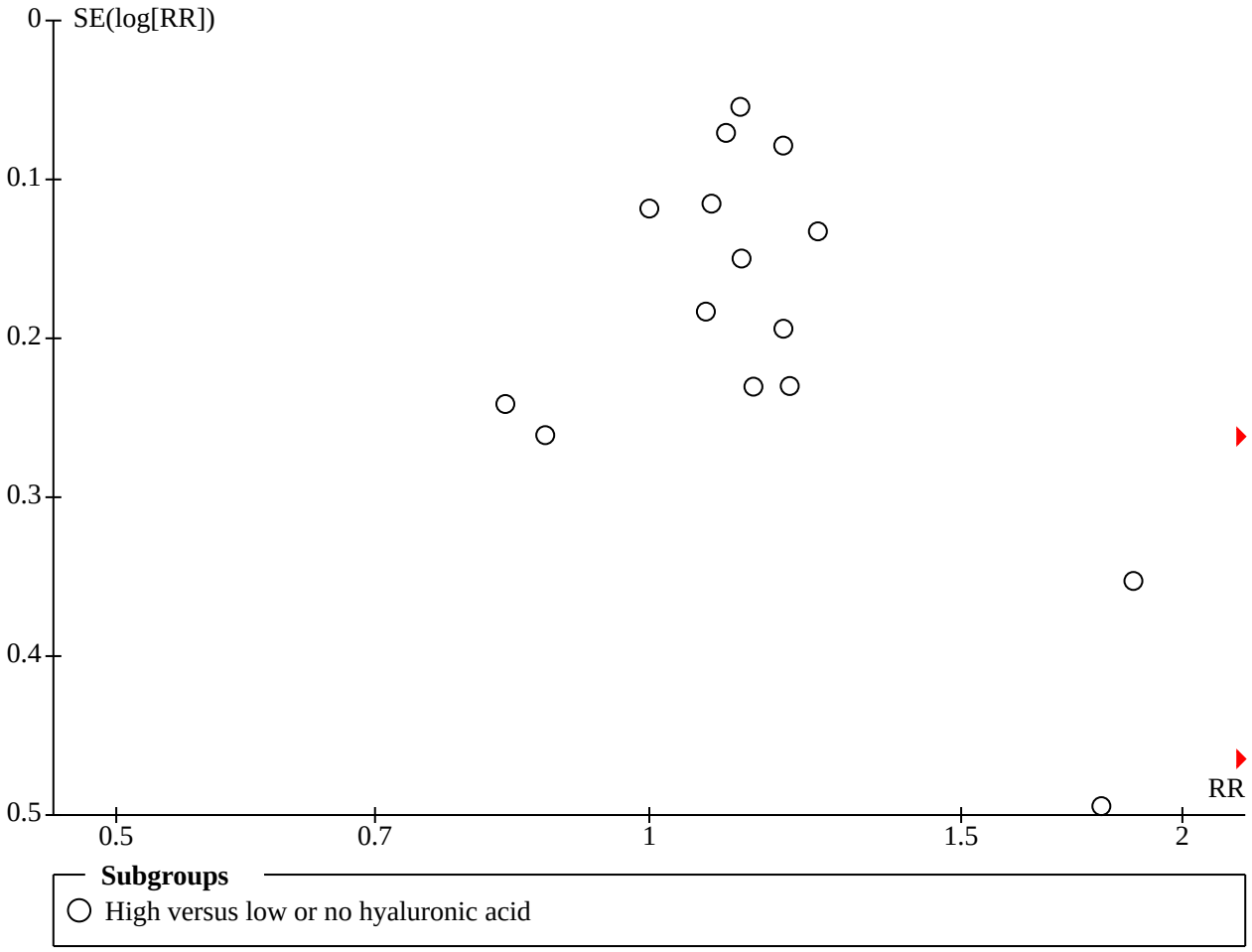
Eleven studies were published as abstracts only (Balaban 2004; Balaban 2011; Chen 2001; Fancsovits 2011; Friedler 2005; Kandari 2019; Ravhon 2005; Schoolcraft 2002; Ten 2019; Yakin 2004; Yung 2019).

Three studies were regarded as free of other sources of bias (Fancsovits 2015; Korošec 2007; Simon 2003). For two studies (Friedler 2007; Kleijkers 2016), we could not determine with certainty whether the culture media were similar between treatment and control groups. Therefore, the risk of other biases was rated as unclear.

Assessment of reporting biases in this review

Seventeen studies were included in the analysis of clinical pregnancy rates for the overall comparison of transfer medium with HA added versus transfer medium with no HA or with a low HA concentration. Therefore, a funnel plot was used to investigate the possibility of small-study effects (see Figure 4). The funnel plot showed most of the studies around the pooled estimate, creating an inverted funnel, which indicates low risk of small-study effects and reporting biases.

Figure 4. Funnel plot of comparison: embryo transfer in medium enriched with hyaluronic acid versus medium devoid of, or with a lower concentration of, hyaluronic acid; outcome, 3.14 Clinical pregnancy rate.



Effects of interventions

See: **Summary of findings 1** High versus low or no hyaluronic acid for assisted reproductive technologies

1. Embryo transfer in medium containing high versus no or low concentration of hyaluronic acid (HA)

1.1 Live birth rate—high HA versus no or low concentration HA

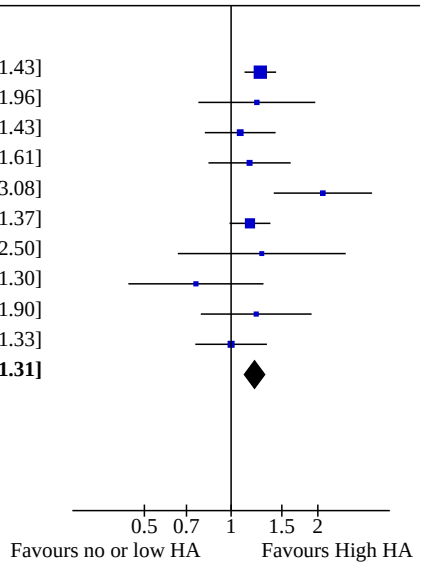
(Analysis 1.1)

Ten of the 20 included studies looking at the effects of HA reported on live birth (Balaban 2011; Fancsovits 2011; Fancsovits 2015;

Hazlett 2008; Kandari 2019; Kleijkers 2016; Korošec 2007; Morbeck 2007; Simon 2003; Yung 2019). The combined results of these studies with a total of 4066 participants were pooled, and evidence showed an increased number of live births for transfer media containing high concentrations of HA compared to no or low concentrations (risk ratio (RR) 1.21, 95% confidence interval (CI) 1.11 to 1.31; 10 studies, N = 4066; I^2 = 33%, moderate-quality evidence; number needed to treat (NNT) 14 (see Figure 5 and Summary of findings 1). This suggests that if the chance of live birth following no HA addition in media is assumed to be 33%, the chance following HA addition would be between 37% and 44%.

Figure 5. Forest plot of comparison: high hyaluronic acid versus low/no hyaluronic acid, outcome: 1.1 Live birth rate.

Study or Subgroup	High HA Events	High HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 High vs low or no hyaluronic acid						
Balaban 2011	310	639	247	643	36.4%	1.26 [1.11 , 1.43]
Fancsovits 2011	30	103	23	97	3.5%	1.23 [0.77 , 1.96]
Fancsovits 2015 (1)	69	208	62	201	9.3%	1.08 [0.81 , 1.43]
Hazlett 2008	49	116	39	107	6.0%	1.16 [0.83 , 1.61]
Kandari 2019	55	153	29	168	4.1%	2.08 [1.41 , 3.08]
Kleijkers 2016	184	417	159	419	23.4%	1.16 [0.99 , 1.37]
Korošec 2007 (2)	12	36	12	46	1.6%	1.28 [0.65 , 2.50]
Morbeck 2007	14	41	19	42	2.8%	0.75 [0.44 , 1.30]
Simon 2003	22	40	18	40	2.7%	1.22 [0.79 , 1.90]
Yung 2019	70	275	70	275	10.3%	1.00 [0.75 , 1.33]
Subtotal (95% CI)		**2028**		**2038**	**100.0%**	**1.21 [1.11 , 1.31]**
Total events:	815		678			
Heterogeneity: Chi² = 13.40, df = 9 (P = 0.15); I² = 33%						
Test for overall effect: Z = 4.57 (P < 0.00001)						
Test for subgroup differences: Not applicable						



Footnotes
(1) Unpublished data retrieved after contacting authors, first cycle data
(2) Only fresh embryo transfer data

Sensitivity analyses

Planned sensitivity analyses were performed and none changed the outcome of the analysis in such a way that the 95% confidence interval crossed the line of no effect. The first sensitivity analysis excluded trials with high risk of bias (Fancsovits 2011; Fancsovits 2015; Kandari 2019; Korošec 2007) (RR 1.17, 95% CI 1.08 to 1.28; N = 3054; I^2 = 2%). The second analysis removed trials with outlier results, which changed heterogeneity (Kandari 2019) (RR 1.17, 95% CI 1.08 to 1.27; N = 3745; I^2 = 0%). In the third analysis, a random-effects model was used (RR 1.19, 95% CI 1.06 to 1.34; N = 4099; I^2 = 33%). The fourth analysis examined studies using only HA concentrations of 0.5 mg/mL. Kleijkers 2016 did not specify the concentration of HA in their treatment group; therefore, this study was excluded from this sensitivity analysis (RR 1.22, 95% CI 1.11 to 1.34; N = 3230; I^2 = 39%).

Two additional sensitivity analyses were added; both resulted in non-significant beneficial effects on live birth rate with the addition of HA to the embryo transfer medium. Due to the large number of studies for which only an abstract was published, a sensitivity analysis was performed examining peer-reviewed, full-text only articles. This excluded four studies (Balaban 2011; Fancsovits 2011; Kandari 2019; Yung 2019) (RR 1.13, 95% CI 1.00 to 1.27; N = 1713; I^2 = 0%). The second added sensitivity analysis examined only studies that used a foetal heartbeat as the method of pregnancy determination, as opposed to gestational sac, or studies that did not specify the method of pregnancy determination used. This excluded four studies (RR 1.11, 95% CI 0.98 to 1.25; N = 1854; I^2 = 0%) (Balaban 2011; Fancsovits 2011; Fancsovits 2015; Kandari 2019). Therefore it is unclear if this result reflects lack of treatment effect or, rather, and more likely, lack of power, given that more than half of the total participants were removed from these two analyses.

1.2 Subgroup analysis, live birth rate (grouped by timing of embryo transfer)

(Analysis 1.2)

Six combined studies with a total of 1759 participants performed mean cleavage embryo transfers (days 2 to 4) (Balaban 2011; Fancsovits 2011; Fancsovits 2015; Hazlett 2008; Morbeck 2007; Simon 2003). An increased live birth rate was noted (RR 1.19, 95% CI 1.05 to 1.35; 6 studies, N = 1759; I^2 = 0%; moderate-quality evidence). Three studies with a total of 600 participants performed transfers at the blastocyst stage (day 5) and showed evidence of increased live birth (RR 1.22, 95% CI 1.05 to 1.42; 3 studies, N = 600; I^2 = 0%; moderate-quality evidence) (Balaban 2011; Hazlett 2008; Korošec 2007). Two studies included both cleavage stage and blastocyst stage embryo transfers (Balaban

2011; Hazlett 2008). These data were extracted separately for this subgroup analysis. Three studies also used both cleavage stage and blastocyst protocols but did not report results separately; therefore these studies could not be included in this subgroup analysis (Kandari 2019; Kleijkers 2016; Yung 2019).

1.3 Subgroup analysis, live birth rate (grouped by frozen-thawed or fresh embryos)

(Analysis 1.3)

Data from three studies with a total of 713 participants with transferred frozen-thawed embryos were pooled (Morbeck 2007; Simon 2003; Yung 2019). No evidence of an effect on live birth rate was found (RR 0.99, 95% CI 0.80 to 1.24; 3 studies, N = 713; I^2 = 0%; moderate-quality evidence).

Six combined studies with a total of 2517 participants transferred fresh embryos and showed evidence of a beneficial treatment effect on live birth from transfer media containing high concentrations of HA (RR 1.28, 95% CI 1.15 to 1.41; 6 studies, N = 2517; I^2 = 36%; moderate-quality evidence) (Balaban 2011; Fancsovits 2011; Fancsovits 2015; Hazlett 2008; Kandari 2019, Korošec 2007). (RR 1.28, 95% CI 1.15 to 1.41; participants = 2517; studies = 6; I^2 = 36%)

1.4 Subgroup analysis, live birth rate (grouped by exposure time to high-concentration HA)

(Analysis 1.4)

Three studies with 689 participants exposed the embryos to HA for up to 10 minutes before transfer (Fancsovits 2011; Fancsovits 2015; Simon 2003), and the combined data show no evidence of a treatment effect (RR 1.14, 95% CI 0.92 to 1.41; 3 studies, N = 689; I^2 = 0%; moderate-quality evidence).

Five combined studies with a total of 2506 participants exposed the embryos to HA for longer than 10 minutes before transfer (Balaban 2011; Hazlett 2008; Kleijkers 2016; Korošec 2007; Morbeck 2007). Evidence of increased live birth with treatment was found (RR 1.20, 95% CI 1.09 to 1.32; 5 studies, N = 2506; I^2 = 0%; moderate-quality evidence).

1.5 Subgroup analysis, live birth rate (grouped by single or multiple embryo transfer policies)

(Analysis 1.5)

Korošec 2007 with 82 participants transferred only one embryo per treatment cycle and found no evidence of a treatment effect (RR 1.28, 95% CI 0.65 to 2.50; 1 study, N = 82; low-quality evidence).

Seven combined studies with a total of 3113 participants transferred multiple embryos per treatment cycle (Balaban 2011; Fancsovits 2011; Fancsovits 2015; Hazlett 2008; Kleijkers 2016; Morbeck 2007; Simon 2003). Evidence shows increased live birth with treatment (RR 1.19, 95% CI 1.09 to 1.29; 7 studies, N = 3113; I^2 = 0%; moderate-quality evidence). Yung 2019 was not included in this subanalysis because the mean number of embryos transferred in this study was 1.4, and separate results for those receiving a single and a multiple embryo transfer protocol were not provided.

1.6 Subgroup analysis, live birth rate (grouped by participant selection)

(Analysis 1.6)

Six combined studies with a total of 1625 participants included only good prognosis participants, showed increased live birth rates with HA addition (RR 1.24, 95% CI 1.09 to 1.40; 6 studies, N = 1625; I^2 = 53%; moderate-quality evidence) (Hazlett 2008; Kandari 2019; Kleijkers 2016; Korošec 2007; Morbeck 2007; Simon 2003). Kandari was added into this group as these researchers looked at women with PCOS, who according to their analyses were a young population, with an average of one previous IVF cycle and a high oocyte number.

Four studies with a total of 2441 participants did not use strict inclusion criteria for participant selection, and the combined data provided evidence of an increased live birth rate (RR 1.19, 95% CI 1.07 to 1.32; 4 studies, N = 2441; I^2 = 0%; moderate-quality evidence) (Balaban 2011; Fancsovits 2011; Fancsovits 2015; Yung 2019).

1.7 Miscarriage rate

Seven studies reported on miscarriage (Fancsovits 2015; Friedler 2007; Kandari 2019; Kleijkers 2016; Korošec 2007; Mahani 2007; Urman 2008). Miscarriage data from Fancsovits 2015 (first cycle data) were obtained by contacting the study authors. The combined results of these studies with a total of 3091 participants were pooled, and trialists found that risk of miscarriage may be reduced after embryos are transferred in high-concentration HA media, compared with no or low-concentration HA media (RR 0.82, 95% CI 0.67 to 1.00; 7 studies, 3091 participants; I^2 = 66%; low-quality evidence; number needed to treat for an additional beneficial outcome (NNTB) 48) (Analysis 1.7) (see Summary of findings 1) (Balaban 2011; Fancsovits 2011; Fancsovits 2015; Yung 2019). This suggests that the risk of miscarriage following no or low-concentration HA media is 12%, and with high-concentration HA, the risk is between 8% and 12%. This analysis had high heterogeneity, which was caused by one study - an abstract on embryo transfer specifically in patients with polycystic ovarian syndrome (PCOS), which had outlier results (Kandari 2019); with removal of this study, no treatment effect with HA and no heterogeneity are shown (see below).

Sensitivity analyses

Of the planned sensitivity analyses, only one did not change the result. When we examined studies using only HA concentrations of 0.5 mg/mL, we removed Kleijkers 2016 because these researchers did not specify the HA concentration used in the treatment group. This analysis showed a reduction in the miscarriage rate from 12% to 8% with the addition of HA to the transfer media (RR 0.64, 95% CI 0.49 to 0.85; N = 2255; I^2 = 55%). It is important to note the high heterogeneity, and that one of the six studies had outlier results.

However, the other sensitivity analyses found no conclusive evidence of a difference in miscarriage rate with the addition of HA to the transfer media. The first sensitivity analysis excluded trials with high risk of bias (Fancsovits 2015; Kandari 2019; Korošec 2007; Mahani 2007) (RR 0.96, 95% CI 0.75 to 1.23; N = 2219; I^2 = 36%). For the second analysis, trials with outlier results were removed, which changed heterogeneity (Kandari 2019) (RR 0.99, 95% CI 0.79 to 1.23; N = 2770; I^2 = 0%). In the third analysis, a random-effects model was used (RR 0.78, 95% CI 0.49 to 1.23; N = 3091; I^2 = 66%). The two additional sensitivity analyses requested during the peer review process showed little or no difference in miscarriage rate with the addition of HA. When we examined peer-reviewed, full-text only articles, we excluded Kandari 2019 (result shown above). The second additional sensitivity analysis examined only studies that

used a foetal heartbeat as the method of pregnancy determination. From this analysis, we excluded five studies (RR 1.15, 95% CI 0.84 to 1.57; N = 918; I^2 = 0%) (Fancsovits 2015; Friedler 2007; Kandari 2019; Mahani 2007; Urman 2008).

1.8 Clinical pregnancy rate - high-concentration HA versus no or low-concentration HA

Seventeen studies with a total of 5247 pooled participants reported on clinical pregnancy rate (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Hazlett 2008; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Urman 2008; Yakin 2004; Yung 2019). The combined result suggests that if the chance of pregnancy in the control group was 40%, then the chance of clinical pregnancy would be between 44% and 49% with the addition of HA to transfer media (RR 1.16, 95% CI 1.09 to 1.23; 17 studies, N = 5247; I^2 = 40%; moderate-quality evidence; NNTB 16) (Analysis 1.8) (see Summary of findings 1). Because more than 10 studies were included in this analysis, a funnel plot was constructed to assess the risk of small-study effects (see Figure 4). The funnel plot shows low risk of small-study effect or reporting bias.

Sensitivity analyses

We added a sensitivity analysis to examine only studies that used a foetal heartbeat as the method of pregnancy determination; this did not change the outcome of the analysis in such a way that the 95% confidence interval crossed the line of no effect. We excluded ten studies (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Mahani 2007; Ravhon 2005; Urman 2008; Yakin 2004) (RR 1.13, 95% CI 1.02 to 1.24; N = 2243; I^2 = 0%).

1.9 Subgroup analysis, clinical pregnancy rate (grouped by timing of embryo transfer)

(Analysis 1.9)

Twelve combined studies with a total of 2513 participants transferred embryos at the cleavage stage of development (days 2 to 3) (Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Hazlett 2008 (day 3);Mahani 2007; Morbeck 2007; Schoolcraft 2002; Simon 2003; Urman 2008 (day 3); Yakin 2004), and evidence showed an increased clinical pregnancy rate (RR 1.24, 95% CI 1.13 to 1.36; 12 studies, N = 2513; moderate-quality evidence). Heterogeneity was moderate, with an I^2 statistic of 52%.

Four combined studies with a total of 1200 participants transferred embryos at the blastocyst stage (day 5) (Balaban 2004; Hazlett 2008; Korošec 2007; Urman 2008). Evidence shows an increased clinical pregnancy rate (P = 0.04; RR 1.10, 95% CI 1.00 to 1.21; 4 studies, N = 1200; I^2 = 0%; moderate-quality evidence). As previously mentioned, data from Kleijkers 2016 and Yung 2019 could not be used for this subgroup analysis because both cleavage stage and blastocyst transfer protocols were used and the results were not reported separately.

1.10 Subgroup analysis, clinical pregnancy rate (grouped by frozen-thawed or fresh embryos)

(Analysis 1.10)

Five studies with a total of 1056 participants transferred frozen-thawed embryos (Korošec 2007; Morbeck 2007; Simon 2003; Yakin 2004; Yung 2019). No evidence of an effect on clinical pregnancy with treatment was found (RR 1.04, 95% CI 0.88 to 1.22; 5 studies, N = 1056; I^2 = 0%; moderate-quality evidence).

Ten studies with a total of 2993 participants transferred fresh embryos and showed evidence of an increased clinical pregnancy rate (RR 1.14, 95% CI 1.06 to 1.23; 10 studies, N = 2993; I^2 = 14%; moderate-quality evidence) (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008; Korošec 2007; Mahani 2007; Ravhon 2005; Urman 2008).

1.11 Subgroup analysis, clinical pregnancy rate (grouped by exposure time to HA)

(Analysis 1.11)

Six combined studies with a total of 1025 participants exposed the embryos to HA for up to 10 minutes before transfer (Fancsovits 2011; Fancsovits 2015; Friedler 2007; Mahani 2007; Schoolcraft 2002; Simon 2003). Evidence shows an increased clinical pregnancy rate (RR 1.21, 95% CI 1.05 to 1.40; 6 studies, N = 1025; I^2 = 32%; moderate-quality evidence).

Seven combined studies with a total of 3208 participants exposed the embryos to HA for longer than 10 minutes before transfer and also found evidence of an increased clinical pregnancy rate (RR 1.14, 95% CI 1.06 to 1.22; 7 trials, N = 3208; I^2 = 0%; moderate-quality evidence) (Balaban 2004; Dittmann-Műller 2009; Hazlett 2008; Kleijkers 2016; Korošec 2007; Morbeck 2007; Urman 2008).

1.12 Subgroup analysis, clinical pregnancy rate (grouped by single or multiple embryo transfer policies)

(Analysis 1.12)

One study with 296 participants transferred only one embryo per treatment cycle and found no evidence of an effect on clinical pregnancy with treatment (RR 1.14, 95% CI 0.73 to 1.80; 1 trial, N = 296; moderate-quality evidence) (Korošec 2007).

Fifteen combined studies with a total of 4401 participants transferred multiple embryos per treatment cycle and found evidence of an increased clinical pregnancy rate (RR 1.18, 95% CI 1.11 to 1.25; 15 studies, N = 4401; moderate-quality evidence) (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Friedler 2005; Friedler 2007; Hazlett 2008; Kleijkers 2016; Mahani 2007; Morbeck 2007; Ravhon 2005; Schoolcraft 2002; Simon 2003; Urman 2008; Yakin 2004). Heterogeneity was moderate, with an I^2 statistic of 45%.

1.13 Subgroup analysis, clinical pregnancy rate (grouped by participant prognosis)

(Analysis 1.13)

Two combined studies with a total of 288 participants included only poor prognosis participants and found evidence of an increased clinical pregnancy rate (RR 3.01, 95% CI 1.92 to 4.71; 2 studies, N = 288; I^2 = 0%; moderate-quality evidence) (Friedler 2005; Friedler 2007).

Six combined studies with a total of 1578 participants with a good prognosis only showed an increased clinical pregnancy rate with

the addition of HA (RR 1.16, 95% CI 1.03 to 1.31; 6 trials, N = 1578; I^2 = 0%; moderate-quality evidence) (Hazlett 2008; Kleijkers 2016; Korošec 2007; Mahani 2007; Morbeck 2007; Simon 2003). In the previous update, this analysis showed no evidence of a treatment effect.

Nine combined studies with a total of 3381 participants did not select participants on the basis of prognosis and showed evidence of an increased clinical pregnancy rate (RR 1.11, 95% CI 1.04 to 1.19; 9 studies, N = 3381; I^2 = 0%; moderate-quality evidence) (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2011; Fancsovits 2015; Ravhon 2005; Schoolcraft 2002; Urman 2008; Yakin 2004; Yung 2019).

1.14 Multiple pregnancy rate

(Analysis 1.14)

Eight studies reported on multiple pregnancy rates (Balaban 2004; Dittmann-Műller 2009; Fancsovits 2015; Friedler 2007; Kleijkers 2016; Simon 2003; Urman 2008; Yung 2019). However, the data from Fancsovits 2015 could not be included in the meta-analysis (see Characteristics of included studies). The combined results of the remaining seven studies with a total of 3337 participants were pooled and showed that if the risk of multiple pregnancy in the control groups was 13%, the risk with HA-enriched transfer media would be between 16% and 21% (RR 1.45, 95% CI 1.24 to 1.70; 7 studies, N = 3337; I^2 = 36%; moderate-quality evidence; number needed to treat for an additional harmful outcome (NNTH) 18) (see Summary of findings 1). This effect is largely driven by one trial, which had a high prevalence of multiple gestations (Urman 2008).

1.15 Implantation rate

(Analysis 1.15)

Implantation rates were also recorded but could not be part of the meta-analysis because the meta-analysis uses as the denominator the number of embryos transferred instead of the number of couples or participants. However, the data are presented in a meta-view without pooling. Results of the 12 studies that reported implantation rates were analysed (Balaban 2004; Fancsovits 2011; Fancsovits 2015; Friedler 2007; Hazlett 2008; Kandari 2019; Kleijkers 2016; Mahani 2007; Morbeck 2007; Simon 2003; Ten 2019; Urman 2008) .

1.16 Adverse events rate

(Analysis 1.16)

Four studies reported on adverse events other than miscarriage (Friedler 2005; Friedler 2007; Kleijkers 2016; Yung 2019). However, the data from one study could not be analysed (Friedler 2005). Two studies reported on ectopic pregnancies (Friedler 2007; Yung 2019). Kleijkers 2016 reported on foetal malformations. The combined results with a total of 1487 participants show no evidence of an effect on adverse events with HA-enriched transfer media (RR 0.86, 95% CI 0.40 to 1.84; 3 studies, N = 1487; I^2 = 0%; low-quality evidence) (see Summary of findings 1).

