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Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)) (Review)

Lacey L, Hassan S, Franik S, Seif MW, Akhtar MA

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[Intervention Review]

Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI))

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ABSTRACT

Background

Failure of implantation and conception may result from inability of the blastocyst to escape from its outer coat, which is known as the zona pellucida. Artificial disruption of this coat is known as assisted hatching and has been proposed as a method for improving the success of assisted conception by facilitating embryo implantation.

Objectives

To determine effects of assisted hatching (AH) of embryos derived from assisted conception on live birth and multiple pregnancy rates.

Search methods

We searched the following databases (from their inception to 27 May 2020), with no language or date restriction: Cochrane Gynaecology and Fertility Group (CGFG) specialised register, CENTRAL, MEDLINE, Embase and PsycINFO. We checked reference lists of relevant studies and searched the trial registers.

Selection criteria

Two review authors identified and independently screened trials. We included randomised controlled trials (RCTs) of AH (mechanical, chemical, or laser disruption of the zona pellucida before embryo replacement) versus no AH that reported live birth or clinical pregnancy data.

Data collection and analysis

We used standard methodological procedures recommended by Cochrane. Two review authors independently performed quality assessments and data extraction.

Main results

We included 39 RCTs (7249 women). All reported clinical pregnancy data, including 2486 clinical pregnancies. Only 14 studies reported live birth data, with 834 live birth events. The quality of evidence ranged from very low to low. The main limitations were serious risk of bias associated with poor reporting of study methods, inconsistency, imprecision, and publication bias. Five trials are currently ongoing.

We are uncertain whether assisted hatching improved live birth rates compared to no assisted hatching (odds ratio (OR) 1.09, 95% confidence interval (CI) 0.92 to 1.29; 14 RCTs, N = 2849; I^2 = 20%; low-quality evidence). This analysis suggests that if the live birth rate in women not using assisted hatching is about 28%, the rate in those using assisted hatching will be between 27% and 34%.

Analysis of multiple pregnancy rates per woman showed that in women who were randomised to AH compared with women randomised to no AH, there may have been a slight increase in multiple pregnancy rates (OR 1.38, 95% CI 1.13 to 1.68; 18 RCTs, N = 4308; I^2 = 48%; low-quality evidence). This suggests that if the multiple pregnancy rate in women not using assisted hatching is about 9%, the rate in those using assisted hatching will be between 10% and 14%.

When all of the included studies (39) are pooled, the clinical pregnancy rate in women who underwent AH may improve slightly in comparison to no AH (OR 1.20, 95% CI 1.09 to 1.33; 39 RCTs, N = 7249; I^2 = 55%; low-quality evidence). However, when a random-effects model is used due to high heterogeneity, there may be little to no difference in clinical pregnancy rate (P = 0.04).

All 14 RCTs that reported live birth rates also reported clinical pregnancy rates, and analysis of these studies illustrates that AH may make little to no difference in clinical pregnancy rates when compared to no AH (OR 1.07, 95% CI 0.92 to 1.25; 14 RCTs, N = 2848; I^2 = 45%).

We are uncertain about whether AH affects miscarriage rates due to the quality of the evidence (OR 1.13, 95% CI 0.82 to 1.56; 17 RCTs, N = 2810; I^2 = 0%; very low-quality evidence).

Authors' conclusions

This update suggests that we are uncertain of the effects of assisted hatching (AH) on live birth rates. AH may lead to increased risk of multiple pregnancy. The risks of complications associated with multiple pregnancy may be increased without evidence to demonstrate an increase in live birth rate, warranting careful consideration of the routine use of AH for couples undergoing in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI).

AH may offer a slightly increased chance of achieving a clinical pregnancy, but data quality was of low grade. We are uncertain about whether AH influences miscarriage rates.

PLAIN LANGUAGE SUMMARY

Assisted hatching of fertilised eggs in assisted conception (IVF and ICSI)

Review question

Does assisted hatching (help to hatch human embryos in the laboratory) during assisted reproduction improve the chance of achieving pregnancy and live birth, and does it affect the risk of multiple pregnancy?

Background

Assisted hatching is a technique that is sometimes used in assisted reproduction for in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). It involves thinning the coat surrounding the fertilised egg or making a hole in it. It is suggested that this may improve the chance of the embryo attaching to the lining of the womb, so that pregnancy can begin.

Study characteristics

Cochrane Review authors included 39 randomised controlled trials (RCTs) of 7249 women. All studies reported clinical pregnancy, but only 14 studies reported live birth, and only 18 reported multiple pregnancy. The evidence is current to May 2020.

Key results

This review of RCTs demonstrates that we are uncertain of the effects of assisted hatching on live birth rate when compared to no assisted hatching. Assisted hatching may increase slightly multiple pregnancy rates when compared to no AH. Assisted hatching may improve slightly the chances of clinical pregnancy in women. We are uncertain about the effects of AH on miscarriage.