2. Embryo transfer in medium containing fibrin sealant versus transfer in medium with no fibrin sealant

Live birth, miscarriage, and multiple pregnancy rates

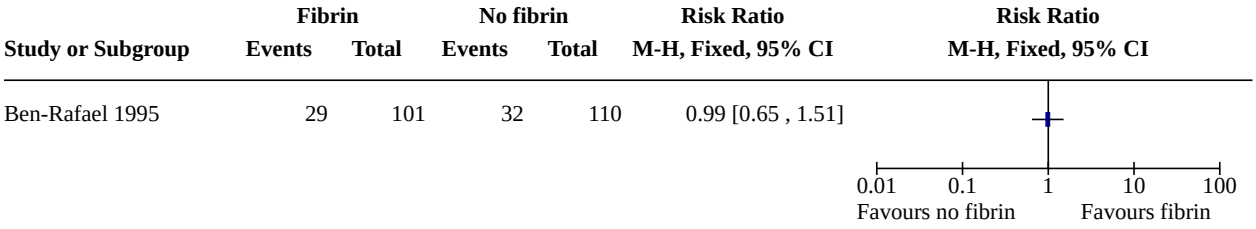
No study was found that examined the effects of fibrin sealant in transfer media on live birth, miscarriage, or multiple pregnancy rates.

2.1 Clinical pregnancy rate

(Analysis 2.1)

One study with a total of 211 participants reported on clinical pregnancies in this comparison (Ben-Rafael 1995). No evidence was found of a treatment effect for transfer media with fibrin sealant (RR 0.99, 95% CI 0.65 to 1.51; 1 study, N = 211; very low-quality evidence) (see Figure 6).

Figure 6. Forest plot of comparison: Fibrin sealant versus no fibrin sealant, outcome: 2.1 Clinical pregnancy rate (per randomly assigned couple).

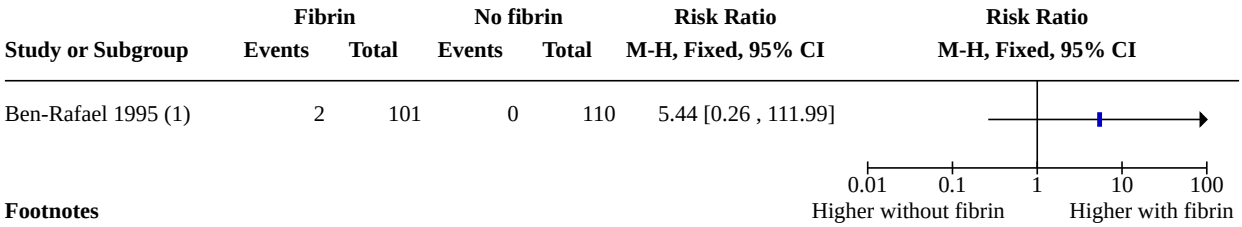


2.2 Adverse events rate

(Analysis 2.2)

One study with a total of 211 participants reported on adverse events (ectopic pregnancies) in this comparison and found no evidence of a treatment effect for transfer media with fibrin sealant (RR 5.44, 95% CI 0.26 to 111.99; 1 study, N = 211; very low-quality evidence) (see Figure 7) (Ben-Rafael 1995).

Figure 7. Forest plot of comparison: Fibrin sealant versus no fibrin sealant, outcome: 2.2 Adverse event rate (per randomly assigned couple).



2.3 Implantation rate

(Analysis 2.3)

Implantation rate was also recorded but could not be part of the meta-analysis because it uses as the denominator the number of embryos transferred instead of the number of couples or participants. However, the data have been presented in a meta-view. Results of this study are presented in Analysis 2.3 (Ben-Rafael 1995).

DISCUSSION

Summary of main results

Widespread use of hyaluronic acid (HA) was introduced into clinical practice in the late 1990s, and HA was initially marketed as an embryo glue. Since that time, a large number of journal papers and proceedings have been published, demonstrating a mixture of positive treatment effects and non-significant results. A funnel plot analysis of results of these trials revealed no evidence of publication bias. Careful consideration of the baseline characteristics resulted in a total of 28 trials that were acceptable for inclusion in this systematic review, and 21 of these, involving 6704 participants, that could be included in the meta-analysis. Of these, 27 trials, including 5568 participants, were included in a meta-analysis examining the effects of HA. A second adherence compound called fibrin sealant was identified. Only one trial met the inclusion criteria, with 211 participants, and due to the paucity of data, conclusions could not be drawn regarding the effects of fibrin.

The systematic analysis of these data has provided a level of confidence in supporting continued use of HA and offers insight into its underlying mechanism of action, given that both cleavage and blastocyst transfers appear to benefit from the addition of HA. The presence of low levels of hyaluronic acid in the culture medium before embryo transfer was a confounding factor that was not anticipated at the outset of this review. For this reason, a post-protocol amendment was initially made to split the trials into two comparisons, whereby one control group received media containing a low concentration of HA (0.125 mg/mL) and another control group had no HA in the transfer media. For this review update, this amendment was revised, and we returned to the comparison of combined data of high HA versus no or low HA for the primary outcomes. Logic suggests that a low concentration of HA in the control group media can reduce the power of differences from the treatment group. However, this has been adequately disproved for both live birth and clinical pregnancy rates, thus supporting the revised protocol. The resulting analysis has provided improved clarity and is less cumbersome to follow.

The most robust outcome of this review lies in its clear beneficial effects on both live birth and clinical pregnancy rates. The chance of live birth following no or low-concentration HA in transfer media is 33% and is increased with the addition of high-concentration HA to 40% (between 37% and 44%; number needed to treat for an additional beneficial outcome (NNTB) 14) (Summary of findings 1). Evidence of this treatment effect was of moderate quality and was based mainly on data from two large randomised controlled trials (Balaban 2011; Kleijkers 2016), although a total of ten studies reported on this outcome measure. In the subanalyses, only a trend towards a beneficial treatment effect on live birth rate could be found when the analysis was confined to studies with embryo transfer after less than 10 minutes of HA exposure (Analysis 1.4). However, this finding was based on just three studies and relatively small numbers of participants. A power deficit is further suggested upon looking at the clinical pregnancy outcome, and this subanalysis showed a treatment effect with the addition of more studies. The addition of HA to frozen embryos did not seem to have a significant beneficial or detrimental effect on live birth and clinical pregnancy rates (Analysis 1.3; Analysis 1.10).

One of the more interesting and perhaps clinically relevant aspects of this analysis is the bigger treatment effect on live birth and clinical pregnancy rates seen after the addition of hyaluronic acid, regardless of the stage of embryo development at the time of transfer (Analysis 1.2; Analysis 1.9). If the primary mechanism of HA action is indeed an adhesive during implantation, one might expect this to be beneficial only for embryos that were transferred close to the time of embryo attachment (days 5 to 6). The fact that this is equally beneficial for cleavage stage embryos transferred on days 2 to 3 supports an additional or facilitating action of HA during implantation.

An elevated multiple pregnancy rate is the expected natural consequence of increased implantation when more than one embryo is transferred, and indeed the results of this comparison reflect this. Moderate-quality evidence supports an increased multiple pregnancy rate from 13% to 18% with the addition of HA in the transfer media (Summary of findings 1; Analysis 1.14). Multiple pregnancies have likely increased as a result of the combination of an adherence compound and a policy of transferring more than one embryo.

The number of studies reporting on live birth rates is limited for reasons that deserve consideration. The most obvious assumption

is that a large proportion of pregnancies ended in miscarriage before birth, yet this is difficult to confirm without more reported data on this event. A more probable explanation can be found in the frequent practice of reporting study findings before the last participant has given birth. Many publications fail to report this important outcome measure, reflecting either inadequate reporting capabilities for deliveries or eagerness to publish. This limitation poses a considerable burden on investigators who intend to maintain the golden standard of 'live births' as the primary outcome in the Cochrane meta-analyses. Nevertheless, any new intervention, such as the addition of adherence compounds, could potentially have an effect on the ultimate outcome of a live baby and remains of paramount importance.

Low-quality evidence suggests that addition of hyaluronic acid can decrease the miscarriage rate. This positive effect however was not seen when one of the studies - an abstract containing outlier results (Kandari 2019) - was removed. One potential explanation is that this study looked only at patients with polycystic ovary syndrome (PCOS) who had a good prognosis. The risk of miscarriage following no or low-concentration HA media is 12%, and with the addition of high-concentration HA, the risk is between 8% and 12% (Summary of findings 1). No treatment effect on total adverse events was identified (low-quality evidence). A disappointing finding of this review is that few studies have reported on miscarriages and ectopic pregnancies. The possibility that an adherence compound could facilitate the implantation of a low-quality embryo, resulting in an increased miscarriage rate, remains unconfirmed. However, current data suggest that this is not a major concern.

This series of meta-analyses of the best available evidence indicates that the addition of HA as an adherence compound to embryo transfer medium is clinically beneficial.

Overall completeness and applicability of evidence

Although we were able to analyse 21 studies across five comparisons, the primary outcome measure - live birth rates - was reported in only ten studies. Of these studies, five actually published the results on live births and the number of deliveries (Fancsovits 2015; Kandari 2019; Kleijkers 2016; Simon 2003; Yung 2019), and one study - Urman 2008 - reported the live birth rate only in an abstract a few years later (Balaban 2011), after reporting only the clinical pregnancy rate in the original publication. The authors of the other four studies reported live births only after they were contacted by the review authors. Data on secondary outcomes and adverse events are also limited. Little is known about the effects of adherence compounds on the incidence of such adverse events as late miscarriages because most of the included studies do not follow up past the stage of clinical pregnancy. Also of note is the lack of reporting on multiple pregnancy rates. All but one of the included studies had a multiple embryo transfer protocol; however only seven reported on multiple pregnancy, indicating that important data may not have been reported.

All original investigators in the 21 analysed studies have been contacted regarding data queries. However, the authoring team of one study could not be found (Chen 2001). In total, we received responses from 12 study authors, which helped to resolve queries regarding data and study characteristics. Ambiguities remain regarding the other nine studies.

Quality of the evidence

We included 28 studies in this review. However, data could be analysed from only 21 of them with a total of 6704 participants. Six outcomes in total were examined (live birth, miscarriage, clinical pregnancy, multiple pregnancy, implantation, and total adverse event rate). It was not possible to perform all six planned subgroup analyses for each outcome measure.

This systematic review demonstrates that the addition of HA probably increases the live birth rate. Included and pooled studies contained methodological limitations and differences in baseline characteristics of participants. Some studies allowed participants to enrol and undertake multiple treatment cycles in the trial. This too has created ambiguities regarding the actual outcomes. Most studies transferred multiple embryos per treatment cycle, which limits the possibility of finding the actual treatment effects of adding adherence compounds to embryo transfer media. Only Korošec 2007 adhered to a single embryo transfer policy. Some studies sampled participants consecutively, and others sampled participants non-consecutively or did not describe their methods. Few included trials performed a power calculation to determine sample size. Causes and durations of subfertility differed between studies but mainly remain unreported. The same goes for the number of previous treatment cycles - a factor that has a big influence on the success rate of assisted reproductive technology (ART). Regarding methods of randomisation and allocation concealment, most studies were not clear in their published articles. A lot of these ambiguities were resolved by contacting the original investigators, although the concept of allocation concealment in particular remains unclear for many of the studies. Most studies performed trials in a double-blind fashion (which means that both participant and physician or embryologist did not know to which treatment arm the participant was allocated) and reported outcomes in a pre-specified way. Few studies reported live birth rates. Furthermore, the overall risk for other sources of bias was high because some studies used very different transfer media in the treatment and control arms, and many studies did not report on multiple pregnancy rates while transferring multiple embryos per treatment cycle.

The number of randomised couples was the denominator for the overall data analysis. Some studies however used the number of randomised embryos as the denominator instead. This discrepancy was addressed by asking study authors to provide first cycle data. The implantation rate could be assessed only per embryo transferred and therefore could not be part of the meta-analysis.

The overall quality of the evidence was rated via GRADE methods, which consider not only study limitations (e.g. risk of bias) but also consistency of effect, imprecision, indirectness, and publication bias. Evidence was rated of moderate quality for live births (downgraded because all studies except for three had high risk of bias in one or more domains), clinical pregnancy (downgraded because of moderate heterogeneity), and multiple pregnancy rates (downgraded because of small numbers of events and a wide confidence interval) (Summary of findings 1. Low-quality evidence suggests that the addition of HA can decrease miscarriage rates. This effect was not seen when one of the studies - an abstract containing outlier results (Kandari 2019) - was removed. This analysis was downgraded for substantial heterogeneity, caused by this study, and for having a wide confidence interval. The analysis of total adverse events was downgraded to low quality because of the

small number of events and the wide confidence intervals. Since only one study analysed fibrin sealant, conclusions regarding its effects could not be drawn.

Potential biases in the review process

During the review process, it appeared that HA was present in standard embryo culture and transfer media of the control groups in many studies. Therefore, in the first review, HA trials were divided into two comparison groups - those with a control group medium without HA and those with a lower concentration of HA. As a result, data on the same adherence compound had to be divided, which led to less significant results, and no difference was seen between low-concentration HA and no HA comparisons. As previously discussed, while performing the current meta-analysis, the review authors decided to pool the data to conduct an overall assessment of treatment effects. Even though the included studies are not completely similar in their intervention and control groups, all compare an embryo transfer medium with a functional concentration of HA as an adherence compound versus a control transfer medium.

Other potential adherence compounds such as heparinase have been identified. However, no randomised controlled trials examining their applicability in human-assisted reproductive technologies could be found.

As stated in the protocol, when data were not reported, we planned on imputing data on the primary outcome as if live births did not occur. We found in the process of data analysis that very few patients were lost to follow-up or withdrew. It became clear that imputing these data would have no influence on the overall treatment effect; therefore this sensitivity analysis was not performed.

As stated in the protocol, the aim was to count multiple live births as a single live birth event. We were able to do this for four studies (Kleijkers 2016; Simon 2003; Urman 2008; Yung 2019).

Agreements and disagreements with other studies or reviews

To the best of our knowledge, one other systematic review has examined the addition of adherence compounds to embryo transfer media (Kolibianakis 2008), and this was published as a conference abstract. This review investigated whether the addition of HA to human embryo culture could increase pregnancy rates after in vitro fertilisation; it included 13 randomised controlled trials with a total of 4476 participants, which, it is interesting to note, accounted for more participants from fewer trials than in our review. Kolibianakis et al might have used different inclusion criteria. Their analysis shows that the addition of HA had a positive treatment effect on the clinical pregnancy rate. This finding is comparable with our results for HA comparison groups, even though participant numbers differed and Kolibianakis et al used a random-effects model rather than a fixed-effect model for data analysis, which we had used. It is unclear whether a full article on this systematic review has been published, and which studies were considered eligible for inclusion. The review authors have been contacted, but no response has been received to date.

This Cochrane Review is an update of the previous review (Bontekoe 2014). Similar conclusions were reached for all analyses.

AUTHORS' CONCLUSIONS

Implications for practice

Moderate-quality evidence shows that hyaluronic acid probably improves the success rate of assisted reproductive technologies such as in vitro fertilisation and intracytoplasmic sperm injection. The addition of hyaluronic acid to transfer medium probably increases the change in live birth from 33% to 40%, with an additional live birth for every 14 embryos transferred with high-HA media. HA addition also probably improves clinical pregnancy rates from 40% to 47%. A treatment effect was noted for both cleavage (days 2 to 4) and blastocyst (day 5) embryo transfers. This implies that the actual working mechanism of adherence compounds may not involve enhancement of adhesion per se. Low-quality evidence suggests that the addition of HA to transfer media may slightly decrease the risk of miscarriage from 12% to 10%. This effect was largely influenced by one study - an abstract with outlier results - and when removed, this treatment effect was no longer seen. Low-quality evidence indicates little or no treatment effect of HA addition on total adverse event rate. The addition of HA probably increases the chance of multiple pregnancy from 13% to 18% but is likely to be a consequence of both the effect of the adherence compound and a policy of transferring multiple embryos per treatment cycle. The combination of HA addition to transfer media and a single embryo transfer policy might yield the best combination with higher clinical pregnancy and live birth rates, without increasing the chance of multiple pregnancies. With each incremental improvement in the in vitro fertilisation technique, a compounding effect on multiple pregnancy rates will further strengthen the drive towards single embryo transfer.

Implications for research

The most important outcome measure that should be addressed is the live birth rate. Only ten of the 21 studies included in this systematic review reported on this outcome measure. The lack of studies reporting on the number of live births may be a result of the large proportion of pregnancies that fail to progress to birth, or it may reflect the frequent practice of reporting studies before the last study participant has given birth, suggesting either inadequate reporting capabilities or eagerness to publish. Other important outcome measures that have not been fully reported include miscarriage, multiple pregnancy, and other adverse events, such as ectopic pregnancy.

Further research on the actual working mechanism of HA might be useful. Additional studies of adherence compounds with single embryo transfer need to be undertaken. Also, randomised controlled trials on other potential adherence compounds should be performed in the future.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Balaban 2004

Study characteristics	
Methods	Parallel randomised controlled trial Prospective recruitment of participants Consecutive participant sampling Single-centre trial at the VKV American Hospital in Istanbul, Turkey Inclusion criterion: blastocyst stage embryos No exclusion criteria No power calculation Participants with multiple treatment cycles were allowed in the trial Participants were enrolled for a period of 4 months; actual length of follow-up per participant was 4 weeks. These times were intentionally kept short to reduce the chance of loss of participants, of which none occurred An intention-to-treat analysis was performed
Participants	Age (years): treatment group 31.2, control group 31.6. No SD given Primary or secondary subfertility: not reported Causes of subfertility: 1. Male factor: treatment group 129, control group 121; 2. Unexplained subfertility: 35 treatments, 30 controls; 3. Endometriosis: 3 treatments, 5 controls More than 1 factor: 26 treatments, 37 controls Mean duration of subfertility (years): treatment group 2.9, control group 3.2 Previous IVF and/or ICSI treatment (mean): treatment group 2.9, control group 3.2 All participants underwent ICSI. No IVF No age analysis 386 blastocyst stage transfers were recruited and randomly assigned: 193 to the treatment group and 193 to the control group. 405 embryos in the treatment group and 424 in the control group were transferred, resulting in a total of 829 transferred embryos. The total number of treatment cycles is unclear because participants were able to enrol multiple times. No loss, so the results of 386 participants were analysed

Balaban 2004 *(Continued)*

Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs embryo transfer in G2.3 (0.125 mg/mL HA). All embryos were cultured in G-III series culture medium. Both culture and transfer media were manufactured by Vitrolife (Gothenburg, Sweden). EmbryoGlue was provided by the American Hospital of Istanbul Randomisation on day of embryo transfer Embryos in treatment group were exposed to EmbryoGlue for 30 minutes before transfer Timing of embryo transfer: blastocyst stage (day 5) Oocyte donation was unclear All transferred embryos were fresh, no frozen-thaw protocol was followed Mean number of embryos transferred: treatment group 2.1, control group 2.1 Pregnancy determination: demonstration of gestational sac on ultrasound scan
Outcomes	Secondary outcomes • Clinical pregnancy rate: defined as number of pregnancies demonstrated on ultrasound divided by group size • Multiple pregnancy rate: defined as number of twin pregnancies divided by number of pregnancies Additional outcomes • Implantation rate: defined as number of demonstrated gestational sacs divided by total number of transferred embryos in group
Notes	Abstract of ASRM conference presentation; no full article has been published regarding this trial Additional data was retrieved after study authors were contacted Although these studies were undertaken by the same research team, there was no cross-over of patients between Balaban 2004 and Urman 2008 because Urman 2008 recruited patients between June 2006 and June 2007. Therefore, data from both Balaban 2004 and Urman 2008 were included in the analysis

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation into treatment or control group was performed by using a computer-generated randomisation list
Allocation concealment (selection bias)	Low risk	Randomisation list was maintained by the chief embryologist, who did not participate in daily laboratory work. The embryologist preparing the transfer was given allocation information immediately before actual transfer
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Clinician and participant were blinded; the scientist was not
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients who were randomised were included in the analysis. No loss of participants was reported

Balaban 2004 *(Continued)*

		To note, live birth rate was not reported, and actual length of follow-up was 4 weeks, which was done intentionally. Intention-to-treat analysis was performed
Selective reporting (reporting bias)	High risk	A published RCT protocol pre-trial was not found. Outcomes were pre-specified in Materials and Methods section
Other bias	High risk	Abstract only. EmbryoGlue was provided by the American Hospital. Transfer media used in both arms of the trial were comparable except for the addition of EmbryoGlue in the treatment arm. Multiple pregnancy rate was reported

Balaban 2011

Study characteristics	
Methods	Same as Urman 2008
Participants	Same as Urman 2008
Interventions	Same as Urman 2008
Outcomes	Live birth rate: reported as the take-home baby rate and defined as the number of live births divided by the number of participants
Notes	Update of live birth rate data resulting from clinical pregnancy rate was reported in Urman 2008, which did not use the live birth rate as an endpoint itself. No new inclusion of participants. Live births reported in the follow-up study - Balaban 2011 - and reported under this publication in the meta-analysis Conference proceeding at 27th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE) in Stockholm, Sweden

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Same as Urman 2008. Participants were randomly assigned to treatment or control group via a computer-generated randomisation list
Allocation concealment (selection bias)	Low risk	Same as Urman 2008. Allocation to study arm was provided after consecutively numbered, sealed opaque envelopes were opened
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Same as Urman 2008. Both clinician and participant were blinded to the group to which the participant was allocated
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Same as Urman 2008. Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	High risk	"Take home baby rate" was added after completion of the trial and does not seem to be part of the pre-specified protocol. Follow-up was long enough, and data were analysed according to the intention-to-treat principle
Selective reporting (reporting bias)	High risk	A published RCT protocol pre-trial was not found. Live birth rate was reported in a pre-specified manner

Balaban 2011 *(Continued)*

Other bias	High risk	Abstract only. Live birth was added after completion of the trial. Same as Urman 2008

Ben-Rafael 1995

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant sampling Multi-centre trial in the Hasharon Hospital of the Rabin Medical Center and in the Sackler School of Medicine of Tel Aviv University in Israel Inclusion criteria: at least 3 embryos ready for transfer; no more than 3 previous treatment cycles Exclusion criteria: fewer than 3 embryos ready for transfer No power calculation was performed Participants with only 1 treatment cycle were included in the trial Participants were recruited over a period of 6 months Follow-up per participant was provided until delivery No intention-to-treat analysis was performed Study was not supported by any commercial funding sources
Participants	Age (years): mean 34.2, SD 4.9 Patients were admitted for oocyte retrieval if 2 or more follicles of at least 18 mm mean diameter were present and the hormone profile was satisfactory Not reported whether primary or secondary subfertility Causes of subfertility were mechanical, male subfertility, and combined causes. Duration of subfertility ranged from 3 to 21 years (mean 8.3 ± 6.9) Previous IVF or ICSI was not reported, although participants could have had no more than 3 previous cycles Participants underwent IVF Age analysis: subgroups of < 31 years, 31 to 38 years, and 39 to 42 years 211 patients who were admitted to the IVF unit were recruited for the trial, and all were randomly assigned: 101 to the treatment group and 110 to the control group. In total, 759 embryos were transferred: 368 in the treatment group and 391 in the control group. No participants were lost to follow-up, so 211 participants were analysed
Interventions	Embryo transfer using a 2-component fibrin sealant vs transfer in regular medium, consisting of EBSS-P-SR2 with 10 mg/mL human serum albumin, manufactured by MediCult. The fibrin sealant was made of 2 components. The first consisted of fibrinogen, fibronectin, and an aprotinin solution. The second component consisted of thrombin and a calcium chloride solution The sealant was manufactured by Immuno AG Randomisation was performed between fertilisation check and day of embryo transfer

Ben-Rafael 1995 *(Continued)*

	Exposure time to fibrin sealant was not stated Timing of transfer was during the cleavage stage - 48 to 50 hours after oocyte retrieval Inclusion of oocyte donations was unclear Unclear whether embryos were frozen-thawed or fresh Two different culture and transfer medium brands: MediCult (culture medium and transfer medium control group) and Immuno AG (treatment group) Mean number of embryos transferred: treatment group 3.64, control group 3.55 Method of pregnancy determination: demonstration of gestational sac on ultrasound scan
Outcomes	Secondary outcomes • Clinical pregnancy rate: stated as percentage with number of transferred embryos as denominator • Adverse event rate: number of ectopic pregnancies, stated per participant Additional outcomes • Implantation rate: stated as percentage of implantations from total number of embryos transferred
Notes	Additional data were retrieved after contact with the original investigators, although raw data such as numbers of multiple pregnancies and live births could no longer be retraced

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly divided into treatment and control groups; method of randomisation was not stated
Allocation concealment (selection bias)	Unclear risk	Centralised randomisation by lab technician, who decided who would go into treatment or control group; unclear how this decision was made
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both participant and doctor were blinded. Embryologist was not blinded
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were analysed
Selective reporting (reporting bias)	Unclear risk	Proposed results were not pre-specified in Materials and Methods section
Other bias	High risk	No commercial funding. Different transfer media brands were used in both arms of the trial. No multiple pregnancy rate was reported, although multiple embryos have been replaced in each treatment cycle

Chen 2001

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Participant sampling unclear Single-centre trial performed at the IVF-Unit of Dr Tsai and Dr Chen's Women Hospital in Chang-Hua, Taiwan Inclusion criterion: patients undergoing IVF/ET who were having a day 3 embryo transfer No exclusion criteria Unclear whether a power calculation was performed Participants were followed for 14 days after embryo transfer Unclear whether participants were able to participate in multiple treatment cycles Unclear whether intention-to-treat analysis was performed, but no mention was made of loss to follow-up. Length of follow-up was 14 days
Participants	Mean age (range): treatment group 31.35 (22 to 43), control group 32.47 (23 to 40) years Primary or secondary subfertility not reported Cause and duration of subfertility not reported Participants underwent IVF. Whether they had been through previous IVF treatments was not reported No age analysis 70 participants were recruited and were randomly assigned to 2 groups: 35 to the treatment group and 35 to the control group. The exact number of embryos transferred is unclear. No loss of participants occurred, so the number of participants analysed was 70
Interventions	Embryo transfer in basal XI HTF(=transfer medium) with 10% human serum albumin (HSA) and 0.125 mg/mL HA vs transfer in basal XI HTF with 10% HSA Exposure time to HA before transfer was not stated Timing of randomisation was unclear, but it most likely occurred on day of embryo transfer because of the inclusion criterion of day 3 transfers Embryo transfer was performed during the cleavage stage (day 3) Frozen-thaw protocol unclear Oocyte donation unclear Culture and transfer medium brands were not stated; however, medium appears to be similar between treatment and control groups, except for the addition of HA to treatment group Mean number of embryos transferred (range): treatment group 2.71 (2 to 5), control group 3 (1 to 5) Pregnancy was determined via a pregnancy test
Outcomes	Other outcomes • Outcome measure of trial was biochemical pregnancy rate, but this was not part of the review

Chen 2001 *(Continued)*

Notes	Abstract of ESHRE conference presentation. Study authors cannot be found to provide additional information

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly assigned to study or control group, but method of randomisation was unclear
Allocation concealment (selection bias)	Unclear risk	Concealment of participant allocation was not clear
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Not stated in text
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not stated in text
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis
Selective reporting (reporting bias)	Low risk	Outcome of biochemical pregnancy test was announced in Methods section
Other bias	High risk	Abstract only. Commercial funding source was unclear. Transfer media used in both arms of the trial were comparable except for the addition of HA to the treatment arm. No multiple pregnancy rate was reported, and multiple embryos were replaced per cycle

Dittmann-Műller 2009

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant sampling Multi-centre study performed at the IVF Unit of the Women's Hospital in Chemnitz, Germany; the IVF/ICSI Centre in Basel, Switzerland; and the Department of Obstetrics and Gynecology of the University Hospital in Würzburg, Germany Inclusion criterion: undergoing IVF or ICSI between January 2006 and March 2007. No further inclusion criteria No exclusion criteria No power calculation was performed Actual length of follow-up per participant was 4 weeks after embryo transfer. Participants were enrolled in the trial between January 2006 and March 2007 and could participate in only 1 treatment cycle

Dittmann-Műller 2009 *(Continued)*

	Study was commercially funded by Vitrolife No intention-to-treat analysis was performed
Participants	Mean age (SD): treatment group 33.4 (4.0), control group 33.6 (4.4) years Primary or secondary subfertility not reported Mean duration of subfertility was 4 ± 2.4 years. Indications for subfertility treatment were tubal factors (21.6%), andrologic factors (69.6%), cycle abnormalities (1%), others (12.7%), and idiopathic causes (17.6%). Some participants had multiple causes Participants underwent both IVF and ICSI; most participants participated for the first time, but some were already in their third (or above) treatment cycle No age analysis was performed 102 participants were recruited and were randomly assigned to a treatment group of 54 or a control group of 48. No loss of participants was reported, so the data on 102 participants were analysed. The exact number of embryos transferred is unclear
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G-2 (0.125 mg/mL HA). Embryos in both groups were cultured in G-1 and G-2 version 3 plus, supplemented with 10% recombinant human albumin Randomisation was performed on the day before oocyte pick-up, which is between commencement of treatment and fertilisation check Exposure time to EmbryoGlue (=higher concentration of HA) before transfer was 30 minutes Cleavage stage embryo transfer on day 3 All transferred embryos were fresh No oocyte donations were included in the trial Both culture and transfer media were manufactured by Vitrolife Mean number of embryos transferred (for both treatment and control groups) was 2.7 Pregnancy was determined by demonstration of gestational sac on ultrasound scan
Outcomes	Secondary outcomes • Clinical pregnancy rate: reported as number of participants who got pregnant divided by group size • Multiple pregnancy rate: reported as number of participants pregnant with twins divided by number of participants from group who got pregnant
Notes	Trial presented at ESHRE Conference. A full article is planned. Additional unpublished data received after contact with original investigators

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to treatment or control group with the use of a cube. Even numbers formed the treatment arm, odd numbers the control arm
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was not reported

Dittmann-Műller 2009 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both participants and clinicians were blinded to treatment; scientists were not
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis
Selective reporting (reporting bias)	Low risk	In Materials and Methods section, it was announced that pregnancy rates will be recorded, and they are accounted for in the Results section
Other bias	High risk	Trial was commercially funded. Transfer media used in both arms of the trial were comparable except for the addition of EmbryoGlue to the treatment arm. A multiple pregnancy rate was reported

Drew 2014

Study characteristics	
Methods	Parallel randomised controlled trial comparing EmbryoGlue (Vitrolife) to standard medium Prospective participant recruitment Sampling unclear Unclear if single-centre or multi-centre study Unclear inclusion and exclusion criteria A power calculation was not performed Length of follow-up per participant was not reported Unclear if intention-to-treat analysis was performed, and unclear if patients were lost to follow-up, or if patients withdrew from the study Randomisation was performed by cycle
Participants	Age characteristics were not reported Causes and duration of subfertility were not specified. Nor was it stated whether study concerned primary or secondary subfertility Unclear whether participants underwent IVF or ICSI or both, and whether they had received previous treatments No age analysis was performed 493 embryo transfer cycles (283 single embryo transfers and 210 double embryo transfers) were randomly assigned to treatment and control groups. No mention in text of number of participants recruited, group sizes, nor any loss to follow-up or numbers of embryos transferred Study authors were contacted, but they declined the invitation to participate in the review
Interventions	Embryo transfer in EmbryoGlue (0.5% mg/mL HA) vs transfer in standard medium (no mention of HA concentration) Timing of randomisation was unclear Embryos in treatment group were exposed to EmbryoGlue for a minimum of 10 minutes before transfer Cleavage and blastocyst stage embryo transfer (on day 3 or day 5) Unclear whether frozen-thawed embryos were included in the trial Unclear whether donor oocytes were included The mean number of embryos transferred was not reported Method of pregnancy determination was not reported

Drew 2014 *(Continued)*

Outcomes	Clinical pregnancy rate - not defined
Notes	Abstract only. From ACE 9th Biennial Conference 2014, United Kingdom Study authors were contacted but declined the invitation to participate in the review Due to randomisation being performed by cycle, rather than by couple, and missing data, this study could not be used in the analysis

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised by random number generation
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment not defined
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Method of blinding unclear
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear number of patients randomised and unclear number included in the analysis. Participation loss unclear Actual length of follow-up per participant was unclear
Selective reporting (reporting bias)	Unclear risk	Outcome measures were reported in a pre-specified way
Other bias	High risk	Abstract only Commercial funding source was unclear Manufacturers of media were unclear Units of randomisation - embryo transfer, not per couple No multiple pregnancy rate was reported, and multiple embryos were transferred per cycle

Fancsovits 2011

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant sampling Single-centre study performed at the Semmelweis University School of Medicine, in Budapest, Hungary No strict inclusion or exclusion criteria

Fancsovits 2011 *(Continued)*

	No power calculation was performed Actual length of follow-up per participant was 4 weeks after embryo transfer. Participants were enrolled in the trial between January 2006 and March 2007 and could participate in only 1 treatment cycle Study was commercially funded by Vitrolife No intention-to-treat analysis was performed
Participants	Mean age (SD): treatment group 35.7 (4.1), control group: 34.2 (4.6) years Type, cause, and duration of subfertility not reported Number of previous IVF or ICSI treatments not reported Included participants could undergo IVF or ICSI for the trial An age analysis was performed; outcome data from participants 40 years of age or younger were compared with those from participants over 40 years of age 200 cycles were randomly assigned to a treatment group of 103 and a control group of 97. The total number of transferred embryos was 467: 238 in the treatment group and 229 in the control group
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G-2 (0.125 mg/mL HA). Embryos were incubated in EmbryoGlue or control medium for 5 to 10 minutes before transfer. Embryos in both groups were cultured in G-1 and G-2 until 2 to 3 days after fertilisation Cleavage stage embryo transfer Randomisation was performed 1 day before embryo transfer. All culture and transfer media were manufactured by the same company - Vitrolife. All embryos were fresh, and oocyte donations were not included. The mean number of transferred embryos was 2.3 (± 0.8) in the treatment group and 2.4 (± 0.7) in the control group. Pregnancy was demonstrated via hCG pregnancy test and demonstration of a gestational sac on ultrasound
Outcomes	Primary outcomes • Live birth rate, reported as the number of born babies. Not reported in original publication Secondary outcomes • Clinical pregnancy rate, reported as the number of clinical pregnancies, demonstrated by positive pregnancy test and on ultrasound, divided by the number of cycles. Reported as percentages in original publication; raw data after contact with study authors Additional outcomes • Implantation rate, defined as the number of implantations divided by the number of transferred embryos. Reported as percentages in original publication; raw data after contact with study authors
Notes	Conference abstract of a trial presented at ESHRE Meeting in 2011. Additional data and study information were provided by the original investigator after contact was made with the authors of this review. See Appendix 7 In this trial, cycles instead of participants were randomly assigned; this is not compatible with data analysis that is part of this systematic review because of the possibility of participants with multiple cycles enrolling. However, after contact was made with the original investigator, it appeared that the number of multiple entries was less than 10% of the total number (7 in the treatment group and 12 in the control group), which was deemed acceptable by the review authors

Risk of bias

Bias	Authors' judgement	Support for judgement

Fancsovits 2011 *(Continued)*

Random sequence generation (selection bias)	Low risk	Random allocation into treatment or control group, with randomisation achieved by a computer-generated randomisation table
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was not reported
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Blinding of participant and clinician/nurse
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear whether any loss to follow-up occurred No intention-to-treat analysis was performed
Selective reporting (reporting bias)	Low risk	Outcome measures were reported in a pre-specified fashion. Of note, certain outcomes such as live birth rate were not reported in the original publication but only after contact was made with the trial authors
Other bias	High risk	Abstract only No commercial funding Culture media/environment in treatment and control groups comparable Multiple pregnancy rate not reported while multiple embryo transfer policy was followed

Fancsovits 2015

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant sampling Single-centre study performed at the Semmelweis University School of Medicine, in Budapest, Hungary Exclusion criteria: oocyte donation No power calculation was performed Length of follow-up per participant was until birth. Participants were enrolled in the trial between January 2010 and August 2012 and could participate in only 1 treatment cycle Funding source unclear An intention-to-treat analysis was performed
Participants	Mean age (SD): treatment group 35.8 (4.5), control group 34.8 (4.9) years Duration of subfertility not reported

Fancsovits 2015 *(Continued)*

	Indication for fertility was reported. The numbers of patients with tubal factors, endometriosis, male factors, and multiple factor and idiopathic for the HA group were 40, 15, 116, 28, and 91, and for the control group 42, 23, 127, 26, and 73, respectively Number of previous IVF or ICSI treatments not reported. However, there was a subgroup analysis of patients with 2 or more IVF failures, with no significant difference between the 2 groups for any outcome Included participants could undergo IVF or ICSI for the trial An age analysis was performed. Patients in the HA group were significantly older (P = 0.011) A subgroup analysis was performed with outcome data from participants 40 years of age or older with no significant differences between the 2 groups on any outcome 581 couples were recruited and randomised: 290 in the intervention arm and 291 in the control group. No patients withdrew or were lost to follow-up To note, randomisation was done by cycle - not by couple, and patients with multiple cycles were included. However, study authors were contacted and provided first cycle data for live birth, miscarriage, and clinical pregnancy. However, multiple pregnancy data were not provided; hence, the data for this measure could not be used in the meta-analysis
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G-2 (0.125 mg/mL HA) Embryos were incubated in EmbryoGlue or control medium for 5 to 10 minutes before transfer. Embryos in both groups were cultured in G-1+ until 2 or 3 days after fertilisation Cleavage stage embryo transfer Randomisation was performed 1 day before embryo transfer All culture and transfer media were manufactured by the same company - Vitrolife All embryos were fresh, and oocyte donations were not included The mean number of transferred embryos was 2.2 (± 0.8) in the treatment group and 2.3 (± 0.7) in the control group. Pregnancy was demonstrated via hCG pregnancy test (> 20 UI/mL) and demonstration of a gestational sac on ultrasound
Outcomes	Primary outcomes • Live birth rate, reported as the number of born babies Secondary outcomes • Clinical pregnancy rate, defined by the appearance of a gestational sac on transvaginal ultrasound 5 weeks after ET Additional outcomes • Implantation rate, defined as the number of gestational sacs by the number of transferred embryos • Multiple pregnancy rate (not first cycle data, so therefore could not be used in the analysis). No significant difference was found between the 2 groups • Abortion rate - not clearly defined
Notes	No overlap in participants with Fancsovits 2011 (different years for recruitment of patients) Contact was made with the study author and data for the first cycle were provided for clinical pregnancy, miscarriage, and live birth rate

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was achieved by a computer-generated randomisation table
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was not stated

Fancsovits 2015 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both the clinician performing the embryo transfer and the patient were blinded to the allocation
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	No loss to follow-up (information provided after study authors contacted)
Selective reporting (reporting bias)	Low risk	Outcome measures were reported in a pre-specified fashion
Other bias	Low risk	Funding source was not stated. However study authors did specify that there were no financial or commercial conflicts of interest Study did not seek institutional review board approval Multiple pregnancy rate was reported

Fasano 2016

Study characteristics	
Methods	Parallel randomised controlled trial comparing Embryo Glue to routine embryo transfer medium Method of randomisation unclear; according to the text a "prospectively randomized list" was used Prospective participant recruitment Unclear if consecutive sampling Unclear if single-centre or multi-centre study Inclusion and exclusion criteria not reported A power calculation was not performed Length of follow-up per participant was not reported An intention-to-treat analysis was not performed, nor was loss to follow-up accounted for Randomised by cycle - not by couple
Participants	Mean age and range were not reported Causes and duration of subfertility not specified, nor was it stated whether the study concerned primary or secondary subfertility Unclear whether participants underwent IVF or ICSI or both, and whether they had received previous treatments No age analysis was performed 253 participants were recruited for this trial. Embryo cycles were randomly assigned to treatment and control groups. Unclear group sizes. No mention of any loss to follow-up
Interventions	Embryo transfer in EmbryoGlue (0.5% mg/mL HA) vs transfer in routine embryo transfer medium (exact components not specified) Timing of randomisation was unclear Duration of exposure to EmbryoGlue before transfer was not specified Transfers were performed during both cleavage stage (on day 2/3) and blastocyst stage (day 5/6) Frozen-thawed embryos were not included in the trial Unclear whether donor oocytes were included

Fasano 2016 *(Continued)*

	The mean number of embryos transferred was not reported, nor was the method of pregnancy determination
Outcomes	Secondary outcomes •Pregnancy rate: no definition given in the text, only percentages given Additional outcomes •Abortion rate: no definition given in the text, only percentages given
Notes	Abstract only from ESHRE 32nd Annual Meeting Study authors contacted but no response was received before publication of the review Randomisation was done per cycle - not per couple. Due to this and missing data, this study could not be included in the analysis

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method of randomisation not specified
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment not defined
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Method of blinding unclear
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear group sizes and unclear number of patients lost to follow-up
Selective reporting (reporting bias)	Unclear risk	Outcome measures not defined
Other bias	High risk	Randomisation done per cycle - not per couple Abstract only

Friedler 2005

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant sampling Single-centre trial performed at the IVF and Infertility Unit of the Assaf Harofeh Medical Center of the University of Tel Aviv, in Israel

Friedler 2005 *(Continued)*

	Inclusion criteria: participants had to be younger than 43 years, undergoing IVF/ICSI, and must have failed to achieve pregnancy after 4 previous embryo transfers Exclusion criteria: 43 years of age or older, more or fewer than 4 previous treatment cycles Unclear whether a power calculation was performed Actual length of follow-up was unclear, yet it appeared to be long enough for outcome measures to be reported Participants were enrolled for only 1 treatment cycle (because of inclusion criterion of 4 previous attempts) Unclear whether an intention-to-treat analysis was performed; neither whether was any loss to follow-up reported
Participants	Mean age (SD): treatment group 33.8 (4.97), control 33.8 (4.97) years Primary and/or secondary subfertility not reported Cause and duration of subfertility not reported Four previous IVF or ICSI treatments Trials in which participants were undergoing both IVF and ICSI were included No age analysis was performed 187 participants were recruited and randomly assigned to treatment group of 94 or control group of 93. No loss of participants was reported; therefore the data on 187 participants were analysed. Exact number of embryos transferred was unclear
Interventions	Embryo transfer with EmbryoGlue (0.5 mg/mL HA) vs transfer with HTF medium enriched with 20% serum substitute supplement (SSS) Timing of randomisation was unclear Exposure time to HA before transfer was not stated Cleavage stage embryo transfer (days 2 to 4) Unclear whether a frozen-thaw protocol was followed Unclear whether oocyte donations were included Transfer medium in treatment group was manufactured by Vitrolife, and transfer medium for the control group was manufactured by Irvine Scientific (Santa Ana, CA, USA) Mean number of embryos transferred: treatment group 3.4 ± 1.05, control group 3.2 ± 1.05 Method of pregnancy determination was not reported
Outcomes	Secondary outcomes • Clinical pregnancy rates: unclear whether defined per participant or per embryo transferred, but it can be assumed to be per participant • Adverse event rate: miscarriage rate was measured - not clear whether per participant, per clinical pregnancy, or per embryo transferred. However, a notably high early spontaneous abortion rate was observed in both groups Additional outcomes • Implantation rate: definition unclear

Friedler 2005 *(Continued)*

Notes	Abstract of an ESHRE Conference presentation

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly allocated to treatment or control group, but method of randomisation was unclear
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was unclear
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Blinding was not reported
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Blinding was not reported
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis
Selective reporting (reporting bias)	Low risk	Outcome measures were reported in a pre-specified way
Other bias	High risk	Abstract only. Commercial funding source was unclear. Transfer media for treatment and control groups were made by different manufacturers. No multiple pregnancy rate was reported, and multiple embryos were transferred per cycle

Friedler 2007

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant sampling Single-centre trial performed at the IVF and Infertility Unit of the Assaf Harofeh Medical Center, Sackler School of Medicine, University of Tel Aviv, Israel Inclusion criteria: patients who failed to achieve an ongoing pregnancy after more than 4 previous embryo transfers, during which 2 to 4 embryos were transferred each time, including at least 1 optimal embryo. Had to be younger than 43 years of age and had to have given informed consent. Undergoing ICSI at the IVF Unit Exclusion criteria: patients older than 43 years of age, suffering from a systemic disease, BMI > 29 kg/m^2, uterine malformation, evidence of low ovarian response, elevated baseline FSH (> 12 IU/L), hydrosalpinx, participation in any other clinical study Power calculation performed but not followed. Group size of 112 in each arm of the study was proposed, but after an interim analysis of 101 participants, the trial was stopped. This was done for ethical reasons, and the study is therefore eligible

Friedler 2007 *(Continued)*

	Participants were enrolled in the trial from June 2005 to November 2006. Actual length of follow-up per participant was up to 9 months. However, 1 of the outcome measures of the study was delivered or ongoing pregnancy rate Participants with only 1 single cycle were able to enrol in the study An intention-to-treat analysis was not performed Study was free from commercial funding
Participants	Mean age (SD): treatment group 33.1 (5.1), control group 31.7 (5.6) years Not reported whether study concerned primary or secondary subfertility Causes of subfertility included male factor, tubal factor, endometriosis, unexplained subfertility, and combination of female and male factors Duration of subfertility was not reported Participants had to have undergone at least 4 previous treatment cycles. Average was 5.5 previous unsuccessful embryo transfers All participants underwent ICSI No age analysis was performed 101 participants were recruited and were randomly assigned to a treatment group of 51 or a control group of 50. 159 embryos in the treatment group were transferred, and 146 in the control group, resulting in a total of 305 embryos transferred. No participants were excluded, withdrawn, or lost to follow-up, so data on 101 participants were analysed
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA and 2.5 mg/mL recombinant human albumin) vs transfer in human tubal fluid (HTF) with gentamycin, enriched with 20% serum substitute supplement Randomisation on day of embryo transfer Exposure time to HA before embryo transfer was 10 minutes Cleavage stage embryo transfer (days 2 and 3) No frozen-thaw protocol was followed Unclear whether oocyte donations were included Transfer media for treatment and control groups were manufactured by 2 different companies: treatment medium by Vitrolife, and control medium by Irvine Scientific Mean number of embryos transferred: treatment group 3.1 ± 0.73, control group 2.9 ± 0.63 Pregnancy was determined by demonstration of gestational sac on ultrasound scan and pregnancy test
Outcomes	Secondary outcomes • Ongoing pregnancy rate: defined as delivered or ongoing pregnancies divided by number of participants per group • Clinical pregnancy rate: defined by ultrasound scan, number of pregnancies divided by group size • Multiple pregnancy rate: defined as number of multiple pregnancies divided by number of pregnancies in group • Adverse event rate: both ectopic pregnancy rate and early spontaneous abortion rate were reported. The rate was defined as the number of events divided by group size. For this review, the data for both types of adverse events have been added up Additional outcomes

Friedler 2007 *(Continued)*

- Implantation rate: defined as the number of implantations per embryo transferred in group

Notes	No overlap in participants with Friedler 2005 Additional data retrieved after contact was made with study authors

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to treatment or control group based on computer-generated random number sequence
Allocation concealment (selection bias)	Low risk	Participant allocation was performed by the chief embryologist just before embryo transfer, according to a computer-generated random number sequence
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both participant and clinician were blinded. The embryologist was not blinded
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis
Selective reporting (reporting bias)	Low risk	Clinical pregnancy rate was announced in the Methods section. However, many other outcomes were reported in the Results section
Other bias	Unclear risk	Free from commercial funding. Embryos in treatment and control groups were transferred in media from different manufacturers, and similarity between culture media was unclear. The multiple pregnancy rate was reported To note, no live births were reported, even though the actual length of follow-up per participant was up to 9 months

Hazlett 2008

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment Participant sampling in consecutive order but stated as non-consecutive in Hazlett 2005 Single-centre trial performed at the Department of Embryology of Karande and Associates, in Hoffman Estates, Illinois, USA Patients who were excluded were diagnosed as having a low success rate, which meant having a diminished ovarian reserve (FSH of at least 10 IU/mL), being older than 40 years of age, or having a hydrosalpinx

Hazlett 2008 *(Continued)*

	Participants were selected for a day 5 embryo transfer if a minimum of 5 embryos with little fragmentation were present on day 3 and/or if they had 8 or more fertilised zygotes A power calculation was performed, which estimated that a 20% difference in clinical pregnancy rate would be found, with 5% significance and 80% power if at least 107 participants were included in each arm of the study Length of follow-up per participant was 11 weeks No intention-to-treat analysis was performed Participants with multiple treatment cycles were able to enrol in the study in case of treatment failure
Participants	Mean age (SD): participants were divided into day 3 and day 5 transfer subgroups Treatment groups: day 3, 33.4 (4.4); day 5, 31.4 (4.2) years; control groups: day 3, 33.4 (5.0); day 5, 33.1 (4.8) years Causes and duration of subfertility not reported. Nor was it reported whether study concerned primary or secondary subfertility. Yet text states that there were no differences between treatment and control groups regarding cause and duration Included participants underwent IVF or ICSI. Unclear whether they underwent previous treatments No age analysis 233 participants appear to be recruited, even though the text states only 224. Of 233 participants, 223 were randomly assigned to a treatment group of 116 or a control group of 107. Five participants were part of a preliminary study and were not randomly assigned. Five others were withdrawn for protocol violations. The treatment group was divided into 84 participants who had a day 3 transfer and 32 who had a day 5 transfer. The control group comprised 78 day 3 transfers and 29 day 5 transfers The total number of transferred embryos was 519: 266 in the treatment group and 253 in the control group
Interventions	Embryo transfer on day 3 or day 5 of development in EmbryoGlue (0.5 mg/mL HA) vs transfer in IVC-1 or IVC-2 + 0.5% HSA (human serum albumin) on day 3 or G2.3 (0.125 mg/mL HA) + 0.5% HSA on day 5. Therefore, for data analysis, this trial is divided into day 3 transfers, which compare HA vs no HA, and day 5 transfers, which compare HA vs low concentrations of HA Embryos were cultured in IVC-1 or IVC-2 until day 3 and in G2.3 for the additional 2 days Timing of randomisation was unclear. It appears that it occurred on the day before embryo transfer, for it is stated this occurred in the Hazlett 2005 trial, which provides data on the same participants Exposure time to EmbryoGlue in the treatment group was 10 to 60 minutes before embryo transfer No frozen-thaw protocol was followed No donor oocytes were included Transfer and culture media were manufactured by 2 different manufacturers: In Vitro Care and Vitrolife Mean number of transferred embryos: treatment group day 3: 2.5 ± 0.9, day 5: 2.1 ± 0.5; control group day 3: 2.4 ± 0.8, day 5: 2.1 ± 0.9 Pregnancy was determined via pregnancy tests and demonstration of gestational sac and foetal heartbeat on ultrasound scan
Outcomes	Primary outcomes • Live birth rate: data received after contact with study author regarding other publication of the same data (Hazlett 2005); reported for the whole study population and for day 3 and day 5 transfers separately. Defined as number of live births divided by number of participants

Hazlett 2008 *(Continued)*

	Secondary outcomes • Clinical pregnancy rate: defined as number of participants with at least 1 intrauterine gestational sac on ultrasound 2 weeks after positive hCG pregnancy test divided by total number of participants Additional outcomes • Implantation rate: defined as total number of intrauterine gestational sacs divided by total number of embryos transferred Other outcomes • Viable pregnancy rate: defined as ongoing pregnancy demonstrated by foetal cardiac activity at 7 weeks' gestation divided by group size
Notes	This study comprises data from previous publications (Hazlett 2004 and Hazlett 2005), but only outcome data were extracted from it In previous versions of this review, this group of studies was counted as 3 separate studies in Figure 1. However, due to these being the same study, this group was included as 1 study only in the current version of the review Additional data were retrieved by contacting study authors

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to treatment or control groups by a computer-generated random numbers sequence
Allocation concealment (selection bias)	Low risk	Randomisation was performed by computer-generated random numbers sequence using sealed envelopes to allocate to the treatment arm
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participant and clinician/nurse were blinded. Embryologist was not
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	High risk	Unclear number of patients recruited. Loss to follow-up was accounted for
Selective reporting (reporting bias)	Low risk	Clinical pregnancy rate and implantation rate were reported in a pre-specified way
Other bias	High risk	Free from commercial funding. Different media brands were used, therefore not comparable. No multiple pregnancy rate was reported, although multiple embryos were transferred per cycle

Kandari 2019

Study characteristics

Kandari 2019 *(Continued)*

Methods	Parallel randomised controlled trial comparing EmbryoGlue (Vitrolife) to CSCM medium (which does not contain hyaluronic acid) Prospective participant recruitment Sampling unclear Multi-centre study including - Cellsure Biotech and Research Centre, Mumbai - ReproGeneX Center, Mumbai - Akruti Fertility Centre, Mumbai Inclusion criteria: time lapse selected; single, fresh embryo transfers in patients with polycystic ovarian syndrome Unclear exclusion criteria A power calculation was not performed Length of follow-up per participant was adequate An intention-to-treat analysis was not performed Information provided on loss to follow-up and patient withdrawals Randomisation was performed by patient - not by cycle
Participants	Age characteristics were reported and analysis performed Causes of subfertility were not specified (other than PCOS) Infertity duration and number of previous IVF cycles were reported. Oocyte number was also reported 428 participants were recruited for this trial and were randomly assigned to treatment - 17 were withdrawn because of early OHSS, given GnRH agonist and freeze-all protocol - 44 withdrew consent before embryo transfer - 12 changed number of transfer embryos - 337 were randomised on day of embryo transfer to either HA or conventional transfer media and underwent embryo transfers - 16 were withdrawn due to protocol non-compliance by staff - 321 were analysed: 153 in HA group and 168 in control group
Interventions	Embryo transfer in EmbryoGlue vs transfer in CSCM medium (containing no HA) Both single cleavage stage and blastocyst stage embryos were transferred Randomisation was performed on the day of embryo transfer Unclear amount of exposure time in EmbryoGlue before transfer Transfers included both cleavage and blastocyst stage embryos Frozen-thawed embryos were not included in the trial Unclear whether donor oocytes were included The mean number of embryos transferred was not reported Method of pregnancy determination was not reported
Outcomes	Outcomes (not mentioned which were primary or secondary) • Live birth • Miscarriage rate - biochemical pregnancy (serum bhCG level of at least 50 IU/L 2 weeks after embryo transfer) not resulting in a live birth • Implantation rate
Notes	Abstract only. From Fertility and Sterility ASRM Abstract, Issue 12 October 2019 to 16 October 2019 Contact was made with the study author and information regarding randomisation, sequence allocation, and patient withdrawals was provided

Kandari 2019 *(Continued)*

This study looked at women with PCOS, who according to their analyses were a young population (32.7 ± 3.6 and 31.7 ± 3.6, respectively), with few previous IVF cycles (1.3 ± 1.5 and 1.1 ± 1) and a good oocyte number(14.4 ± 5.2 and 13.2 ± 5). Therefore, this analysis was added to the good prognosis group

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed using a programme based on Wichmann-Hill random number generator on randomization.com Randomisation number was serially allotted to every patient posted for fresh embryo transfer on day of embryo transfer
Allocation concealment (selection bias)	Low risk	Sequence allocation was concealed by providing a sealed envelope with patient's name to laboratory personnel and clinician before embryo transfer
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Method of blinding unclear
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	On contact with study authors, details on number of patients recruited, number of withdrawals, and number lost to follow-up was specified Length of follow-up per participant was adequate
Selective reporting (reporting bias)	Low risk	Outcome measures were reported in a pre-specified way
Other bias	High risk	Abstract only data Manufacturers of media specified Commercial funding source was unclear No multiple pregnancy rate was reported, and multiple embryos were transferred per cycle

Khan 2004

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment Sampling unclear Single-centre trial performed at IVF Michigan in Rochester Hills, in the USA Participants with all types of subfertility diagnosis were included, but they had to be younger than 39 years of age Unclear whether a power calculation was performed Length of follow-up per participant is unclear

Khan 2004 *(Continued)*

	Unclear whether an intention-to-treat analysis was performed, or whether any loss to follow-up occurred Unclear whether participants on multiple treatment cycles were able to enrol in the trial
Participants	Mean age (range): treatment group 32.7 (24 to 39), control group 33.8 (23 to 39) years Causes and duration of subfertility not specified. Nor was it stated whether study concerned primary or secondary subfertility Unclear whether participants underwent IVF or ICSI or both, and whether they had received previous treatments No age analysis was performed 165 participants were recruited for this trial and were randomly assigned to treatment and control groups. No mention in text of group sizes, nor of any loss to follow-up or numbers of embryos transferred. Study authors were contacted, but no response has been received yet
Interventions	Embryo transfer in EmbryoGlue (0.5% mg/mL HA) vs transfer in P1 Complete Medium (contains gentamycin, taurine, and 10% protein supplement; no HA) All embryos were cultured in P1 Complete Medium Timing of randomisation was unclear Embryos in treatment group were exposed to EmbryoGlue for approximately 10 minutes before transfer Cleavage stage embryo transfer (day 3) Unclear whether frozen-thawed embryos were included in the trial Unclear whether donor oocytes were included All embryos were cultured in medium manufactured by Irvine Scientific, and control group was transferred in same medium; treatment group was transferred in EmbryoGlue manufactured by Vitrolife Mean number of embryos transferred: treatment group 3.3, control group 3.1 Method of pregnancy determination was not reported
Outcomes	Secondary outcomes • Ongoing pregnancy rate: no definition given in the text, only percentages given; no raw data Additional outcomes • Implantation rate: no definition given in the text, only percentages given; no raw data
Notes	Abstract of an ESHRE Conference presentation

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly assigned to treatment or control group, but method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was unclear

Khan 2004 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Unclear whether participants, clinicians, and/or embryologists were blinded
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No information on actual participant numbers, group sizes, or loss to follow-up
Selective reporting (reporting bias)	Low risk	Ongoing pregnancy and implantation rates were reported in a pre-specified way
Other bias	High risk	Abstract only. Commercial funding source was unclear. Different transfer media brands were used. However, culture and transfer media for both arms were comparable, with the exception of EmbryoGlue added to the medium in the treatment arm. No multiple pregnancy rate was reported, although multiple embryos were transferred per cycle

Kleijkers 2016

Study characteristics	
Methods	Randomised, double-blind trial comparing human tubal fluid (HTF) medium and G5 medium Parallel randomised controlled trial Prospective participant recruitment Consecutive sampling Multi-centre study, Netherlands Included participants: couples who were scheduled for IVF or ICSI treatment for their first IVF/ICSI cycle ever, or first IVF/ICSI cycle after a previous successful pregnancy, were eligible to participate in the study Excluded participants: couples undergoing a modified natural cycle, couples for whom IVF was used to prevent transmission of human immunodeficiency virus, couples undergoing PGD, and couples using ART for fertility preservation A power calculation was performed Length of follow-up per participant is sufficient An intention-to-treat analysis was performed, and loss to follow-up accounted for Randomised by couple - not by cycle
Participants	Mean age (range): treatment group 33.9 (29.6 to 38.2), control group 33.8 (29.4 to 37.2) years Causes and duration of subfertility were reported. The numbers of couples with tubal, male subfertility, unexplained infertility, and other causes of infertility for the treatment group was 47, 211, 100, and 59, and for the control group 37, 225, 84, and 73, respectively Duration of infertility for the treatment group was 3.1 ± 1.9, and for the control group 3.1 ± 2.3

Kleijkers 2016 *(Continued)*

	Included couples undergoing their first IVF/ICSI cycle or their first IVF cycle after a successful pregnancy An age analysis was performed with no significant differences between the 2 groups 836 participants were recruited for this trial and were randomly assigned to treatment (417 participants) and control groups (419 participants) One participant was lost to follow-up, and 1 withdrew
Interventions	Tubal fluid (HTF), no concentrations were mentioned in the text Timing of randomisation was 1 day before oocyte retrieval Embryos in treatment group were exposed to G1 PLUS media from days 1 to 3 and G2 PLUS from days 3 to 4. The control group HTF was transferred to another culture dish (also containing HTF) on day 3 Cleavage stage embryo transfer (day 2 or 3 and, in the case of cryopreservation, day 3 or 5) Both fresh and frozen-thawed embryos were included in the trial. Separate analyses comparing these groups were not performed Donor oocytes were included Mean number of embryos transferred: treatment group 2.8, control group 2.3 Method of pregnancy determination • Serum bhCG level of at least 50 IU/L 2 weeks after embryo transfer • Gestational sac and foetal heartbeat on transvaginal ultrasound examination
Outcomes	Primary outcome: live birth rate Secondary outcomes • Clinical pregnancy rate - presence of a gestational sac and foetal heartbeat confirmed by transvaginal ultrasound examination at 6 to 8 weeks' gestation • Ongoing pregnancy rate - viable intrauterine pregnancy after 12 weeks of gestational viable intrauterine pregnancy after 12 weeks' gestation Additional outcomes • Miscarriage rate - biochemical pregnancy (serum bhCG level of at least 50 IU/L 2 weeks after embryo transfer) not resulting in a live birth • Multiple pregnancy rate • Number of implantations - determined by the number of foetal sacs as identified by transvaginal ultrasound examination at 6 to 8 weeks' gestation. From this, together with number of embryos transferred, the implantation rate could be determined • Major and minor foetal congenital defects - major malformations were defined as those causing functional impairment or requiring surgical correction. The remaining congenital malformations were considered minor
Notes	To note, there were differences in the media used other than the addition of hyaluronan (e.g. lipoic acid) Study authors were contacted about the concentration of hyaluronan in the G5 culture; however a reply was not received before publication Additionally, among the embryos transferred after cryopreservation, 1 hospital transferred these embryos during the blastocyst stage, on day 5. However, the rest of embryos in this study were transferred during the cleavage stage (between days 2 and 4). Data for the hospital with the blastocyst transfer protocol were not provided separately; therefore this study was excluded from the subgroup analyses on timing of embryo transfer (Analysis 1.2; Analysis 1.9)

Kleijkers 2016 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed centrally by an online computer program with 1:1 allocation using random block sizes of 2 and 4 couples, 1 day before oocyte retrieval of the first cycle
Allocation concealment (selection bias)	Low risk	Only at the end of the study, when data collection was completed, the allocation sequence was revealed to the primary investigators
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Allocation sequence and allocated treatment were fully blinded to participating couples, attending gynaecologists, fertility doctors, and outcome assessors. Blinding of the embryologists was not possible because they performed the procedures in the laboratory
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	Loss to follow-up and withdrawn patients accounted for
Selective reporting (reporting bias)	Low risk	Outcome measures were reported in a pre-specified way
Other bias	Unclear risk	Unclear whether culture media were similar between treatment and control groups. Concentration of HA not specified Source of funding: The NutsOhra Foundation (Grant 1203-061) and the March of Dimes (Grant 6-FY13-153). Funders had no role in development, interpretation, or writing of the study

Korošec 2007

Study characteristics

Methods	Parallel randomised controlled trial Prospective participant recruitment and consecutive sampling Multi-centre trial performed at the Department of Obstetrics and Gynecology of the University Medical Centre of Ljubljana, Slovenia, and at the Institute for Reproductive Medicine and Endocrinology, in Bregenz, Austria Inclusion criteria: women had to be younger than 37 years of age and within their first 3 treatment cycles, resulting in selection of twin-prone women A power calculation that estimated 80% power was performed according to preliminary results before the entire study population was randomly assigned Length of follow-up per participant was 30 days. However, a subgroup of participants undergoing a fresh embryo transfer was checked for live births a year later. These data have not been published No intention-to-treat analysis was performed Participants with multiple cycles were able to enrol in the study

Korošec 2007 *(Continued)*

Participants	328 women were recruited for this study; it included a group of women who had received a single fresh embryo transfer and a group who had received a single frozen-thawed embryo transfer. Participants were randomly assigned to a treatment group of 138 and a control group of 158. 32 women declined participation after randomisation was carried out. Because only a single embryo was transferred per woman, 296 (138 + 158) transfers were performed and analysed. The tables in the Results section of the article present only the data on 279 transfers, but in the text, the data on 17 women who had received a compulsory single transfer were reported Mean age (SD): treatment group fresh 31.3 (3.7), frozen-thawed 32.1 (3.5); control group fresh 31.9 (3.7), frozen-thawed 32.7 (3.2) Causes of subfertility included tubal factor, endometriosis, endocrine disorders, idiopathic causes, male factors, combined female factors, and combined female and male factors Duration of subfertility was not reported, nor whether primary and/or secondary subfertility was present Included women underwent IVF or ICSI and could have received up to 2 previous treatments No age analysis was performed
Interventions	Fresh and frozen-thawed embryo transfers in EmbryoGlue (0.5 mg/mL HA) vs fresh and frozen-thawed transfers in M2 medium Embryos were cultured to the blastocyst stage sequentially in M1 and M2 culture medium, which contains no HA Randomisation was performed on the day of embryo transfer Embryos in the treatment group were exposed to HA for at least 4 hours Transfers were performed in the blastocyst stage of embryo development, which occurs on day 5 Donor oocyte inclusion was unclear Culture and transfer media were manufactured by MediCult and Vitrolife Only 1 embryo was transferred per cycle Pregnancy was determined by hCG pregnancy test, and gestational sacs and foetal heartbeat were demonstrated on ultrasound scan
Outcomes	Primary outcomes • Live birth rate: retrieved after the study author was contacted; it was measured only in the fresh embryo transfer group and was reported as a percentage of the number of clinical pregnancies Secondary outcomes • Clinical pregnancy rate: defined by ultrasound observation of a positive heartbeat 30 days after embryo transfer. Reported as a percentage of the number of transfers • Adverse event rate: concerns miscarriage rate. Data were retrieved by contacting study author and were reported only for the fresh embryo transfer group. They were reported as a percentage of the number of clinical pregnancies Other outcomes • Pregnancy rate in cycles after previous implantation failure
Notes	Additional data were retrieved by contacting study authors. Important note: only single embryos were transferred, and no multiple pregnancies occurred

Risk of bias

Korošec 2007 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to a treatment or control group by a computerised randomisation table
Allocation concealment (selection bias)	High risk	Allocation was performed at the site by the investigator just before the intervention was provided, according to the computerised randomisation table. Information was retrieved by contacting the study author
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Clinician and participants were blinded; the scientist was not
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were accounted for. Loss to follow-up was accounted for
Selective reporting (reporting bias)	Low risk	Clinical pregnancy rates were reported in a pre-specified way. Live birth rate was retrieved via study author contact
Other bias	Low risk	The trial was free from commercial funding. Different transfer media brands were used in treatment and control groups, but culture media were comparable up to the moment of embryo transfer. No multiple pregnancy rate was reported, but only singleton embryo transfers

Mahani 2007

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Participant sampling unclear Multi-centre trial performed at the Department of Obstetrics and Gynaecology of the Afzallipour Hospital, Kerman University of Medical Sciences, in Kerman, Iran; and the Research and Clinical Centre for Infertility of the Sadoughi University of Medical Sciences, in Yazd, Iran Inclusion criteria: 35 years of age or younger, at least 3 embryos suitable for transfer, and no previous IVF/ICSI cycles Unclear whether a power calculation was performed Participants were included from September 2003 to January 2004; length of follow-up per participant appears to be 10 weeks Unclear whether participants with multiple treatment cycles could enrol Unclear whether an intention-to-treat analysis was performed; no mention of loss to follow-up

Mahani 2007 *(Continued)*

Participants	60 women were recruited and were randomly assigned to a treatment group of 30 and a control group of 30. No loss of participants was reported, so data on all 60 women were analysed. 183 embryos were transferred: 85 in the treatment group and 98 in the control group Mean age (SD): treatment group 27.5 (4.26), control group 28.6 (3.68) years Causes of subfertility and whether it concerned primary or secondary subfertility not reported Mean duration of subfertility was 7.24 (3.68) years in treatment group and 6.93 (3.6) years in control group Both IVF and ICSI participants were included, but they could not have received previous treatment No age analysis was performed
Interventions	Embryo transfers in EmbryoGlue (0.5 mg/mL HA) vs transfers in standard medium containing 20% albumin Culture medium was not stated Randomisation occurred on day of embryo transfer Embryos in treatment group were exposed to HA for 10 minutes before transfer Transfer was performed on day 3 of embryo development All embryos were fresh; no frozen-thaw protocol was followed Donor oocyte inclusion was unclear Transfer medium from treatment group was manufactured by Vitrolife, transfer medium from control group by Bayer Corporation Mean number of embryos transferred (SD): treatment group 2.68 (0.66), control group 2.7 (0.79) Pregnancy demonstrated by hCG pregnancy test 14 days after transfer; gestational sac and foetal viability were demonstrated on ultrasound scan
Outcomes	Secondary outcomes • Clinical pregnancy rate: defined as number of demonstrated pregnancies divided by number of implantations • Adverse events rate: defined as number of miscarriages divided by number of implantations Additional outcomes • Implantation rate: defined as number of demonstrated gestational sacs divided by number of participants
Notes	Article was translated by Interlibrary Loans and Document Delivery

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly assigned to treatment or control group. However, method of randomisation was unclear
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was not stated

Mahani 2007 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both clinician and participant were blinded to treatment. The scientist was not
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised patients were analysed
Selective reporting (reporting bias)	Low risk	Clinical pregnancy and implantation rates were reported in a pre-specified way. Miscarriage was not announced but was added to the Results section
Other bias	High risk	Commercial funding source was unclear. Different transfer media brands were used for both study groups. However, it is unclear whether 2 different transfer media were used, or if EmbryoGlue was added to the same transfer medium used in the control group. No multiple pregnancy rate was reported, although multiple embryos were transferred per cycle

Morbeck 2007

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Consecutive participant group sampling Single-centre trial performed at the Mayo Clinic, in Rochester, Minnesota, USA Inclusion criteria: frozen-thawed embryo transfers; men over the age of 18 years and women from 18 to 42 years (if using their own oocytes and embryos frozen before 39 completed years) or from 18 to 50 years (if using donor oocytes) Exclusion criteria: participation in prior study, blastocyst transfers, single embryo transfers for medical reasons, prior embryo transfer with large amount of blood on outside of the catheter, 3 or more previous treatment failures A power calculation was performed; no further information was provided Participants were enrolled in the study from May 2003 to June 2005. Follow-up per participant was provided up to time of delivery Unclear whether an intention-to-treat analysis was performed Participants with only 1 treatment cycle were allowed in the study
Participants	150 participants were scheduled to enrol in the trial, but only 121 were recruited. Of these, 38 were excluded (reasons unknown), resulting in 83 participants randomly assigned to a treatment group of 41 and a control group of 42. 92 embryos were transferred per group, resulting in a total of 184 embryos transferred Participant mean age (SD): treatment group 31.4 (3.8), control group 30.5 (4.2) years Causes, duration, and kind of subfertility not reported

Morbeck 2007 *(Continued)*

	Participants could not have had more than 2 previous treatment failures, but no further information on previous IVF/ICSI treatments was provided All participants appeared to undergo IVF; ICSI was not stated Age analysis: 2 sets: participants < 35 years vs ≥ 35 years, both in blocks of 4
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G2 culture medium (0.125 mg/mL HA). All embryos were cultured in G2 culture medium Randomisation was performed before commencement of the treatment cycle Treatment group was exposed to EmbryoGlue for an average of 15 minutes before transfer, with a range of 6 to 44 minutes (one transfer 62 minutes, and another 131 minutes) Cleavage stage embryo transfer (day 3) All embryos were frozen-thawed Donor oocytes were included, but outcomes were not reported as a comparison between donor oocytes and non-donor oocytes Culture and transfer media for both groups were manufactured by Vitrolife Mean number of transferred embryos per participant was 2.2 in both treatment and control groups (numbers calculated from unpublished data provided by the study author) Pregnancy was determined by foetal heartbeat monitoring and demonstration of gestational sac on ultrasound scan
Outcomes	Primary outcomes • Live birth rate: number of deliveries divided by number of treatment cycles. Data on both donor oocytes and non-donor oocytes were reported and were retrieved via contact with the study author Secondary outcomes • Ongoing pregnancy rate: number of ongoing pregnancies demonstrated by foetal heartbeat monitoring per participant • Clinical pregnancy rate: number of pregnancies demonstrated by gestational sac on ultrasound per participant Additional outcomes • Implantation rate: number of gestational sacs demonstrated on ultrasound divided by number of embryos transferred
Notes	From ClinicalTrial.gov This study was suspended because the implantation rate was significantly lower in the treatment group than in the control group. Outcome data originally were not published but were received by contacting the principal investigator

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to treatment or control groups via a random numbers table
Allocation concealment (selection bias)	Low risk	Allocation concealment was performed with sealed, opaque, numbered envelopes

Morbeck 2007 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both participant and clinician were blinded to treatment. The scientist was not
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	Follow-up was provided up to time of delivery. Outcomes originally were not reported because the study was suspended, but data were retrieved by contacting the original investigators. Unclear whether an intention-to-treat analysis was performed. No loss to follow-up was apparent, apart from the 38 participants who were excluded, so loss of participants is accounted for
Selective reporting (reporting bias)	Low risk	Clinical pregnancy and implantation rates were pre-specified. Ongoing pregnancy rate was reported when the original study author was contacted
Other bias	High risk	Trial was funded by and was performed at the Mayo Clinic, but this is not considered to be commercial funding. Transfer media in both study groups were comparable, with the exception of EmbryoGlue added to the medium in the treatment group. Multiple pregnancy was not reported, although multiple embryos were transferred per cycle

Ravhon 2005

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Participant sampling unclear Single-centre trial performed at the Edith Wolfson Medical Center, in Holon, Israel Only fresh embryo transfers were included in the study Unclear whether a power calculation was performed Participants were enrolled in the study between July 2004 and November 2004, but the actual length of follow-up was not reported An intention-to-treat analysis was not mentioned in the text, so unclear whether it was performed Unclear whether multiple treatment cycles per participant were included in the study
Participants	148 participants were recruited and randomly assigned to a treatment group of 79 or a control group of 69. No loss to follow-up was apparent, so the data on all 148 participants were analysed The number of embryos transferred was unclear, for only mean numbers per participant were given Mean age (SD): treatment group 34.8 (5.8), control group 34.3 (5.9) years Causes of subfertility and whether study concerned primary or secondary subfertility not reported Subfertility duration (SD): treatment group 3.9 (4.9) years, control group 3.6 (2.8) years All participants underwent IVF

Ravhon 2005 *(Continued)*

	Number of previous cycles (SD): treatment group 4.5 (4.1), control group 5.4 (4.9) No age analysis was performed
Interventions	Fresh embryo transfers in EmbryoGlue (0.5 mg/mL HA) vs fresh transfers in G1 medium (0.125 mg/mL HA) All embryos were cultured in G1 medium Randomisation was performed on day of embryo transfer Exposure time to EmbryoGlue before transfer was not reported Timing of transfer during embryo development was not reported All embryos were fresh; no frozen-thaw protocol was followed Inclusion of donor oocytes was unclear Culture and transfer media were manufactured by Vitrolife Mean number of embryos transferred per participant: treatment group 2.3 ± 0.8, control group 2.2 ± 0.8 Method of pregnancy determination was not reported
Outcomes	Secondary outcomes • Clinical pregnancy rate: reported as a percentage of group size. No further definitions given Additional outcomes • Implantation rate: reported as a percentage, but total number of embryos transferred was unclear, so implantation rate cannot be calculated. No further definitions given
Notes	Abstract of a ASRM Conference presentation

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly allocated to a treatment or control group, but method of randomisation was unclear
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was unclear
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Unclear whether participants, clinicians, and/or scientists were blinded
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were analysed
Selective reporting (reporting bias)	Low risk	Clinical pregnancy and implantation rates were reported in a pre-specified way

Ravhon 2005 *(Continued)*

Other bias	High risk	Abstract only. No commercial funding. Same transfer media brand in treatment and control groups. Transfer media were comparable, with the addition of EmbryoGlue to the treatment group. No multiple pregnancy rate was reported, although multiple embryos were transferred per cycle

Schoolcraft 2002

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Method of sampling of participants was unclear Single-centre trial performed at the Colorado Center for Reproductive Medicine, in Englewood, Colorado, USA IVF patients with their own oocytes and oocyte donors were included Unclear whether a power calculation was performed Participants were enrolled in this trial from January 2001 to February 2002. The exact length of follow-up per participant was not stated, but it was long enough to permit measurement of the trial's proposed outcomes Unclear whether an intention-to-treat analysis was performed Unclear whether multiple treatment cycles or only 1 treatment cycle per participant was included in the trial Trial was supported by Vitrolife
Participants	A total of 175 IVF participants and oocyte donors were recruited for this trial. 141 of them were IVF patients, and 34 were oocyte donors. 91 participants were randomly assigned to the treatment group and 84 to the control group. No loss was reported, so the data on all 175 participants were analysed. Number of transferred embryos was unclear; only the mean number per group was published Age not reported Not reported whether the study concerned primary and/or secondary subfertility Cause and duration of subfertility not reported Trial studied only IVF participants, not participants receiving ICSI. Not reported whether participants had received previous IVF treatment No age analysis was reported
Interventions	Embryo transfer of participants' own or donated fertilised oocytes in G2.3 medium supplemented with EmbryoGlue (0.5 mg/mL HA) vs transfer in G2.3 medium (0.125 mg/mL HA) All embryos were cultured in G1.3 medium Timing of randomisation was unclear Embryos were exposed to the higher concentration of HA just before transfer Transfer was performed on day 3 of embryo development Donor oocytes were included

Schoolcraft 2002 *(Continued)*

	Unclear whether embryos had to be fresh, or if frozen-thawed embryos were also included All culture and transfer media were manufactured by Vitrolife Mean number of embryos transferred: treatment IVF group 3.9, treatment donor oocytes 3.9; control IVF group 3.3, control donor oocytes 3.2 Pregnancy and implantation rates were determined by demonstration of foetal heartbeat
Outcomes	Secondary outcomes • Clinical pregnancy rate: presented as percentage, with number of participants in study group as the denominator Additional outcomes • Implantation rate: presented as percentage; denominator was unclear. No raw data available
Notes	Abstract of ASRM Conference presentation The primary author was contacted regarding unclear details in published abstract, but further participation was declined. So some uncertainty cannot be resolved

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation into treatment or control group via a computer-generated randomisation sheet
Allocation concealment (selection bias)	Unclear risk	Allocation was correctly performed, but concealment was unclear
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Method of blinding unclear
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised patients were analysed
Selective reporting (reporting bias)	Low risk	Clinical pregnancy and implantation rates were reported in a pre-specified way
Other bias	High risk	Abstract only. Trial was commercially funded by Vitrolife. Same transfer media brand was used in treatment and control groups. Transfer media were comparable, with the addition of EmbryoGlue to the treatment group. No multiple pregnancy rate was reported, although multiple embryos were transferred per cycle

Simon 2003

Study characteristics

Simon 2003 *(Continued)*

Methods	Parallel randomised controlled trial Prospective participant recruitment Non-consecutive group sampling Single-centre trial performed at the IVF Unit of the Department of Obstetrics and Gynecology of the Hadassah University Hospital Ein Kerem, in Jerusalem, Israel Inclusion criteria: women had to be 35 years of age or younger with at least 3 embryos suitable for transfer and 3 or fewer previous treatment failures No power calculation was performed, and information was received by contacting the study author Participants were followed up until the pregnancy had ended No intention-to-treat analysis was performed Participants with only a single cycle were enrolled in the study The trial received no commercial funding
Participants	80 participants were recruited and were randomly assigned to a treatment group of 40 or a control group of 40. No loss of participants was reported, so all were analysed A total of 200 embryos were transferred: 103 in the treatment group, 97 in the control group Mean age (SD): treatment group 28.7 (3.3), control group 29.7 (3.8) years Primary or secondary subfertility not reported Cause and duration of subfertility not reported Both IVF and ICSI cases were included. Participants could not have received more than 3 previous treatments No age analysis was performed
Interventions	Embryo transfers in culture medium were supplemented with 0.5 mg/mL HA vs transfer in culture medium Embryos were cultured in P1 medium containing 10% synthetic serum substitute (SSS) Embryos in treatment group were exposed to HA for 5 to 10 minutes before transfer Randomisation was performed on the day of embryo transfer Transfer was performed on day 3 of embryo development Contact with study authors indicated that a frozen-thaw protocol was followed No donor oocytes were included in the trial The P1 culture/transfer medium was manufactured by Irvine Scientific. The HA was manufactured by Biolon, Bio-Technology Ltd (Amring Pharmaceuticals, Berwyn, PA, USA) Mean number of embryos transferred (SD): treatment group 2.6 (0.6), control group 2.4 (0.5) Methods of pregnancy determination: hCG pregnancy test, demonstration of a gestational sac on transvaginal ultrasound scan, and determination of foetal viability (foetal heartbeat) on serial ultrasounds
Outcomes	Primary outcomes

Simon 2003 *(Continued)*

- Live birth rate: defined as number of pregnancies resulting in a delivery divided by number of participants in the group. Unclear regarding why actual results for data stated in the Results table are not the same as those reported in the article

Secondary outcomes

- Ongoing pregnancy rate: defined as number of pregnancies not ended by abortion at time of manuscript submission
- Clinical pregnancy rate: defined as number of pregnant participants divided by group size
- Multiple pregnancy rate: defined as number of twin pregnancies divided by number of pregnancies

Additional outcomes

- Implantation rate: defined as number of gestation sacs divided by number of embryos transferred

Other outcomes

- Deliveries
- Ongoing pregnancy rate per embryo transfer
- Singleton pregnancy rate
- Clinical pregnancy rate per embryo transfer

Notes	Additional data retrieved by contacting study authors

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were allocated to treatment or control arm of the trial based on what was stated in a random sealed envelope. Actual method of randomisation was not clarified, but it appears to be correct
Allocation concealment (selection bias)	Low risk	A sealed envelope was drawn at the laboratory when a suitable participant arrived for transfer. According to what was stated on the envelope, the participant was allocated to either arm of the trial
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both clinician and participant were blinded to treatment received by the participant
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for the outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised patients were analysed. Follow-up was provided until pregnancy ended
Selective reporting (reporting bias)	Low risk	Clinical pregnancy and implantation rates were reported in a pre-specified way. On top of this, live birth, ongoing pregnancy, and multiple pregnancy were reported
Other bias	Low risk	Study received no commercial funding. Transfer media in both arms of the trial were comparable, with the exception of EmbryoGlue added to the medium in the treatment group. Multiple pregnancy rate was reported

Ten 2019

Study characteristics	
Methods	Parallel randomised controlled trial comparing EmbryoGlue to 2 different standard mediums in 2 different analyses Prospective participant recruitment Sampling unclear Unclear whether single-centre or multi-centre study Inclusion criteria: donor oocytes Exclusion criteria: patients with recurrent implantation failure A power calculation was not performed Length of follow-up per participant was not reported Unclear if intention-to-treat analysis was performed; unclear if patients were lost to follow-up, or if patients withdrew from the study Randomisation was performed by cycle, rather than by patient
Participants	Age characteristics were not reported Causes and duration of subfertility not specified. Nor was it stated whether study concerned primary or secondary subfertility Unclear whether participants underwent IVF or ICSI or both, or whether they had received previous IVF treatments No age analysis was performed 226 participants were recruited for this trial. There were 2 separate analyses with 2 types of control media. The first randomisation occurred between 48 in the treatment and 61 in the control GLOBAL® TOTAL®, LifeGlobal® group. The second randomisation was between 81 in the treatment and 76 in the control Single Culture® Complete, IrvineScientific® group No mention in text of any participants who withdrew or were lost to follow-up The mean numbers of embryos transferred is stated
Interventions	Embryo transfer in EmbryoGlue (0.5% mg/mL HA) vs 2 types of standard medium (no mention of HA concentration) Timing of randomisation was unclear No mention of exposure time of embryos to EmbryoGlue before transfer Transfers were performed at the blastocyst stage of embryo development (day 5) Unclear whether frozen-thawed embryos were included in the trial Donor oocytes were included The mean number of embryos transferred was not reported Method of pregnancy determination was not reported
Outcomes	Outcomes (not mentioned which were primary or secondary) include implantation, clinical pregnancy, and clinical miscarriage rates
Notes	Abstract only. From 35th Congresso ESHRE, Vienna, June 2019 Study authors were contacted multiple times but no response was received. Randomised was performed by cycle, rather than by patient; therefore only data on implantation rate could be used in the meta-analysis

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed via a randomisation programme (RNDSEQ V2011.09.09)

Ten 2019 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment not defined
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blind trial
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Double-blind trial
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Participant loss is unclear Actual length of follow-up per participant is unclear
Selective reporting (reporting bias)	Unclear risk	Outcome measures were reported in a pre-specified way. Method of pregnancy determination was not reported
Other bias	High risk	Abstract only data Commercial funding source was unclear Units of randomisation - per embryo transfer, not per participant

Urman 2008

Study characteristics	
Methods	Parallel randomised controlled trial Prospective patient recruitment Consecutive group sampling Single-centre trial performed at the Assisted Reproduction Unit of the American Hospital of Istanbul, Turkey Participants with treatment cycles reaching embryo transfer were included in this study. The IVF/ICSI cycles had to be fresh and had to use the participant's own oocytes An a priori power calculation revealed that 537 participants in each study group would be necessary to detect a 15% increase in clinical pregnancy rate The maximum length of follow-up per participant was 16 weeks, but the average length of follow-up per participant was unclear All analyses were done according to the intention-to-treat principle Only 1 treatment cycle per participant was included in the trial
Participants	A total of 1282 couples undergoing IVF/ICSI were recruited and were randomly assigned to a treatment group of 639 and a control group of 643. 825 of the 1282 received an embryo transfer on day 3 of embryo development, and 457 on day 5. No loss of participants was reported, so the data on 1282 couples were analysed. A total of 3487 embryos were transferred: 1718 in the treatment group and 1769 in the control group Mean age: treatment group 32.8, control group 32.9 years Primary and/or secondary subfertility not reported

Urman 2008 *(Continued)*

	Causes of subfertility included male factor, ovarian, endometriosis, tubal factor, and unexplained causes Mean duration of subfertility: treatment group 6.9 years, control group 7.2 years Participants underwent both IVF and ICSI Mean number of previous treatment cycles: treatment group 2.0, control group 2.1 Age analysis: women < 35 years vs women ≥ 35 years of age
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G2 version 3 (0.125 mg/mL HA) supplemented with HSA (human serum albumin) All embryos were cultured in G1 version 3 until day 3 and in G2 version 3 from day 3 onwards EmbryoGlue used for the trial was provided by the American Hospital of Istanbul Randomisation was performed on day of embryo transfer Embryos in treatment group were exposed to EmbryoGlue for 30 minutes before transfer Transfer was performed on day 3 or day 5 of embryo development All embryos were fresh; no frozen-thaw protocol was followed No donor oocytes were included All culture and transfer media were manufactured by Vitrolife Mean number of embryos transferred: treatment group 2.69, control group 2.75 Method of pregnancy determination: hCG pregnancy test and demonstration of gestational sac on transvaginal ultrasound
Outcomes	Secondary outcomes • Clinical pregnancy rate: defined as the presence of at least 1 gestational sac on ultrasound divided by group size of participants • Multiple pregnancy rate: number of multiple pregnancies divided by group size • Adverse event rate: number of abortions divided by group size Additional outcomes • Implantation rate: number of gestational sacs divided by number of embryos transferred and multiplied by 100 Other outcomes studied • Implantation and clinical pregnancy rates stratified for the following groups: women < 35 years. of age, women ≥ 35 years of age, women without previous implantation failure, women with previous implantation failure, good quality embryos, and poor quality embryos
Notes	Embryo transfer was performed on day 3 or day 5 of embryo development. Outcomes were reported for all embryo transfers and for day 3 and day 5 transfers separately. When necessary (for instance, for subgroup analyses), data were analysed separately

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to treatment or control group via a computer-generated randomisation list

Urman 2008 *(Continued)*

Allocation concealment (selection bias)	Low risk	Allocation to study arm was performed after consecutively numbered, sealed opaque envelopes were opened
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both clinician and participant were blinded to the group to which the participant was allocated
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Clinician was blinded; not relevant for the outcome of live birth
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised patients were analysed
Selective reporting (reporting bias)	High risk	A published RCT protocol pre-trial was not found. Clinical pregnancy, implantation, adverse events, and multiple pregnancy rates were reported in a prespecified way. However, ongoing pregnancy was announced but was not reported on
Other bias	Low risk	EmbryoGlue was provided by the American hospital where the trial was performed. All media were manufactured by the same company (Vitrolife) and were therefore comparable, with the exception of EmbryoGlue added to the medium in the treatment group. Multiple pregnancy rate was reported

Walker 2005

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Group sampling unclear Single-centre trial performed at the Mayo Clinic College of Medicine in Rochester, Minnesota, USA. However, 1 of the study authors was based at the London Bridge Fertility Clinic, in London, UK Exclusion criteria: prior participation in this study, blastocyst stage embryos, single embryo transfer for medical reasons, prior embryo transfer with a large amount of blood on the catheter, 3 or more consecutive failed embryo transfers. Participants appear to have a maximum age of 39 years Unclear whether a power calculation was performed, or if the number of included participants was planned before trial commencement Trial received no commercial funding Actual length of follow-up per participant was unclear Unclear whether an intention-to-treat analysis was performed Unclear whether participants could partake in multiple treatment cycles
Participants	Of a planned total of 250 participants, 98 were recruited and randomly assigned. By the time of publication of this interim analysis, only 68 of these 98 completed treatment: 34 in the treatment group and 34 in the control group. The text was not clear on whether all 98 participants were randomly assigned, or just the 68. It appears that 30 participants were lost, so data on the 68 participants were analysed. For the data analysis of the review, the group size of 34 was used as the denominator

Walker 2005 *(Continued)*

	Total number of embryos transferred was unclear Mean age of women (SD): treatment group 31.1 (4.0), control group 30.6 (4.4) years Not reported whether study concerned primary and/or secondary subfertility, although no difference between treatment and control groups was reported regarding previous live births Causes and duration of subfertility were not reported The trial appears to focus only on IVF participants, not on participants given ICSI. Participants could not have had more than 2 consecutive previous treatment failures, but actual data per study group were not reported Age analysis: participants were stratified by age (< 35 and 35 to < 39 years)
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G1 version 3 (0.125 mg/mL HA) All embryos were cultured in G1 version 3 Timing of randomisation was unclear Timing of transfer during embryo development was unclear Embryos in treatment group were exposed to EmbryoGlue just before transfer All transferred embryos were frozen-thawed Unclear whether donor oocytes were included All transfer and culture media were manufactured by Vitrolife Mean number of embryos transferred (SD): treatment group 2.2 (0.7), control group 2.2 (0.6) Method of pregnancy demonstration was not reported
Outcomes	Secondary outcomes • Clinical pregnancy rate: stated as percentage with the number of participants as the denominator • Multiple pregnancy rate: stated as percentage, defined as multiple gestations. Denominator was unclear Additional outcomes • Implantation rate: stated as percentage, denominator unclear (total of transferred embryos was unclear) Other outcomes • Biochemical pregnancy rate: defined as positive pregnancy rate • Previous live birth
Notes	Abstract of ASRM Conference presentation of an interim analysis of a bigger study. Contact with the study authors of Morbeck 2007 revealed that the bigger study appeared to be theirs. Therefore, the data from this study of Walker et al were not analysed, although the study remains included for additional information

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly assigned to treatment or control group, but method of randomisation was not reported

Walker 2005 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment was unclear
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Method of blinding unclear
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear
Incomplete outcome data (attrition bias) All outcomes	High risk	Length of follow-up was unclear. Unclear cause of loss of 30 of the 98 included participants; whether they were randomly assigned; and if so, whether an intention-to-treat analysis was performed
Selective reporting (reporting bias)	Low risk	All outcomes were reported in a pre-specified way
Other bias	High risk	Abstract only. No commercial funding. All media used were obtained from the same manufacturer and therefore are comparable, with the exception of EmbryoGlue added to the medium in the treatment group. Multiple pregnancy rate was reported

Yakin 2004

Study characteristics	
Methods	Parallel randomised controlled trial Prospective participant recruitment Method of participant sampling was unclear Single-centre trial performed at the Assisted Reproduction Unit of the VKV American Hospital, in Istanbul, Turkey Included embryos had to be frozen-thawed Unclear whether a power calculation was performed Length of follow-up was not reported. Not clear whether an intention-to-treat analysis was performed, nor whether any loss to follow-up occurred Only 1 treatment cycle per participant was included in the trial
Participants	Supernumerary embryos were cryopreserved in 204 cycles; only 1 cycle per patient was included, so this means that 204 participants were recruited. Only 129 embryos were thawed and randomly assigned to a treatment group of 64 or a control group of 65. Some confusion exists regarding the group size (see Notes). No further loss was reported, so the data on 129 participants were analysed Total number of embryos transferred was unclear Mean age: treatment group 31.6, control group 32.1 years Not reported whether study concerned primary or secondary subfertility Cause and duration of subfertility not reported

Yakin 2004 *(Continued)*

	Not reported whether study concerned IVF or ICSI participants, or both, nor whether participants had received previous subfertility treatments No age analysis was performed
Interventions	Embryo transfer in EmbryoGlue (0.5 mg/mL HA) vs transfer in G2 version 3 culture medium (0.125 mg/mL HA) All embryos were cultured in G1 version 3 medium, followed by G2 version 3 on day 3 of embryo development Randomisation was performed on day of embryo transfer Exposure time to EmbryoGlue before transfer was not reported All embryos were frozen on day 3 of development and were transferred after thawing, which means that transfer was also performed on day 3 of development Unclear whether donor oocytes were included in the trial All culture and transfer media were manufactured by Vitrolife Mean number of embryos transferred: treatment group 3.1, control group 3.2 Method of pregnancy determination not reported
Outcomes	Secondary outcomes • Clinical pregnancy rate: reported as a percentage, appears to be percentage of the group size. No definitions given Additional outcomes • Implantation rate: reported as a percentage, but unclear of what. No definitions given Other outcomes • Cryosurvival rate
Notes	Abstract of ESHRE Conference presentation. In the text of the abstract, it is stated that the treatment group consisted of 65 participants and the control group of 64 participants, although data are presented the other way around in the Results table

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomly assigned to treatment or control group, but it is unclear in what way
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was unclear
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Method of blinding unclear
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Method of blinding unclear

Yakin 2004 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	High risk	204 cycles were frozen, but only 129 were thawed. It remains unclear why. Length of follow-up was unclear. No intention-to-treat analysis was reported. Some confusion exists regarding the group sizes
Selective reporting (reporting bias)	Low risk	Implantation and clinical pregnancy rates were reported in a pre-specified way
Other bias	High risk	Abstract only. Unclear whether trial received any commercial funding. All media were manufactured by Vitrolife; study groups were therefore comparable, with the exception of EmbryoGlue added to the medium in the treatment group. No multiple pregnancy rate was reported, although multiple embryos were transferred per cycle

Yung 2019

Study characteristics	
Methods	Parallel randomised controlled trial comparing hyaluronan-enriched embryo transfer medium (HETM) and a conventional medium Prospective participant recruitment Sampling unclear Multi-centre study Inclusion criteria: women under the age of 43 at the time of IVF undergoing frozen-thawed embryo transfer Exclusion criteria: women with donor oocyte/embryo treatment or pre-implantation genetic testing A power calculation was performed Length of follow-up per participant was sufficient Intention-to-treat analysis was performed The number of patients that withdrew from the study was stated Randomisation was performed by patient, not by cycle
Participants	An age analysis was performed, but specific characteristics were not reported Causes and duration of subfertility were not specified. Nor was it stated whether study concerned primary or secondary subfertility, or whether participants had received previous treatments Participants undergoing IVF or ICSI were included 550 participants were recruited for this trial and were randomly assigned to treatment (N = 275) and control groups (N = 2875). Thirteen women (8 in HETM group, 5 in control group) did not undergo embryo transfer because the embryos did not survive upon thawing. One woman in the HETM group cancelled due to fever, and another in the HETM group withdrew after randomisation
Interventions	Embryo transfer in hyaluronan-enriched embryo transfer medium (HETM) (hyaluronan concentration 0.5 mg/mL), while the control group used a conventional medium (hyaluronan concentration 0.125 mg/mL medium Randomisation was performed 1 day before embryo transfer Exposure time of the embryos to the treatment was not stated Transfers were performed with both cleavage stage (day 3) and blastocyst stage (day 6) embryos Frozen-thawed embryos were included in the trial Donor oocytes were excluded The mean number of embryos transferred was reported - 1.4 embryos/blastocysts transferred in both groups Method of pregnancy determination was reported: fetal heartbeat
Outcomes	Primary outcome: live birth rate Secondary outcomes.

Yung 2019 *(Continued)*

	• Clinical pregnancy rate - presence of a foetal heartbeat • Multiple pregnancy rate • Ectopic pregnancy rate Other secondary outcomes were mentioned, but specific numbers or percentages were not reported in the abstract. These include ongoing pregnancy rate, miscarriage rate, and incidence of obstetric complications
Notes	Abstract only. From 35th Annual Meeting of the European Society of Human Reproduction and Embryology, Vienna, Austria, June 2019 Contact was made with the study author and information regarding randomisation, sequence allocation, day of embryo transfer, and method of pregnancy identification was provided

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed via a randomisation list generated by an online programme
Allocation concealment (selection bias)	Unclear risk	Allocations were placed in opaque envelopes, but it is not mentioned if they were sealed
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blind study; both participants and clinicians were not aware of the allocation
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Double-blind study; both participants and clinicians were not aware of the allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	Number of patients randomised, number included in the analysis, and participation loss were stated. Length of follow-up clear
Selective reporting (reporting bias)	Low risk	Outcome measures were reported in a pre-specified way
Other bias	High risk	Abstract only data Commercial funding source was unclear Manufacturers of media unclear

ART: assisted reproductive technology.
BMI: body mass index.
CSCM: continuous single culture medium.
ET: embryo transfer.
GnRH: gonadotropin-releasing hormone.
HA: hyaluronic acid.
hCG: human chorionic gonadotropin.
HETM: hyaluronan-enriched embryo transfer medium.
HSA: human serum albumin.
HTF: human tubal fluid.
ICSI: intracytoplasmic sperm injection.
IVF: in vitro fertilisation.
OHSS: ovarian hyperstimulation syndrome.
PCOS: polycystic ovary syndrome.

PGD: pre-implantation genetic diagnosis.
RCT: randomised controlled trial.
SD: standard deviation.

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

Study	Reason for exclusion
Balaban 2005	Quasi-randomised trial. Randomisation was undertaken according to alternating weekdays
Bungum 2003	Randomised controlled trial comparing implantation and pregnancy rates between 2 different culture media, both containing HA. Not suitable for this systematic review because this RCT did not compare a treatment group with addition of an adherence compound versus a control group devoid of, or with a lower concentration of, such a compound
Chao 2008	Quasi-randomised trial. Allocation to treatment or control group was based on the consecutive participant list. Every other participant was placed in the treatment group. Information was retrieved by contacting study authors
Chatziioannou 2010	RCT comparing different embryo culture media. Not suitable for this systematic review because this RCT did not compare a treatment group with addition of an adherence compound versus a control group devoid of, or with a lower concentration of, such a compound
Check 2012	A matched controlled study - not an RCT
de Moura 2017	Investigating the effect of hyaluronidase on the process of oocyte denudation - not embryo transfer
Feichtinger 1990	Preliminary trial - not an RCT
Feichtinger 1992	Quasi-randomised trial. Randomisation to treatment or control arm of the trial was based on the week in which embryo transfer took place
Hambiliki 2010	Quasi-randomised trial. Randomisation to treatment or control arm of the trial according to alternating weeks
Karimian 2004	Duplication of data from Valojerdi 2006, which was a quasi-randomised trial as well
Loutradi 2008	Review on trials studying the effect of hyaluronic acid on embryo implantation rates. However, not all reviewed trials were RCTs
Nakagawa 2012	Quasi-randomised trial. Allocation to different treatment groups was based on odd or even identification numbers
Nakagawa 2012-II	Conference abstract of a quasi-randomised trial (Nakagawa 2012)
Nishihara 2017	Quasi-randomised trial. Randomisation to treatment or control arm of the trial according to days of the week. Information obtained via contact with study authors
Perez 2019	Quasi-randomisation. Patient ID number used for randomisation
Romano 2004	Randomised controlled trial comparing implantation and pregnancy rates between 3 different culture media. Not suitable for this systematic review because this RCT did not compare a treatment group with addition of an adherence compound versus a control group devoid of, or with a lower concentration of, such a compound
Sallam 2010	Meta-analysis of different methods of assisted reproductive technology, including the use of EmbryoGlue. No data were reported - only lack of evidence of a beneficial treatment effect

Study	Reason for exclusion
Schiewe 2013	Not an RCT. Quasi-randomisation. Day 5 embryos acted as controls and day 6 embryos had the added HA solution. Information obtained via contact with study authors
Sieren 2006	Randomised controlled trial comparing implantation and pregnancy rates between 2 different culture media without studying the specific effects of the addition of HA. This RCT did not compare a treatment group with addition of an adherence compound versus a control group devoid of, or with a lower concentration of, such a compound
Sifer 2009	Randomisation of oocytes instead of participants
Singh 2015	A prospective case control study - not an RCT
Sun 2010	Retrospective analysis
Thornton 2018	A case control study - not an RCT. Information obtained via contact with study authors
Tomari 2014	Quasi-randomisation. Randomised according to days of the week. Randomisation was done per cycle - not per couple. Information was obtained via contact with study authors
Valojerdi 2006	Quasi-randomisation. Randomisation to treatment or control group was based on consecutive weekdays
Venetis 2009	RCT comparing different embryo culture media, with similar levels of hyaluronic acid at the time of embryo transfer

HA: hyaluronic acid.
RCT: randomised controlled trial.

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

Mowafy 2016

Study name	Comparative Study of Three Different Embryo Transfer Media in ICSI Cycles
Methods	Prospective randomised clinical trial. Two or three embryos will be transferred at day 5 after ovum pick-up. Patients will be randomised by a closed envelope method into 3 groups (see interventions)
Participants	150 patients undergoing IVF and ICSI
Interventions	Patients will be randomised by a closed envelope method into 3 groups containing 50 patients - Group A: embryos transferred using 0.5 mg/mL of hyaluronic acid (EmbryoGlue) for 20 minutes before intrauterine transfer - Group B: embryos transferred using a medium of 30% protein-supplemented culture medium (Global total) for 20 minutes - Group C: embryos transferred using a medium of autologous follicular fluid for 20 minutes
Outcomes	• "Chemical and clinical" pregnancy rates • Implantation rates • Ongoing pregnancy • Miscarriage rates
Starting date	May 2016
Contact information	Ahmed Mowafy, South Valley University

Mowafy 2016 *(Continued)*

Notes	Clinical Trials Identifier: NCT02792673

Oxford Fertility 2017

Study name	EmbryoGlue as an Embryo Transfer Medium
Methods	Randomised controlled trial with parallel assignment with a single-site 2-arm parallel design. There was blinding of the physician, nurses, and patients. The embryologist was not blinded Inclusion criteria: • Couples having embryo transfer for fertility treatment Exclusion criteria: • Cycles in which elective freezing of all embryos is clinically indicated (e.g. severe risk of OHSS, biopsy patients) • Use of donor gametes • Having endometrial scratch technique this cycle • Concurrent participation in clinical trial(s) • Previously randomised to this study • Planned self-fund of EmbryoGlue as transfer
Participants	730 participants
Interventions	Patients are randomised into 2 groups - EmbryoGlue and standard control medium. Embryos are placed in these media before transfer into the uterus via embryo transfer procedure
Outcomes	Primary outcome measure: • Live birth rate Secondary outcome measures: • Implantation rate • Clinical pregnancy rate • Adverse IVF events
Starting date	16 June 2017
Contact information	University of Oxford
Notes	ClinicalTrials.gov Identifier: NCT03332680

ICSI: intracytoplasmic sperm injection.
IVF: in vitro fertilisation.
OHSS: ovarian hyperstimulation syndrome.

DATA AND ANALYSES

Comparison 1. High versus low or no hyaluronic acid

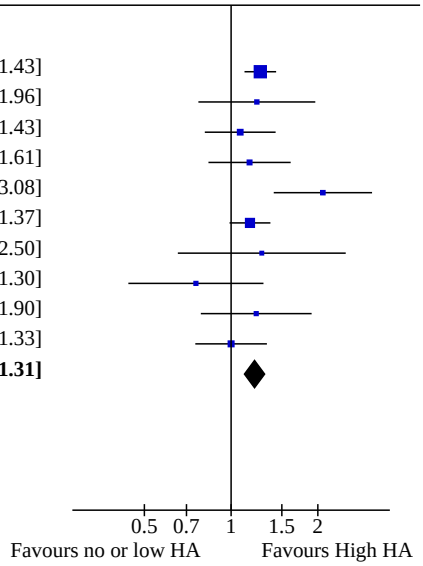
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Live birth rate	10		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1.1 High vs low or no hyaluronic acid	10	4066	Risk Ratio (M-H, Fixed, 95% CI)	1.21 [1.11, 1.31]
1.2 Live birth rate (grouped by timing of embryo transfer)	7	2359	Risk Ratio (M-H, Fixed, 95% CI)	1.20 [1.09, 1.32]
1.2.1 Cleavage stage transfers	6	1759	Risk Ratio (M-H, Fixed, 95% CI)	1.19 [1.05, 1.35]
1.2.2 Blastocyst stage transfers	3	600	Risk Ratio (M-H, Fixed, 95% CI)	1.22 [1.05, 1.42]
1.3 Live birth rate (grouped by frozen-thawed or fresh embryos)	9	3230	Risk Ratio (M-H, Fixed, 95% CI)	1.22 [1.11, 1.34]
1.3.1 Frozen-thawed embryos	3	713	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.80, 1.24]
1.3.2 Fresh embryos	6	2517	Risk Ratio (M-H, Fixed, 95% CI)	1.28 [1.15, 1.41]
1.4 Live birth rate (grouped by exposure time to HA)	8	3195	Risk Ratio (M-H, Fixed, 95% CI)	1.19 [1.09, 1.30]
1.4.1 Exposure time ≤ 10 minutes	3	689	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.92, 1.41]
1.4.2 Exposure time > 10 minutes	5	2506	Risk Ratio (M-H, Fixed, 95% CI)	1.20 [1.09, 1.32]
1.5 Live birth rate (grouped by embryo transfer policy)	8	3195	Risk Ratio (M-H, Fixed, 95% CI)	1.19 [1.09, 1.30]
1.5.1 Single embryo transfer	1	82	Risk Ratio (M-H, Fixed, 95% CI)	1.28 [0.65, 2.50]
1.5.2 Multiple embryo transfer	7	3113	Risk Ratio (M-H, Fixed, 95% CI)	1.19 [1.09, 1.29]
1.6 Live birth rate (grouped by participant prognosis)	10	4066	Risk Ratio (M-H, Fixed, 95% CI)	1.21 [1.11, 1.31]
1.6.1 Good prognosis	6	1625	Risk Ratio (M-H, Fixed, 95% CI)	1.24 [1.09, 1.40]
1.6.2 Unselected	4	2441	Risk Ratio (M-H, Fixed, 95% CI)	1.19 [1.07, 1.32]
1.7 Miscarriage	7	3091	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.67, 1.00]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.8 Clinical pregnancy rate	17		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.8.1 High versus low or no hyaluronic acid	17	5247	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [1.09, 1.23]
1.9 Clinical pregnancy rate (grouped by timing of embryo transfer)	14	3713	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [1.10, 1.27]
1.9.1 Cleavage stage transfers	12	2513	Risk Ratio (M-H, Fixed, 95% CI)	1.24 [1.13, 1.36]
1.9.2 Blastocyst stage transfers	4	1200	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [1.00, 1.21]
1.10 Clinical pregnancy rate (grouped by frozen-thawed or fresh embryos)	14	4049	Risk Ratio (M-H, Fixed, 95% CI)	1.12 [1.05, 1.20]
1.10.1 Frozen-thawed embryos	5	1056	Risk Ratio (M-H, Fixed, 95% CI)	1.04 [0.88, 1.22]
1.10.2 Fresh embryos	10	2993	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [1.06, 1.23]
1.11 Clinical pregnancy rate (grouped by exposure time to HA before transfer)	13	4233	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [1.08, 1.23]
1.11.1 Exposure time ≤ 10 minutes	6	1025	Risk Ratio (M-H, Fixed, 95% CI)	1.21 [1.05, 1.40]
1.11.2 Exposure time > 10 minutes	7	3208	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [1.06, 1.22]
1.12 Clinical pregnancy rate (grouped by embryo transfer policy)	16	4697	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [1.11, 1.25]
1.12.1 Single embryo transfer	1	296	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.73, 1.80]
1.12.2 Multiple embryo transfer	15	4401	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [1.11, 1.25]
1.13 Clinical pregnancy rate (grouped by participant prognosis)	17	5247	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [1.09, 1.23]
1.13.1 Poor prognosis	2	288	Risk Ratio (M-H, Fixed, 95% CI)	3.01 [1.92, 4.71]
1.13.2 Good prognosis	6	1578	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [1.03, 1.31]
1.13.3 Unselected participants	9	3381	Risk Ratio (M-H, Fixed, 95% CI)	1.11 [1.04, 1.19]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.14 Multiple pregnancy rate	7	3337	Risk Ratio (M-H, Fixed, 95% CI)	1.45 [1.24, 1.70]
1.15 Implantation rate	12		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
1.16 Total adverse event rate	3	1487	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.40, 1.84]

Analysis 1.1. Comparison 1: High versus low or no hyaluronic acid, Outcome 1: Live birth rate

Study or Subgroup	High HA Events	High HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 High vs low or no hyaluronic acid						
Balaban 2011	310	639	247	643	36.4%	1.26 [1.11 , 1.43]
Fancsovits 2011	30	103	23	97	3.5%	1.23 [0.77 , 1.96]
Fancsovits 2015 (1)	69	208	62	201	9.3%	1.08 [0.81 , 1.43]
Hazlett 2008	49	116	39	107	6.0%	1.16 [0.83 , 1.61]
Kandari 2019	55	153	29	168	4.1%	2.08 [1.41 , 3.08]
Kleijkers 2016	184	417	159	419	23.4%	1.16 [0.99 , 1.37]
Korošec 2007 (2)	12	36	12	46	1.6%	1.28 [0.65 , 2.50]
Morbeck 2007	14	41	19	42	2.8%	0.75 [0.44 , 1.30]
Simon 2003	22	40	18	40	2.7%	1.22 [0.79 , 1.90]
Yung 2019	70	275	70	275	10.3%	1.00 [0.75 , 1.33]
Subtotal (95% CI)		**2028**		**2038**	**100.0%**	**1.21 [1.11 , 1.31]**
Total events:	815		678			
Heterogeneity: Chi² = 13.40, df = 9 (P = 0.15); I² = 33%						
Test for overall effect: Z = 4.57 (P < 0.00001)						
Test for subgroup differences: Not applicable						



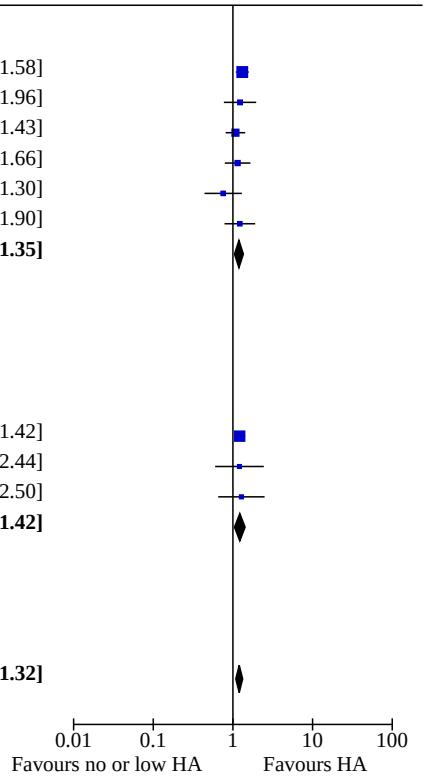
Footnotes

(1) Unpublished data retrieved after contacting authors, first cycle data

(2) Only fresh embryo transfer data

Analysis 1.2. Comparison 1: High versus low or no hyaluronic acid, Outcome 2: Live birth rate (grouped by timing of embryo transfer)

Study or Subgroup	HA Events	HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.2.1 Cleavage stage transfers						
Balaban 2011	166	412	127	413	30.2%	1.31 [1.09 , 1.58]
Fancsovits 2011	30	103	23	97	5.6%	1.23 [0.77 , 1.96]
Fancsovits 2015 (1)	69	208	62	201	15.0%	1.08 [0.81 , 1.43]
Hazlett 2008 (2)	37	84	30	78	7.4%	1.15 [0.79 , 1.66]
Morbeck 2007 (3)	14	41	19	42	4.5%	0.75 [0.44 , 1.30]
Simon 2003	22	40	18	40	4.3%	1.22 [0.79 , 1.90]
Subtotal (95% CI)		**888**		**871**	**66.9%**	**1.19 [1.05 , 1.35]**
Total events:	338		279			
Heterogeneity: Chi² = 4.32, df = 5 (P = 0.50); I² = 0%						
Test for overall effect: Z = 2.67 (P = 0.008)						
1.2.2 Blastocyst stage transfers						
Balaban 2011	144	227	120	230	28.3%	1.22 [1.04 , 1.42]
Hazlett 2008 (4)	12	32	9	29	2.2%	1.21 [0.60 , 2.44]
Korošec 2007 (5)	12	36	12	46	2.5%	1.28 [0.65 , 2.50]
Subtotal (95% CI)		**295**		**305**	**33.1%**	**1.22 [1.05 , 1.42]**
Total events:	168		141			
Heterogeneity: Chi² = 0.02, df = 2 (P = 0.99); I² = 0%						
Test for overall effect: Z = 2.55 (P = 0.01)						
Total (95% CI)		**1183**		**1176**	**100.0%**	**1.20 [1.09 , 1.32]**
Total events:	506		420			
Heterogeneity: Chi² = 4.40, df = 8 (P = 0.82); I² = 0%						
Test for overall effect: Z = 3.61 (P = 0.0003)						
Test for subgroup differences: Chi² = 0.06, df = 1 (P = 0.80), I² = 0%						

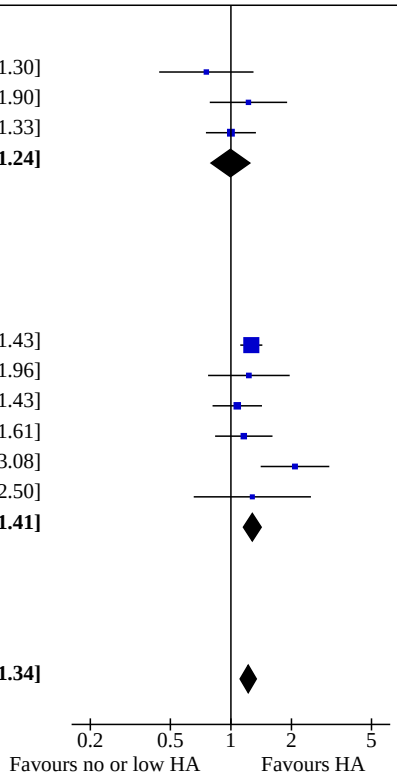


Footnotes

(1) Unpublished data retrieved after contacting authors, first cycle data
(2) Day 3 transfers only
(3) Data retrieved after contact author
(4) Day 5 transfers only
(5) Only fresh embryo transfer data

Analysis 1.3. Comparison 1: High versus low or no hyaluronic acid, Outcome 3: Live birth rate (grouped by frozen-thawed or fresh embryos)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.3.1 Frozen-thawed embryos						
Morbeck 2007	14	41	19	42	3.6%	0.75 [0.44 , 1.30]
Simon 2003	22	40	18	40	3.5%	1.22 [0.79 , 1.90]
Yung 2019	70	275	70	275	13.5%	1.00 [0.75 , 1.33]
Subtotal (95% CI)		**356**		**357**	**20.6%**	**0.99 [0.80 , 1.24]**
Total events:	106		107			
Heterogeneity: Chi² = 1.84, df = 2 (P = 0.40); I² = 0%						
Test for overall effect: Z = 0.05 (P = 0.96)						
1.3.2 Fresh embryos						
Balaban 2011	310	639	247	643	47.5%	1.26 [1.11 , 1.43]
Fancsovits 2011	30	103	23	97	4.6%	1.23 [0.77 , 1.96]
Fancsovits 2015 (1)	69	208	62	201	12.2%	1.08 [0.81 , 1.43]
Hazlett 2008	49	116	39	107	7.8%	1.16 [0.83 , 1.61]
Kandari 2019	55	153	29	168	5.3%	2.08 [1.41 , 3.08]
Korošec 2007	12	36	12	46	2.0%	1.28 [0.65 , 2.50]
Subtotal (95% CI)		**1255**		**1262**	**79.4%**	**1.28 [1.15 , 1.41]**
Total events:	525		412			
Heterogeneity: Chi² = 7.76, df = 5 (P = 0.17); I² = 36%						
Test for overall effect: Z = 4.73 (P < 0.00001)						
Total (95% CI)		**1611**		**1619**	**100.0%**	**1.22 [1.11 , 1.34]**
Total events:	631		519			
Heterogeneity: Chi² = 13.17, df = 8 (P = 0.11); I² = 39%						
Test for overall effect: Z = 4.21 (P < 0.0001)						
Test for subgroup differences: Chi² = 4.06, df = 1 (P = 0.04), I² = 75.4%						

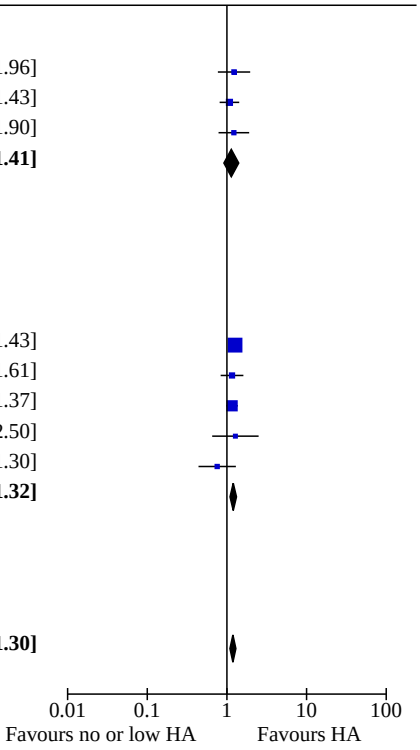


Footnotes

(1) Unpublished data retrieved after contacting authors, first cycle data

Analysis 1.4. Comparison 1: High versus low or no hyaluronic acid, Outcome 4: Live birth rate (grouped by exposure time to HA)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.4.1 Exposure time ≤ 10 minutes						
Fancsovits 2011	30	103	23	97	4.1%	1.23 [0.77 , 1.96]
Fancsovits 2015 (1)	69	208	62	201	10.9%	1.08 [0.81 , 1.43]
Simon 2003	22	40	18	40	3.1%	1.22 [0.79 , 1.90]
Subtotal (95% CI)		**351**		**338**	**18.1%**	**1.14 [0.92 , 1.41]**
Total events:	121		103			
Heterogeneity: Chi² = 0.36, df = 2 (P = 0.84); I² = 0%						
Test for overall effect: Z = 1.16 (P = 0.25)						
1.4.2 Exposure time > 10 minutes						
Balaban 2011	310	639	247	643	42.5%	1.26 [1.11 , 1.43]
Hazlett 2008	49	116	39	107	7.0%	1.16 [0.83 , 1.61]
Kleijkers 2016	184	417	159	419	27.4%	1.16 [0.99 , 1.37]
Korošec 2007 (2)	12	36	12	46	1.8%	1.28 [0.65 , 2.50]
Morbeck 2007	14	41	19	42	3.2%	0.75 [0.44 , 1.30]
Subtotal (95% CI)		**1249**		**1257**	**81.9%**	**1.20 [1.09 , 1.32]**
Total events:	569		476			
Heterogeneity: Chi² = 3.68, df = 4 (P = 0.45); I² = 0%						
Test for overall effect: Z = 3.85 (P = 0.0001)						
Total (95% CI)		**1600**		**1595**	**100.0%**	**1.19 [1.09 , 1.30]**
Total events:	690		579			
Heterogeneity: Chi² = 4.25, df = 7 (P = 0.75); I² = 0%						
Test for overall effect: Z = 3.97 (P < 0.0001)						
Test for subgroup differences: Chi² = 0.22, df = 1 (P = 0.64), I² = 0%						



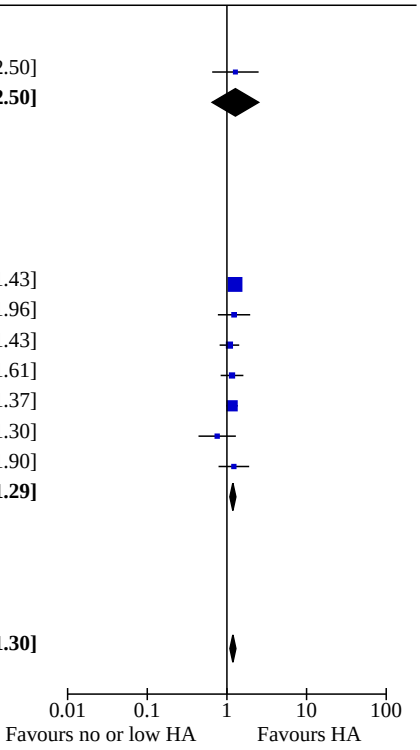
Footnotes

(1) Unpublished data retrieved after contacting authors, first cycle data

(2) Only fresh embryo transfer data

Analysis 1.5. Comparison 1: High versus low or no hyaluronic acid, Outcome 5: Live birth rate (grouped by embryo transfer policy)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.5.1 Single embryo transfer						
Korošec 2007 (1)	12	36	12	46	1.8%	1.28 [0.65 , 2.50]
Subtotal (95% CI)		**36**		**46**	**1.8%**	**1.28 [0.65 , 2.50]**
Total events:	12		12			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.72 (P = 0.47)						
1.5.2 Multiple embryo transfer						
Balaban 2011	310	639	247	643	42.5%	1.26 [1.11 , 1.43]
Fancsovits 2011	30	103	23	97	4.1%	1.23 [0.77 , 1.96]
Fancsovits 2015 (2)	69	208	62	201	10.9%	1.08 [0.81 , 1.43]
Hazlett 2008	49	116	39	107	7.0%	1.16 [0.83 , 1.61]
Kleijkers 2016	184	417	159	419	27.4%	1.16 [0.99 , 1.37]
Morbeck 2007	14	41	19	42	3.2%	0.75 [0.44 , 1.30]
Simon 2003	22	40	18	40	3.1%	1.22 [0.79 , 1.90]
Subtotal (95% CI)		**1564**		**1549**	**98.2%**	**1.19 [1.09 , 1.29]**
Total events:	678		567			
Heterogeneity: $Chi^2 = 4.21$, df = 6 (P = 0.65); $I^2 = 0\%$						
Test for overall effect: Z = 3.90 (P < 0.0001)						
Total (95% CI)		**1600**		**1595**	**100.0%**	**1.19 [1.09 , 1.30]**
Total events:	690		579			
Heterogeneity: $Chi^2 = 4.25$, df = 7 (P = 0.75); $I^2 = 0\%$						
Test for overall effect: Z = 3.97 (P < 0.0001)						
Test for subgroup differences: $Chi^2 = 0.05$, df = 1 (P = 0.83), $I^2 = 0\%$						



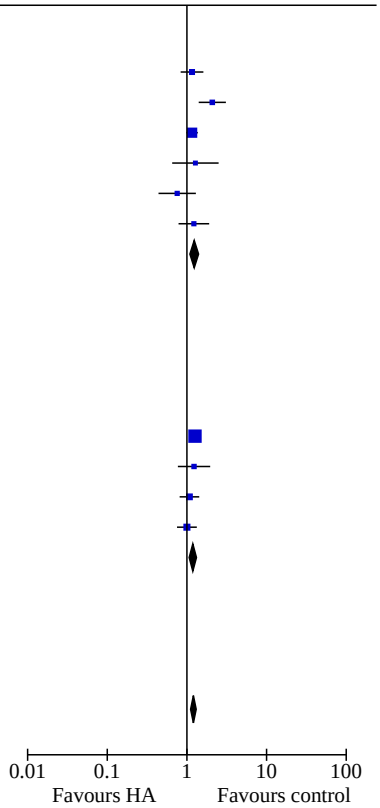
Footnotes

(1) Only fresh embryo transfer data

(2) Unpublished data retrieved after contacting authors, first cycle data

Analysis 1.6. Comparison 1: High versus low or no hyaluronic acid, Outcome 6: Live birth rate (grouped by participant prognosis)

Study or Subgroup	HA Events	HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.6.1 Good prognosis						
Hazlett 2008	49	116	39	107	6.0%	1.16 [0.83 , 1.61]
Kandari 2019	55	153	29	168	4.1%	2.08 [1.41 , 3.08]
Kleijkers 2016	184	417	159	419	23.4%	1.16 [0.99 , 1.37]
Korošec 2007 (1)	12	36	12	46	1.6%	1.28 [0.65 , 2.50]
Morbeck 2007	14	41	19	42	2.8%	0.75 [0.44 , 1.30]
Simon 2003	22	40	18	40	2.7%	1.22 [0.79 , 1.90]
Subtotal (95% CI)		**803**		**822**	**40.5%**	**1.24 [1.09 , 1.40]**
Total events:	336		276			
Heterogeneity: Chi² = 10.67, df = 5 (P = 0.06); I² = 53%						
Test for overall effect: Z = 3.31 (P = 0.0009)						
1.6.2 Unselected						
Balaban 2011	310	639	247	643	36.4%	1.26 [1.11 , 1.43]
Fancsovits 2011	30	103	23	97	3.5%	1.23 [0.77 , 1.96]
Fancsovits 2015 (2)	69	208	62	201	9.3%	1.08 [0.81 , 1.43]
Yung 2019	70	275	70	275	10.3%	1.00 [0.75 , 1.33]
Subtotal (95% CI)		**1225**		**1216**	**59.5%**	**1.19 [1.07 , 1.32]**
Total events:	479		402			
Heterogeneity: Chi² = 2.80, df = 3 (P = 0.42); I² = 0%						
Test for overall effect: Z = 3.19 (P = 0.001)						
Total (95% CI)		**2028**		**2038**	**100.0%**	**1.21 [1.11 , 1.31]**
Total events:	815		678			
Heterogeneity: Chi² = 13.40, df = 9 (P = 0.15); I² = 33%						
Test for overall effect: Z = 4.57 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.24, df = 1 (P = 0.62), I² = 0%						



Footnotes

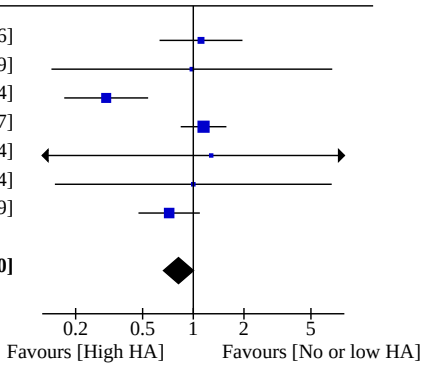
(1) Only fresh embryo transfer data

(2) Unpublished data retrieved after contacting authors, first cycle data

Analysis 1.7. Comparison 1: High versus low or no hyaluronic acid, Outcome 7: Miscarriage

Study or Subgroup	High HA Events	High HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Fancsovits 2015 (1)	23	208	20	201	11.3%	1.11 [0.63 , 1.96]
Friedler 2007	2	51	2	50	1.1%	0.98 [0.14 , 6.69]
Kandari 2019	13	153	47	168	24.8%	0.30 [0.17 , 0.54]
Kleijkers 2016	71	417	62	419	34.2%	1.15 [0.84 , 1.57]
Korošec 2007 (2)	1	36	1	46	0.5%	1.28 [0.08 , 19.74]
Mahani 2007	2	30	2	30	1.1%	1.00 [0.15 , 6.64]
Urman 2008	35	639	49	643	27.0%	0.72 [0.47 , 1.09]
Total (95% CI)		**1534**		**1557**	**100.0%**	**0.82 [0.67 , 1.00]**
Total events:	147		183			

Heterogeneity: Chi² = 17.71, df = 6 (P = 0.007); I² = 66%
Test for overall effect: Z = 1.94 (P = 0.05)
Test for subgroup differences: Not applicable



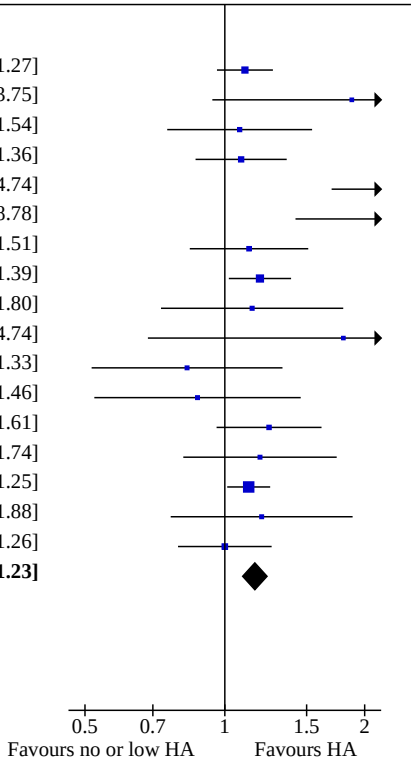
Footnotes
(1) Unpublished data retrieved after contacting authors, first cycle data
(2) Unpublished data retrieved after contacting author, only concerns fresh embryo transfers

Analysis 1.8. Comparison 1: High versus low or no hyaluronic acid, Outcome 8: Clinical pregnancy rate

Study or Subgroup	HA Events	HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.1 High versus low or no hyaluronic acid						
Balaban 2004	137	193	124	193	11.7%	1.10 [0.96 , 1.27]
Dittmann-Műller 2009	19	54	9	48	0.9%	1.88 [0.94 , 3.75]
Fancsovits 2011	40	103	35	97	3.4%	1.08 [0.75 , 1.54]
Fancsovits 2015 (1)	92	208	82	201	7.9%	1.08 [0.86 , 1.36]
Friedler 2005	43	94	15	93	1.4%	2.84 [1.70 , 4.74]
Friedler 2007	18	51	5	50	0.5%	3.53 [1.42 , 8.78]
Hazlett 2008	55	116	45	107	4.4%	1.13 [0.84 , 1.51]
Kleijkers 2016	199	417	168	419	15.9%	1.19 [1.02 , 1.39]
Korošec 2007	30	138	30	158	2.6%	1.14 [0.73 , 1.80]
Mahani 2007	9	30	5	30	0.5%	1.80 [0.68 , 4.74]
Morbeck 2007	17	41	21	42	2.0%	0.83 [0.52 , 1.33]
Ravhon 2005	21	79	21	69	2.1%	0.87 [0.52 , 1.46]
Schoolcraft 2002	58	91	43	84	4.2%	1.25 [0.96 , 1.61]
Simon 2003	25	40	21	40	2.0%	1.19 [0.81 , 1.74]
Urman 2008	349	639	312	643	29.4%	1.13 [1.01 , 1.25]
Yakin 2004	26	64	22	65	2.1%	1.20 [0.76 , 1.88]
Yung 2019	94	275	94	275	8.9%	1.00 [0.79 , 1.26]
Subtotal (95% CI)		**2633**		**2614**	**100.0%**	**1.16 [1.09 , 1.23]**
Total events:	1232		1052			

Heterogeneity: Chi² = 26.51, df = 16 (P = 0.05); I² = 40%
Test for overall effect: Z = 4.83 (P < 0.00001)

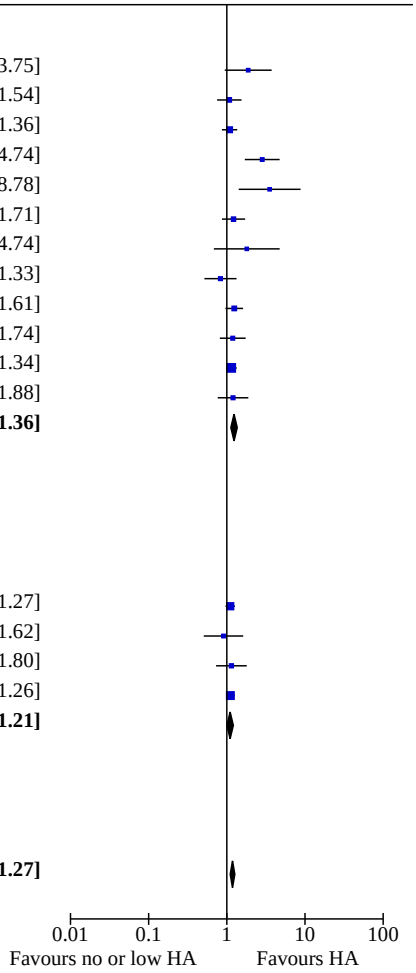
Test for subgroup differences: Not applicable



Footnotes
(1) Unpublished data retrieved after contacting authors, first cycle data

Analysis 1.9. Comparison 1: High versus low or no hyaluronic acid, Outcome 9: Clinical pregnancy rate (grouped by timing of embryo transfer)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.9.1 Cleavage stage transfers						
Dittmann-Műller 2009	19	54	9	48	1.2%	1.88 [0.94 , 3.75]
Fancsovits 2011	40	103	35	97	4.7%	1.08 [0.75 , 1.54]
Fancsovits 2015 (1)	92	208	82	201	10.8%	1.08 [0.86 , 1.36]
Friedler 2005	43	94	15	93	2.0%	2.84 [1.70 , 4.74]
Friedler 2007	18	51	5	50	0.7%	3.53 [1.42 , 8.78]
Hazlett 2008 (2)	42	84	32	78	4.3%	1.22 [0.87 , 1.71]
Mahani 2007	9	30	5	30	0.6%	1.80 [0.68 , 4.74]
Morbeck 2007	17	41	21	42	2.7%	0.83 [0.52 , 1.33]
Schoolcraft 2002	58	91	43	84	5.8%	1.25 [0.96 , 1.61]
Simon 2003	25	40	21	40	2.7%	1.19 [0.81 , 1.74]
Urman 2008 (2)	188	412	165	413	21.3%	1.14 [0.97 , 1.34]
Yakin 2004	26	64	22	65	2.8%	1.20 [0.76 , 1.88]
Subtotal (95% CI)		**1272**		**1241**	**59.6%**	**1.24 [1.13 , 1.36]**
Total events:	577		455			
Heterogeneity: Chi² = 22.78, df = 11 (P = 0.02); I² = 52%						
Test for overall effect: Z = 4.44 (P < 0.00001)						
1.9.2 Blastocyst stage transfers						
Balaban 2004 (3)	137	193	124	193	16.1%	1.10 [0.96 , 1.27]
Hazlett 2008 (4)	13	32	13	29	1.8%	0.91 [0.51 , 1.62]
Korošec 2007 (3)	30	138	30	158	3.6%	1.14 [0.73 , 1.80]
Urman 2008 (4)	161	227	147	230	18.9%	1.11 [0.98 , 1.26]
Subtotal (95% CI)		**590**		**610**	**40.4%**	**1.10 [1.00 , 1.21]**
Total events:	341		314			
Heterogeneity: Chi² = 0.47, df = 3 (P = 0.92); I² = 0%						
Test for overall effect: Z = 2.02 (P = 0.04)						
Total (95% CI)		**1862**		**1851**	**100.0%**	**1.18 [1.10 , 1.27]**
Total events:	918		769			
Heterogeneity: Chi² = 25.20, df = 15 (P = 0.05); I² = 40%						
Test for overall effect: Z = 4.83 (P < 0.00001)						
Test for subgroup differences: Chi² = 2.89, df = 1 (P = 0.09), I² = 65.4%						

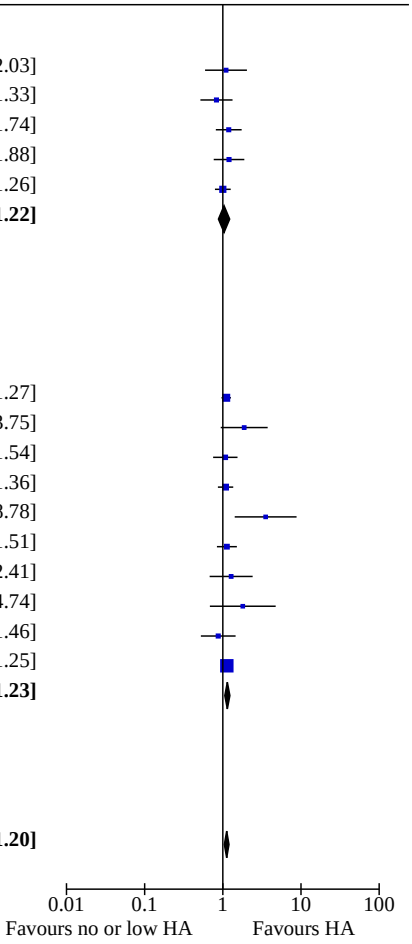


Footnotes

(1) Unpublished data retrieved after contacting authors, first cycle data
(2) Day 3 transfers only
(3) Day 5 transfers
(4) Day 5 transfers only

Analysis 1.10. Comparison 1: High versus low or no hyaluronic acid, Outcome 10: Clinical pregnancy rate (grouped by frozen-thawed or fresh embryos)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.10.1 Frozen-thawed embryos						
Korošec 2007 (1)	17	102	17	112	2.0%	1.10 [0.59 , 2.03]
Morbeck 2007	17	41	21	42	2.5%	0.83 [0.52 , 1.33]
Simon 2003	25	40	21	40	2.5%	1.19 [0.81 , 1.74]
Yakin 2004	26	64	22	65	2.6%	1.20 [0.76 , 1.88]
Yung 2019	94	275	94	275	11.3%	1.00 [0.79 , 1.26]
Subtotal (95% CI)		**522**		**534**	**21.0%**	**1.04 [0.88 , 1.22]**
Total events:	179		175			
Heterogeneity: Chi² = 1.90, df = 4 (P = 0.75); I² = 0%						
Test for overall effect: Z = 0.43 (P = 0.67)						
1.10.2 Fresh embryos						
Balaban 2004	137	193	124	193	15.0%	1.10 [0.96 , 1.27]
Dittmann-Műller 2009	19	54	9	48	1.2%	1.88 [0.94 , 3.75]
Fancsovits 2011	40	103	35	97	4.4%	1.08 [0.75 , 1.54]
Fancsovits 2015 (2)	92	208	82	201	10.1%	1.08 [0.86 , 1.36]
Friedler 2007	18	51	5	50	0.6%	3.53 [1.42 , 8.78]
Hazlett 2008	55	116	45	107	5.7%	1.13 [0.84 , 1.51]
Korošec 2007 (3)	13	36	13	46	1.4%	1.28 [0.68 , 2.41]
Mahani 2007	9	30	5	30	0.6%	1.80 [0.68 , 4.74]
Ravhon 2005	21	79	21	69	2.7%	0.87 [0.52 , 1.46]
Urman 2008	349	639	312	643	37.5%	1.13 [1.01 , 1.25]
Subtotal (95% CI)		**1509**		**1484**	**79.0%**	**1.14 [1.06 , 1.23]**
Total events:	753		651			
Heterogeneity: Chi² = 10.51, df = 9 (P = 0.31); I² = 14%						
Test for overall effect: Z = 3.50 (P = 0.0005)						
Total (95% CI)		**2031**		**2018**	**100.0%**	**1.12 [1.05 , 1.20]**
Total events:	932		826			
Heterogeneity: Chi² = 13.08, df = 14 (P = 0.52); I² = 0%						
Test for overall effect: Z = 3.27 (P = 0.001)						
Test for subgroup differences: Chi² = 1.10, df = 1 (P = 0.30), I² = 8.7%						



Footnotes

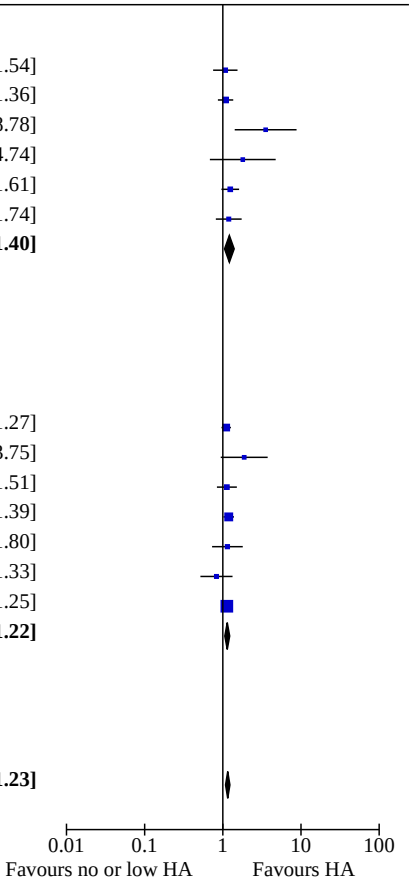
(1) Data on frozen/thawed transfers

(2) Unpublished data retrieved after contacting authors, first cycle data

(3) Data on fresh transfers

Analysis 1.11. Comparison 1: High versus low or no hyaluronic acid, Outcome 11: Clinical pregnancy rate (grouped by exposure time to HA before transfer)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.11.1 Exposure time ≤ 10 minutes						
Fancsovits 2011	40	103	35	97	4.0%	1.08 [0.75 , 1.54]
Fancsovits 2015 (1)	92	208	82	201	9.2%	1.08 [0.86 , 1.36]
Friedler 2007	18	51	5	50	0.6%	3.53 [1.42 , 8.78]
Mahani 2007	9	30	5	30	0.6%	1.80 [0.68 , 4.74]
Schoolcraft 2002	58	91	43	84	5.0%	1.25 [0.96 , 1.61]
Simon 2003	25	40	21	40	2.3%	1.19 [0.81 , 1.74]
Subtotal (95% CI)		**523**		**502**	**21.6%**	**1.21 [1.05 , 1.40]**
Total events:	242		191			
Heterogeneity: Chi² = 7.34, df = 5 (P = 0.20); I² = 32%						
Test for overall effect: Z = 2.67 (P = 0.008)						
1.11.2 Exposure time > 10 minutes						
Balaban 2004	137	193	124	193	13.7%	1.10 [0.96 , 1.27]
Dittmann-Műller 2009	19	54	9	48	1.1%	1.88 [0.94 , 3.75]
Hazlett 2008	55	116	45	107	5.2%	1.13 [0.84 , 1.51]
Kleijkers 2016	199	417	168	419	18.6%	1.19 [1.02 , 1.39]
Korošec 2007	30	138	30	158	3.1%	1.14 [0.73 , 1.80]
Morbeck 2007	17	41	21	42	2.3%	0.83 [0.52 , 1.33]
Urman 2008	349	639	312	643	34.4%	1.13 [1.01 , 1.25]
Subtotal (95% CI)		**1598**		**1610**	**78.4%**	**1.14 [1.06 , 1.22]**
Total events:	806		709			
Heterogeneity: Chi² = 4.29, df = 6 (P = 0.64); I² = 0%						
Test for overall effect: Z = 3.58 (P = 0.0003)						
Total (95% CI)		**2121**		**2112**	**100.0%**	**1.16 [1.08 , 1.23]**
Total events:	1048		900			
Heterogeneity: Chi² = 11.96, df = 12 (P = 0.45); I² = 0%						
Test for overall effect: Z = 4.43 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.59, df = 1 (P = 0.44), I² = 0%						

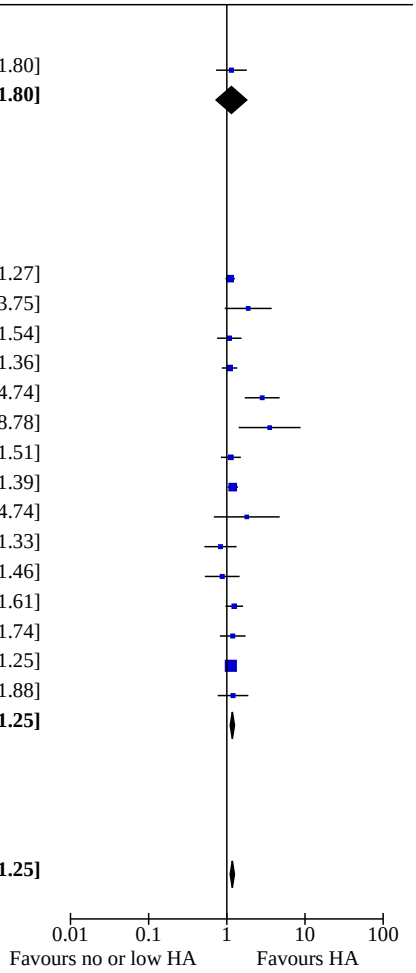


Footnotes

(1) Unpublished data retrieved after contacting authors, first cycle data.

Analysis 1.12. Comparison 1: High versus low or no hyaluronic acid, Outcome 12: Clinical pregnancy rate (grouped by embryo transfer policy)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.12.1 Single embryo transfer						
Korošec 2007	30	138	30	158	2.9%	1.14 [0.73 , 1.80]
Subtotal (95% CI)		**138**		**158**	**2.9%**	**1.14 [0.73 , 1.80]**
Total events:	30		30			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.59 (P = 0.56)						
1.12.2 Multiple embryo transfer						
Balaban 2004	137	193	124	193	12.9%	1.10 [0.96 , 1.27]
Dittmann-Műller 2009	19	54	9	48	1.0%	1.88 [0.94 , 3.75]
Fancsovits 2011	40	103	35	97	3.7%	1.08 [0.75 , 1.54]
Fancsovits 2015 (1)	92	208	82	201	8.7%	1.08 [0.86 , 1.36]
Friedler 2005	43	94	15	93	1.6%	2.84 [1.70 , 4.74]
Friedler 2007	18	51	5	50	0.5%	3.53 [1.42 , 8.78]
Hazlett 2008	55	116	45	107	4.9%	1.13 [0.84 , 1.51]
Kleijkers 2016	199	417	168	419	17.4%	1.19 [1.02 , 1.39]
Mahani 2007	9	30	5	30	0.5%	1.80 [0.68 , 4.74]
Morbeck 2007	17	41	21	42	2.2%	0.83 [0.52 , 1.33]
Ravhon 2005	21	79	21	69	2.3%	0.87 [0.52 , 1.46]
Schoolcraft 2002	58	91	43	84	4.6%	1.25 [0.96 , 1.61]
Simon 2003	25	40	21	40	2.2%	1.19 [0.81 , 1.74]
Urman 2008	349	639	312	643	32.3%	1.13 [1.01 , 1.25]
Yakin 2004	26	64	22	65	2.3%	1.20 [0.76 , 1.88]
Subtotal (95% CI)		**2220**		**2181**	**97.1%**	**1.18 [1.11 , 1.25]**
Total events:	1108		928			
Heterogeneity: Chi² = 25.29, df = 14 (P = 0.03); I² = 45%						
Test for overall effect: Z = 5.09 (P < 0.00001)						
Total (95% CI)		**2358**		**2339**	**100.0%**	**1.18 [1.11 , 1.25]**
Total events:	1138		958			
Heterogeneity: Chi² = 25.27, df = 15 (P = 0.05); I² = 41%						
Test for overall effect: Z = 5.10 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.01, df = 1 (P = 0.91), I² = 0%						

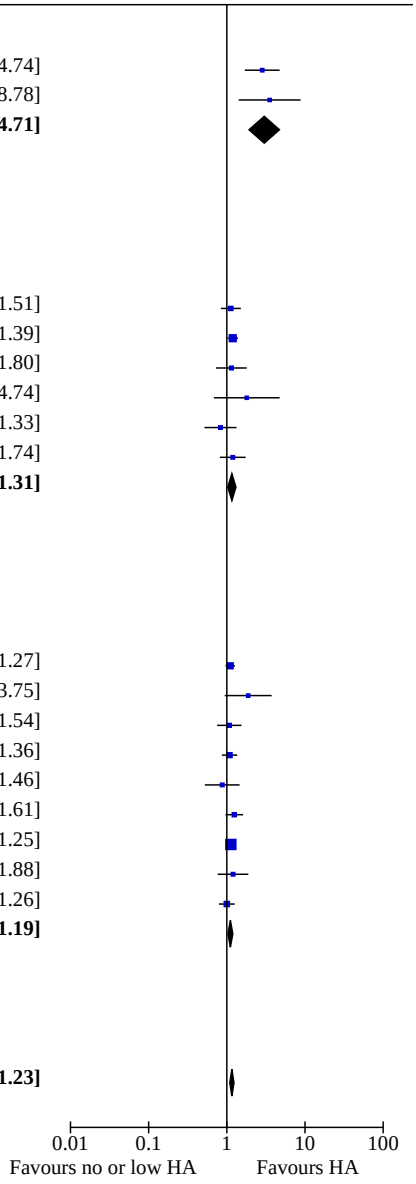


Footnotes

(1) Unpublished data retrieved after contacting author, first cycle data

Analysis 1.13. Comparison 1: High versus low or no hyaluronic acid, Outcome 13: Clinical pregnancy rate (grouped by participant prognosis)

Study or Subgroup	HA Events	HA Total	Low or no HA Events	Low or no HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.13.1 Poor prognosis						
Friedler 2005	43	94	15	93	1.4%	2.84 [1.70 , 4.74]
Friedler 2007	18	51	5	50	0.5%	3.53 [1.42 , 8.78]
Subtotal (95% CI)		**145**		**143**	**1.9%**	**3.01 [1.92 , 4.71]**
Total events:	61		20			
Heterogeneity: Chi² = 0.17, df = 1 (P = 0.68); I² = 0%						
Test for overall effect: Z = 4.82 (P < 0.00001)						
1.13.2 Good prognosis						
Hazlett 2008	55	116	45	107	4.4%	1.13 [0.84 , 1.51]
Kleijkers 2016	199	417	168	419	15.9%	1.19 [1.02 , 1.39]
Korošec 2007	30	138	30	158	2.6%	1.14 [0.73 , 1.80]
Mahani 2007	9	30	5	30	0.5%	1.80 [0.68 , 4.74]
Morbeck 2007	17	41	21	42	2.0%	0.83 [0.52 , 1.33]
Simon 2003	25	40	21	40	2.0%	1.19 [0.81 , 1.74]
Subtotal (95% CI)		**782**		**796**	**27.4%**	**1.16 [1.03 , 1.31]**
Total events:	335		290			
Heterogeneity: Chi² = 2.89, df = 5 (P = 0.72); I² = 0%						
Test for overall effect: Z = 2.44 (P = 0.01)						
1.13.3 Unselected participants						
Balaban 2004	137	193	124	193	11.7%	1.10 [0.96 , 1.27]
Dittmann-Műller 2009	19	54	9	48	0.9%	1.88 [0.94 , 3.75]
Fancsovits 2011	40	103	35	97	3.4%	1.08 [0.75 , 1.54]
Fancsovits 2015 (1)	92	208	82	201	7.9%	1.08 [0.86 , 1.36]
Ravhon 2005	21	79	21	69	2.1%	0.87 [0.52 , 1.46]
Schoolcraft 2002	58	91	43	84	4.2%	1.25 [0.96 , 1.61]
Urman 2008	349	639	312	643	29.4%	1.13 [1.01 , 1.25]
Yakin 2004	26	64	22	65	2.1%	1.20 [0.76 , 1.88]
Yung 2019	94	275	94	275	8.9%	1.00 [0.79 , 1.26]
Subtotal (95% CI)		**1706**		**1675**	**70.7%**	**1.11 [1.04 , 1.19]**
Total events:	836		742			
Heterogeneity: Chi² = 4.84, df = 8 (P = 0.77); I² = 0%						
Test for overall effect: Z = 2.92 (P = 0.004)						
Total (95% CI)		**2633**		**2614**	**100.0%**	**1.16 [1.09 , 1.23]**
Total events:	1232		1052			
Heterogeneity: Chi² = 26.51, df = 16 (P = 0.05); I² = 40%						
Test for overall effect: Z = 4.83 (P < 0.00001)						
Test for subgroup differences: Chi² = 18.67, df = 2 (P < 0.0001), I² = 89.3%						



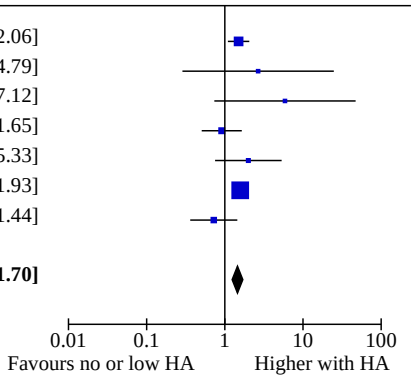
Footnotes

(1) Unpublished data retrieved after contacting author, first cycle data

Analysis 1.14. Comparison 1: High versus low or no hyaluronic acid, Outcome 14: Multiple pregnancy rate

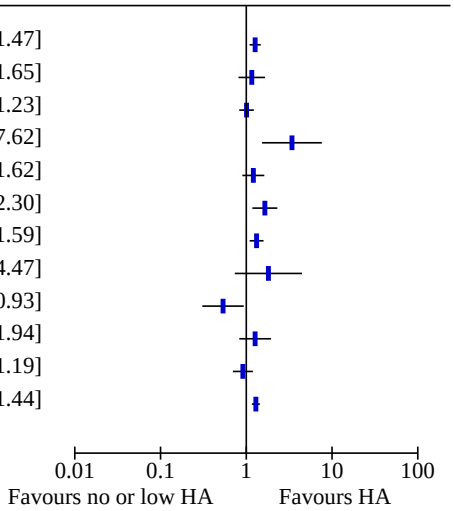
Study or Subgroup	HA Events	HA Total	No or low HA Events	No or low HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Balaban 2004	69	193	46	193	21.9%	1.50 [1.09 , 2.06]
Dittmann-Műller 2009	3	54	1	48	0.5%	2.67 [0.29 , 24.79]
Friedler 2007	6	51	1	50	0.5%	5.88 [0.73 , 47.12]
Kleijkers 2016	20	417	22	419	10.5%	0.91 [0.51 , 1.65]
Simon 2003	10	40	5	40	2.4%	2.00 [0.75 , 5.33]
Urman 2008	183	639	117	643	55.6%	1.57 [1.28 , 1.93]
Yung 2019	13	275	18	275	8.6%	0.72 [0.36 , 1.44]
Total (95% CI)		**1669**		**1668**	**100.0%**	**1.45 [1.24 , 1.70]**
Total events:	304		210			

Heterogeneity: Chi² = 9.33, df = 6 (P = 0.16); I² = 36%
Test for overall effect: Z = 4.67 (P < 0.00001)
Test for subgroup differences: Not applicable



Analysis 1.15. Comparison 1: High versus low or no hyaluronic acid, Outcome 15: Implantation rate

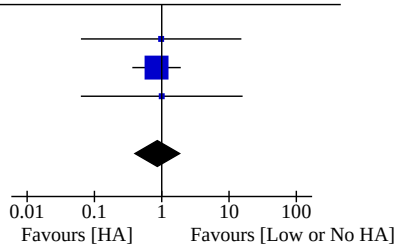
Study or Subgroup	HA Events	HA Total	No or low HA Events	No or low HA Total	Risk Ratio M-H, Fixed, 95% CI
Balaban 2004	206	405	170	424	1.27 [1.09 , 1.47]
Fancsovits 2011	53	238	44	229	1.16 [0.81 , 1.65]
Fancsovits 2015	151	647	155	669	1.01 [0.83 , 1.23]
Friedler 2007	26	159	7	146	3.41 [1.53 , 7.62]
Hazlett 2008	75	266	59	253	1.21 [0.90 , 1.62]
Kandari 2019	60	153	40	168	1.65 [1.18 , 2.30]
Kleijkers 2016	195	966	171	1117	1.32 [1.09 , 1.59]
Mahani 2007	11	85	7	98	1.81 [0.74 , 4.47]
Morbeck 2007	15	92	28	92	0.54 [0.31 , 0.93]
Simon 2003	35	103	26	97	1.27 [0.83 , 1.94]
Ten 2019	55	129	64	137	0.91 [0.70 , 1.19]
Urman 2008	549	1718	437	1769	1.29 [1.16 , 1.44]



Analysis 1.16. Comparison 1: High versus low or no hyaluronic acid, Outcome 16: Total adverse event rate

Study or Subgroup	HA Events	HA Total	Low or No HA Events	Low or No HA Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Friedler 2007	1	51	1	50	7.2%	0.98 [0.06 , 15.25]
Kleijkers 2016	10	417	12	419	85.6%	0.84 [0.37 , 1.92]
Yung 2019	1	275	1	275	7.2%	1.00 [0.06 , 15.91]
Total (95% CI)		**743**		**744**	**100.0%**	**0.86 [0.40 , 1.84]**
Total events:	12		14			

Heterogeneity: Chi² = 0.02, df = 2 (P = 0.99); I² = 0%
Test for overall effect: Z = 0.39 (P = 0.70)
Test for subgroup differences: Not applicable

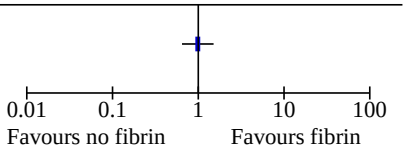


Comparison 2. Fibrin sealant versus no fibrin sealant

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Clinical pregnancy rate (per randomly assigned couple)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
2.2 Adverse event rate (per randomly assigned couple)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
2.3 Implantation rate (per embryos transferred)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

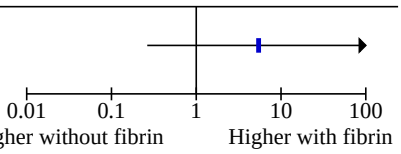
Analysis 2.1. Comparison 2: Fibrin sealant versus no fibrin sealant, Outcome 1: Clinical pregnancy rate (per randomly assigned couple)

Study or Subgroup	Fibrin Events	Fibrin Total	No fibrin Events	No fibrin Total	Risk Ratio M-H, Fixed, 95% CI
Ben-Rafael 1995	29	101	32	110	0.99 [0.65 , 1.51]



Analysis 2.2. Comparison 2: Fibrin sealant versus no fibrin sealant, Outcome 2: Adverse event rate (per randomly assigned couple)

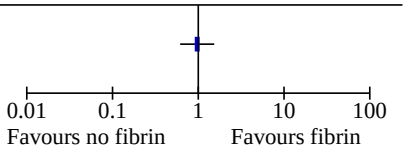
Study or Subgroup	Fibrin Events	Fibrin Total	No fibrin Events	No fibrin Total	Risk Ratio M-H, Fixed, 95% CI
Ben-Rafael 1995 (1)	2	101	0	110	5.44 [0.26 , 111.99]



Footnotes
(1) Ectopic pregnancy

Analysis 2.3. Comparison 2: Fibrin sealant versus no fibrin sealant, Outcome 3: Implantation rate (per embryos transferred)

Study or Subgroup	Fibrin sealant Events	Fibrin sealant Total	No fibrin Events	No fibrin Total	Risk Ratio M-H, Fixed, 95% CI
Ben-Rafael 1995	32	368	35	391	0.97 [0.61 , 1.54]



APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Group (CGFG) specialised register search strategy

ProCite platform

Searched 7 January 2020

Keywords CONTAINS "ivf" or "icsi" or "Embryo" or "IVF-ET" or "in-vitro fertilisation " or "in vitro fertilization" or "intracytoplasmic sperm injection" or "Sperm Injections, Intracytoplasmic" or "ART" or "assisted reproduction" or "Blastocyst" or "blastocyst transfer" or "embryo coculture system" or "embryo culture" or "embryo culture media" or "embryo transfer" or "embryo transfer media" or Title CONTAINS "ivf" or "icsi" or "Embryo" or "IVF-ET" or "in-vitro fertilisation " or "in vitro fertilization" or "intracytoplasmic sperm injection" or "Sperm Injections, Intracytoplasmic" or "ART" or "assisted reproduction" or "Blastocyst" or "blastocyst transfer" or "embryo coculture system" or "embryo culture" or "embryo culture media" or "embryo transfer" or "embryo transfer media"
AND
Keywords CONTAINS "hyaluronan" or "hyaluronic acid" or "EmbryoGlue" or "fibrin sealant" or "G3 culture media" or "adherence" or "adhesion" or "HA-ICSI" or "hyaluronan enriched transfer media" or "hyaluronic acid intracytoplasmic sperm injection" or "embryo glue" or Title CONTAINS "hyaluronan" or "hyaluronic acid" or "EmbryoGlue" or "fibrin sealant" or "G3 culture media" or "adherence" or "adhesion" or "HA-ICSI" or "hyaluronan enriched transfer media" or "hyaluronic acid intracytoplasmic sperm injection" or "embryo glue" (84 records)

Appendix 2. CENTRAL via Cochrane Register of Studies Online (CRSO) search strategy

Web platform

Searched 7 January 2020

#1 MESH DESCRIPTOR Reproductive Techniques, Assisted EXPLODE ALL TREES 3081

#2 (embryo* OR blastocyst*):TI,AB,KY 7550

#3 (in vitro fertilization or IVF):TI,AB,KY 6298

#4 (intracytoplas* adj5 sperm*):TI,AB,KY 1936

#5 ICSI:TI,AB,KY 2604

#6 (Assisted reproduct*):TI,AB,KY 1378

#7 #1 OR #2 OR #3 OR #4 OR #5 OR #6 11527

#8 MESH DESCRIPTOR Hyaluronic Acid EXPLODE ALL TREES 1458

#9 hyalur*:TI,AB,KY 3966

#10 HA:TI,AB,KY 2901

#11 (embryo glue*):TI,AB,KY 8

#12 embryoglue*:TI,AB,KY 17

#13 G5:TI,AB,KY 267

#14 GIII:TI,AB,KY 106

#15 MESH DESCRIPTOR Fibrin Tissue Adhesive EXPLODE ALL TREES 458

#16 Fibrin:TI,AB,KY 3058

#17 (adhesive compound*):TI,AB,KY 5

#18 adherence:TI,AB,KY 24640

#19 sealant:TI,AB,KY 1308

#20 #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 34795

#21 #7 AND #20 186

Appendix 3. MEDLINE search strategy

OVID platform

Searched from 1946 to 7 January 2020

1 exp embryo transfer/ or exp fertilization in vitro/ (40685)
2 exp Intracytoplasmic Sperm Injection/ (6455)
3 (embryo$ adj2 transfer$).tw. (15773)
4 in vitro fertilization.tw. (20857)
5 (intracytoplas$ adj5 sperm).tw. (7275)
6 (ivf or icsi).tw. (26762)
7 or/1-6 (55954)
8 exp Hyaluronic Acid/ (20943)
9 hyalur$.tw. (34768)
10 HA.tw. (68614)
11 embryo glue$.tw. (7)
12 embryoglue$.tw. (11)
13 G5.tw. (2841)
14 GIII.tw. (936)
15 ver$ 5.tw. (4426)
16 exp Fibrin Tissue Adhesive/ (4707)
17 Fibrin.tw. (36815)
18 adherence compound$.tw. (6)
19 or/8-18 (141749)
20 randomized controlled trial.pt. (498039)
21 controlled clinical trial.pt. (93504)
22 randomized.ab. (465647)
23 placebo.tw. (209606)
24 clinical trials as topic.sh. (189735)
25 randomly.ab. (324625)
26 trial.ti. (210686)
27 cross over.ab. (21590)
28 or/20-27 (1268398)
29 (animals not (humans and animals)).sh. (4627438)
30 28 not 29 (1166486)
31 7 and 19 and 30 (68)

Appendix 4. Embase search strategy

OVID platform

Searched from 1980 to 7 January 2020

1 exp embryo transfer/ or exp fertilization in vitro/ or exp Intracytoplasmic Sperm Injection/ (67428)
2 (embryo$ adj2 transfer$).tw. (25212)
3 in vitro fertilization.tw. (27021)
4 (intracytoplas$ adj5 sperm).tw. (9847)
5 (ivf or icsi).tw. (46293)
6 or/1-5 (93073)
7 exp Hyaluronic Acid/ (38754)
8 hyalur$.tw. (39631)
9 embryo glue$.tw. (18)
10 embryoglue$.tw. (39)
11 exp Fibrin Glue/ (10592)
12 Fibrin.tw. (43806)
13 HA.tw. (74912)
14 (G5 or ver$ 5).tw. (10589)
15 GIII.tw. (1304)
16 adherence compound$.tw. (12)
17 or/7-16 (168745)
18 Clinical Trial/ (951076)

19 Randomized Controlled Trial/ (581281)
20 exp randomization/ (85573)
21 Single Blind Procedure/ (37519)
22 Double Blind Procedure/ (165361)
23 Crossover Procedure/ (61654)
24 Placebo/ (331236)
25 Randomi?ed controlled trial$.tw. (218630)
26 Rct.tw. (35319)
27 random allocation.tw. (1963)
28 randomly allocated.tw. (33923)
29 allocated randomly.tw. (2492)
30 (allocated adj2 random).tw. (808)
31 Single blind$.tw. (23836)
32 Double blind$.tw. (197882)
33 ((treble or triple) adj blind$).tw. (1069)
34 placebo$.tw. (295180)
35 prospective study/ (572364)
36 or/18-35 (2121573)
37 case study/ (66187)
38 case report.tw. (388727)
39 abstract report/ or letter/ (1081432)
40 or/37-39 (1526339)
41 36 not 40 (2069485)
42 6 and 17 and 41 (171)

Appendix 5. PsycINFO search strategy

OVID platform

Searched from 1806 to 7 January 2020

1 exp Reproductive Technology/ (1783)
2 (ivf or icsi).tw. (581)
3 (intracytoplas$ adj5 sperm).tw. (59)
4 (in vitro adj5 fertili$).tw. (746)
5 (embryo$ adj5 transfer$).tw. (174)
6 or/1-5 (2194)
7 hyaluron$.tw. (188)
8 embryo glue$.tw. (0)
9 embryoglue$.tw. (0)
10 G5.tw. (38)
11 GIII.tw. (11)
12 Fibrin.tw. (164)
13 adherence compound$.tw. (0)
14 or/7-13 (401)
15 6 and 14 (2)

Appendix 6. Trials with non-useable data

Trial	Non-useable data
Chen 2001	Only biochemical pregnancy rate reported
Drew 2014	Clinical pregnancy rate
Fancsovits 2015	Birth weight, multiple pregnancy rate
Fasano 2016	Clinical pregnancy rate, miscarriage rate

(Continued)

Friedler 2005	Adverse event rate, implantation rate
Khan 2004	Ongoing pregnancy rate, implantation rate
Kleijkers 2016	Birth weight
Ravhon 2005	Implantation rate
Schoolcraft 2002	Implantation rate
Yakin 2004	Implantation rate

Appendix 7. Responses to data queries

Trial	Additional data supplied by original investigator
Balaban 2004	No power calculation performed, causes of subfertility, types of treatments, number of previous treatment cycles, exposure time to HA before embryo transfer, only fresh embryo transfers, method of pregnancy determination, raw data on clinical pregnancy, multiple pregnancy and implantation rates, number of embryos transferred, allocation concealment, blinding, length of follow-up per participant, loss of participants, intention-to-treat analysis, no funding, number of treatment cycles per participant
Ben-Rafael 1995	Method of pregnancy determination, participant enrolment, number of previous treatment cycles, timing of randomisation, methods of randomisation and allocation concealment, no intention-to-treat analysis, no power calculation, length of follow-up, no funding, 1 treatment cycle per participant
Dittmann-Műller 2009	Participant enrolment, no power calculation, participant age, subfertility causes, subfertility duration and number of previous treatment cycles, timing of randomisation, only fresh embryos transferred, no donor oocytes, method of pregnancy determination, number of embryos; transferred, method of randomisation, length of follow-up, blinding, no loss of participants, no intention-to-treat analysis, funding source, 1 treatment cycle per participant
Fancsovits 2011	Method and frequency of participant enrolment, no power calculation, method of pregnancy demonstration, oocyte donation, raw data live birth rate, clinical pregnancy rate, implantation rate, number of transferred embryos, method of randomisation, method of blinding, length of follow-up, lack of intention-to-treat analysis, no commercial funding
Fancsovits 2015	Randomisation was performed per cycle. Therefore, first cycle data were provided for clinical pregnancy, live birth, and miscarriage rates Regarding concealment, medical doctors and patients did not know the results of randomisation. Only the embryologist who prepared the ET dishes knew which medium was used No patients withdrew from the study or were lost to follow-up
Friedler 2007	Participant enrolment, ongoing pregnancy rate determination, length of follow-up, no overlap in data with Friedler 2005, no intention-to-treat analysis, no funding, 1 treatment cycle per participant
Hazlett 2008	Overlap in data with Hazlett 2004 and Hazlett 2005, participant enrolment, live birth rate data, length of follow-up, blinding, number of treatment cycles, no intention-to-treat analysis, no funding

(Continued)

Kandari 2019	Centres involved in the research include Cellsure Biotech and Research Centre, Mumbai, ReproGeneX Center, Mumbai and Akruti Fertility Centre, Mumbai Randomisation was performed via a programme based on Wichmann-Hill random number generator on randomisation.com. Randomisation number was serially allotted to every patient posted for fresh embryo transfer on day of embryo transfer. Sequence allocation was concealed by providing sealed envelope with patient name on it to laboratory personnel and clinician before embryo transfer. The control medium (Irvine Scientific; Santa Ana, CA, USA). Continuous single-step culture medium) did not contain hyaluronic acid Seventeen withdrew due to early OHSS, were given an GnRH agonist protocol. Forty-four patients withdrew consent before embryo transfer. Sixteen patients were withdrawn during interim analysis for protocol non-compliance by staff. A total of 321 patients were analysed, with 153 in the HA group and 168 in the conventional medium group
Korošec 2007	Participant enrolment, timing of randomisation, methods of allocation concealment and blinding, raw data divided for fresh and frozen-thawed embryo transfers, live birth rate in fresh embryo transfer group, number of treatment cycles per participant, no intention-to-treat analysis, no funding
Morbeck 2007	Participant enrolment, power calculation performed, participant age, number of participants, number of embryos transferred, number of participant exclusions, number of donor oocytes, timing of randomisation, exposure time to HA before transfer, method of pregnancy determination, raw data on live birth, clinical pregnancy, and implantation rates, methods of randomisation and allocation concealment, blinding, length of follow-up, number of treatment cycles per participant
Simon 2003	Participant enrolment, no power calculation performed, methods of allocation concealment and blinding, definition of ongoing pregnancy rate, data implantation rate, length of follow-up, no intention-to-treat analysis, no funding, 1 treatment cycle per participant
Yung 2019	Randomisation was performed via a randomisation list that was generated by an online programme and was placed in opaque envelopes. This was done 1 day before embryo transfer so that the laboratory staff had enough time to prepare the medium. It was a double-blind study as the participants and the clinicians were not aware of the allocation. Transfer was done on day 3 for cleavage stage embryos and on day 6 for blastocysts. Pregnancy was determined first by urine pregnancy test, followed by foetal pole with heartbeat on pelvic ultrasound

ET: embryo transfer.

GnRH: gonadotropin-releasing hormone.

HA: hyaluronic acid.

OHSS: ovarian hyperstimulation syndrome.

Appendix 8. Data extraction form

Assessment		Final
Assessor	DH LV	Inclusion
Date		Exclusion; because: .....
		Awaiting; because: .....
Study information		
1. Ref ID		
2. First author		
3. Year		
4. Published	Yes No	
5. Language		
6. Retrieval	Electronic search Handsearched	After citation tracking After contacting author in the field
Notes:		
Criteria for eligibility		
Participants	Couples undergoing embryo transfer after IVF, ICSI, and/or an embryo thaw cycle	Yes No
Intervention	Embryo transfer with media containing hyaluronic acid or fibrin sealant for embryos · Grown in vitro for 2 to 4 days • Grown in vitro for 5 to 6 days • Frozen-thawed	Yes No

(Continued)

	· Both fresh and frozen-thawed	
Comparison	Embryo transfer with standard media for embryos	Yes
	· Grown in vitro for 2 to 4 days	No
	• Grown in vitro for 5 to 6 days • Frozen-thawed	
	· Both fresh and frozen-thawed	
Outcome	**Primary**	
	Live birth rate (per randomly assigned couple)	Yes
		No
	Secondary	
	Ongoing pregnancy rate (per randomly assigned couple) *(12+ weeks viable, fetal heartbeat positive, pregnancy)*	Yes
		No
	Clinical pregnancy rate (per randomly assigned couple) *(positive pregnancy test, gestational sac on ultrasound)*	Yes
		No
	Multiple pregnancy rate (per randomly assigned couple)	Yes
		No
	Additional	
	Implantation rate (per randomly assigned couple) *(gestational sac per embryo transfer)*	Yes
		No
	Adverse events (ectopic pregnancies, miscarriage, fetal/congenital defects, pelvic inflammation, or other) (per randomly assigned couple)	Yes
		No
Notes:		
Study characteristics		

(Continued)

Design	
1. Study design	RCT Parallel *(intervention vs control)* Cross-over *(participants used as intervention and control groups)* ..... **Quotes:** .....
2. Participant recruitment	Prospective Retrospective Unclear **Quotes:** .....
3. Sampling (How was the sampling group formed?)	Consecutive Non-consecutive Unclear **Quotes: .....**
4. Setting	Single-centre Multi-centre **Country** .....
Participants: included and excluded	
5. Study criteria for participant inclusion	
6. Study criteria for participant exclusion	
7. Description of control/comparison treatment	

(Continued)

8. Power calculation performed and followed	Yes No Unclear **Quotes:**.....	
Notes:		
Participants		
Baseline characteristics		
Age (of female): Not reported	Mean:	SD:
	Intervention:	
	Control:	
Subfertility	Primary Secondary Both Not reported	
Cause and duration of subfertility	Reported Not reported	
Previous IVF and/or ICSI treatment	Reported Not reported	
Undergoing IVF or ICSI, or both	IVF ICSI Both	
Age group analysis	Yes, define: No	

(Continued)

Notes:	
Flow chart of participants	
Remarks:	
Intervention	
Embryo transfer after IVF, ICSI, and/or frozen-thaw cycle	
1. Time of randomisation during cycle	Before commencement of treatment cycle After commencement of treatment and before fertilisation check From fertilisation check to day of embryo transfer On day of embryo transfer
2. Nature of intervention	Addition of hyaluronic acid to embryo transfer medium; concentration was ..... Addition of fibrin sealant to embryo transfer medium; concentration was .....
3. Exposure time to hyaluronic acid or fibrin sealant before ET	.....
4. Timing of intervention	Early in embryo development: mean cleavage stage (day 2 to and including day 4) Late in embryo development: blastocyst stage (days 5 and 6) Both cleavage and blastocyst stages in embryo development
5. Frozen-thaw protocol	Yes No Unclear
6. Including oocyte donations	Yes No Unclear

(Continued)

7. Culture and transfer (with and without adherence compound) medium brand	.....	
8. Mean number of embryos transferred	.....	Not reported
9. Pregnancy determination	Foetal heartbeat	
	Demonstration of gestational sac on ultrasound scan	
	Pregnancy test	
	Not reported	
Notes:		
Primary outcomes		
Total occurrence N = Total non-occurrence N =		
Notes:		
Secondary outcomes		
Total occurrence N = Total non-occurrence N =		
Notes:		
Total occurrence N = Total non-occurrence N =		
Notes:		
Total occurrence N = Total non-occurrence N =		

(Continued)

Notes:				
Additional outcomes				
Implantation rate (gestational sacs per embryos transferred)		Occurrence of outcome	Non-occurrence of outcome	
	Treatment			
	Control			
	Total (by event)			
Notes:				
Adverse events				
Ectopic pregnancy		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			
	Total (by event)			
Notes:				
Miscarriage		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			
	Total (by event)			
Notes:				

(Continued)

Foetal/congenital defects		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			
	Total (by event)			
Notes:				
Pelvic inflammation		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			
	Total (by event)			
Notes:				
Other adverse events		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			
	Total (by event)			
Notes:				
Other outcomes studied				
Miscarriage		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			

(Continued)

	Total (by event)			
Notes:				
Miscarriage		Occurrence of outcome	Non-occurrence of outcome	Total (by group)
	Treatment			
	Control			
	Total (by event)			
Notes:				
Risk of bias assessment				
Selection bias	Was the allocation sequence adequately generated? Explain the method used by the study authors to assess whether it should produce comparable groups	Yes No Unclear		
	Was participant allocation concealment adequate? Explain. (adequate: central computer randomisation, on-site assignment can be determined only after participant data are entered; serially numbered, sealed opaque envelopes)	Yes No Unclear		
	How was randomisation performed?	Computer generated Random numbers table Not stated		
Selective outcome reporting	Are reports of the study free of the suggestion of selective outcome reporting? Explain (compare Methods with Results)	Yes No Unclear		
Detection bias	Was follow-up long enough?	Yes No Unclear		
	Was the clinician or nurse blinded?	Yes No		

(Continued)

		Unclear
	Was the scientist blinded?	Yes No Unclear
	Was the participant blinded?	Yes No Unclear
Attrition bias	Was loss to follow-up accounted for? (Is it stated in the study?)	Yes No Unclear
	Was an intention-to-treat analysis performed?	Yes No Unclear
Source of funding	Was the source of funding stated?	Yes No Unclear
Other remarks on quality		

WHAT'S NEW

Date	Event	Description
8 January 2020	New citation required but conclusions have not changed	New studies added but no change in conclusions
8 January 2020	New search has been performed	New search performed. 7 studies added Author team changed Title changed to "Hyaluronic acid in embryo transfer media for assisted reproductive technologies" Risk ratio used instead of odds ratio. Number needed to treat/ harm column added to the 'Summary of findings' table

HISTORY

Protocol first published: Issue 4, 2008
Review first published: Issue 7, 2010

Date	Event	Description
10 November 2014	Amended	Correction: in the updated review, published in issue 2, 2014, there was a change to the conclusion for the primary outcome live birth. There was evidence of an increased number of live births with transfer media containing high concentrations of hyaluronic acid
13 November 2013	New citation required but conclusions have not changed	Two studies added; no change made to conclusions
13 November 2013	New search has been performed	Meta-analyses on hyaluronic acid have been grouped together. Instead of division into 3 different comparison groups, now only 1 group with a subgroup analysis of HA vs low HA and HA vs no HA for live birth rate and clinical pregnancy rate. Furthermore, ongoing pregnancy rate has been removed as a secondary outcome measure, and subgroup analyses have been removed from secondary outcome measures of multiple pregnancy rate and adverse events rate
12 May 2010	Amended	Post-protocol change: originally, implantation rate was not planned for analysis but was to be presented in an additional table. However, we decided to present this outcome measure without pooling
8 July 2009	Amended	Changed title and author team Changes to protocol: inclusion of all types of adherence compounds, different outcome measures, multiple comparison groups, additional subgroup analyses

CONTRIBUTIONS OF AUTHORS

Devorah Heymann (DH) and Liat Vidal (LV) performed searches, selected studies, and extracted and analysed data. Devorah Heymann wrote the review.

Zeev Shoham (ZS) and Yuval Or (YO) were independent advisors and reviewed included studies.

ZS, YO, and LV reviewed the manuscript.

In previous published versions:
Debbie Blake (DB) was an independent advisor, reviewed included studies, resolved discrepancies, and wrote the original protocol. Furthermore, she wrote much of the main text of the review.
Stephan Bontekoe (SB) and Maas Jan Heineman (MJH) performed searches, selected studies, and extracted and analysed data. Stephan Bontekoe wrote the review, together with Debbie Blake (DB).
Neil Johnson (NJ) was an independent advisor who reviewed included studies and co-wrote the protocol. Eleanor Williams (EM) wrote the protocol.

DECLARATIONS OF INTEREST

No declarations of interest to declare.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The original title was changed in the previous review from "Hyaluronic acid inclusion in embryo transfer media for assisted reproductive technologies" to "Adherence compounds in embryo transfer media for assisted reproductive technologies" to permit inclusion of all kinds of 'embryo glues' in the review. However, based on the single identified study that used fibrin sealant, no evidence indicates improved pregnancy outcomes by adding this compound to the embryo transfer medium and data were insufficient data to justify conclusions. Based on peer review and editorial discussion, because of this paucity of data, fibrin sealant was removed from the summary of findings table and the title changed back from "adhesion compounds" to "hyaluronic acid". We acknowledge the Cochrane Review entitled "Post-embryo transfer interventions for in vitro fertilisation and intracytoplasmic sperm injection patients" (Abou-Setta 2014), which covers one of the interventions included in this review. This review looked at methods to prevent post-transfer embryo expulsion including bed rest, fibrin sealant in the embryo transfer fluid, and mechanical pressure to close the cervical canal.
A second primary outcome measure of miscarriage, separate from total adverse event rate, was added. In the previous update, the additional outcome measures of live birth rate per oocyte pickup (OPU) and embryo transfer (ET), clinical pregnancy rate per OPU and ET, and the proportion of women in whom at least one embryo has implanted were replaced by the outcome measure of "implantation rate". For this review, it was decided to use a risk ratio instead of an odds ratio due to easier clinical interpretation. For the previous update, the number needed to treat for an additional beneficial outcome (NNTB) was calculated for live births only. We decided to calculate the NNTB and the number needed to treat for an additional harmful effect (NNTH) for all main results that showed a treatment effect, and as such we added a column with these figures to the summary of findings tables.
In the previous update, certain baseline characteristics changed, for example, 'over the age of 37 years and undergoing IVF or ICSI, or both' changed to 'age group analysis', and the interventions of ovarian stimulation and luteal support were removed. The subgroup analyses of oocyte donation, exposure time to adherence compounds, different prognosis groups, and different embryo transfer policies were added in the last update. However, for this update, based on peer review and editorial discussion, it was decided that oocyte donation should be removed.
After the meta-analysis was performed on the HA per concentration comparison, it was decided to pool the data to get an overall view of the treatment effect. In the previous review, all three analyses were performed - high-concentration HA compared to low-concentration HA, high-concentration HA compared to no HA, and high-concentration HA compared to a combination of low or no HA. Because there was no difference between these analyses and for a more succinct and readable review, only the combined analysis was performed in this update. Even though the included studies are not completely similar in their intervention and control groups, all do compare the addition of HA as an adherence compound to the embryo transfer medium versus a control transfer medium.
Two additional sensitivity analyses were added. The first examined only peer-reviewed, full-text only articles due to the large number of studies for which only an abstract was published. The second added sensitivity analysis was performed to examine only studies that used a foetal heartbeat as the method of pregnancy determination, as opposed to gestational sac, or studies that did not specify the method of pregnancy determination. The sensitivity analysis of alternative imputation strategies was not performed because we found in the data analysis process that there was very little patient loss. It became clear that imputation of these data would have no influence on the overall treatment effect; therefore this sensitivity analysis was not performed.

INDEX TERMS

Medical Subject Headings (MeSH)

Abortion, Spontaneous [epidemiology]; Culture Media [*chemistry]; Embryo Implantation [*drug effects] [physiology]; Fibrin Tissue Adhesive [*pharmacology]; Hyaluronic Acid [*pharmacology]; Live Birth [epidemiology]; Pregnancy, Multiple [statistics & numerical data]; Randomized Controlled Trials as Topic; *Reproductive Techniques, Assisted; Tissue Adhesives [*pharmacology]

MeSH check words

Adult; Female; Humans; Pregnancy