Only studies that report live birth and multiple pregnancy as their primary outcome measures should be performed and funded in the future.

Quality of the evidence

The quality of the evidence is low to very low. The main limitations are serious risk of bias associated with poor reporting of study methods, inconsistency, imprecision, and publication bias.

SUMMARY OF FINDINGS

Summary of findings 1. Assisted hatching compared to no assisted hatching for women undergoing assisted conception

Assisted hatching compared to no assisted hatching for women undergoing assisted conception

Patient or population: women undergoing assisted conception

Setting: clinic
Intervention: assisted hatching
Comparison: no assisted hatching

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of evidence (GRADE)	Comments
	Risk with no assisted hatching	Risk with assisted hatching				
Live births per woman randomised	283 per 1000	301 per 1000 (267 to 338)	OR 1.09 (0.92 to 1.29)	2849 (14 RCTs)	⊕⊕⊖⊖ LOW[a]	
Multiple pregnancy rate per woman randomised	91 per 1000	121 per 1000 (102 to 144)	OR 1.38 (1.13 to 1.68)	4308 (18 RCTs)	⊕⊕⊖⊖ LOW[b]	
Clinical pregnancy rate per woman randomised	322 per 1000	363 per 1000 (341 to 387)	OR 1.20 (1.09 to 1.33)	7249 (39 RCTs)	⊕⊕⊖⊖ LOW[b]	
Miscarriage rate per woman randomised	53 per 1000	60 per 1000 (44 to 81)	OR 1.13 (0.82 to 1.56)	2810 (17 RCTs)	⊕⊖⊖⊖ VERY LOW[c]	

*__The risk in the intervention group__ (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; OR: odds ratio; RCT: randomised controlled trial.

GRADE Working Group grades of evidence.
High quality: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded two levels for serious risk of bias and publication bias: in many studies, the method was unclear and information was incomplete. The main limitation was serious risk of bias associated with poor reporting of study methods.
[b]Downgraded two levels for serious risk of bias and for serious inconsistency.

[c]Downgraded three levels for serious risk of bias, serious inconsistency, and serious imprecision (only 158 events).

BACKGROUND

Description of the condition

The World Health Organization estimates that one in four couples in developing countries have been found to be affected by infertility (Mascarenhas 2012). Increasing numbers of couples require treatment by the assisted conception (AC) procedures of in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) - types of assisted reproductive technology (ART). In the UK in 2018, 54,000 women had 68,727 fresh and frozen IVF cycles at Human Fertilisation and Embryology Authority (HFEA)-licensed fertility clinics (HFEA 2020). In 2016, across 40 European countries, a total of 156,002 cycles of IVF and 407,222 cycles of ICSI were performed, with ART infants contributing to 2.9% of all births. Following IVF and ICSI, clinical pregnancy rates per transfer were 34.8% and 33.2%, respectively (ESHRE- European IVF monitoring consortium 2020).

The European Society of Human Reproduction and Embryology (ESHRE) reported that the most important indicator of success of ART treatment is the birth of a single healthy child (Land 2003). Following this, the Harbin Consensus Conference Workshop Group developed a modified Consolidated Standards of Reporting Trials (CONSORT) statement for infertility trials and recommended that the preferred primary outcome of all infertility trials is live birth (defined as any delivery of a live infant at ≥ 20 weeks' gestation) (Harbin Consensus Conference Workshop Group 2014).

Numerous innovations have improved assisted reproduction outcomes; these include laboratory technologies to improve fertilisation, cryopreservation techniques, blastocyst transfers to improve success (Glujovsky 2016), pre-genetic testing (Sengupta 2012), and time lapse embryo imaging, which provides a better selection of embryos for transfer (Freour 2012).

To improve ART outcomes, a receptive endometrium in the presence of a good quality embryo is important for achieving a successful pregnancy. The quality of the transferred embryos may be affected by genetic disorders, issues with hatching (zona pellucida harding), and poor laboratory cultural conditions.

The human oocyte and early embryo is surrounded by a 13- to 15-µm-thick acellular matrix, the zona pellucida (ZP) (Bleil 1980), which is composed of glycoproteins, carbohydrates, and zona pellucida-specific proteins (ZP1, ZP2, ZP3, ZP4) (Lefievre 2004). The zona pellucida is bi-layered; the outer layer is thick, whereas the inner layer is thin but resilient. It is involved in sperm binding and induction of the acrosome reaction, and it promotes oocyte fusion (Gupta 2015). Following fertilisation, the zona pellucida blocks polyspermy, prevents blastomere dispersal, and helps in oviductal transport. It avoids contact with other cells (epithelial lining of the reproductive tract, leucocytes, spermatozoa, and other cells of the embryo). It is essential for maintaining the integrity of the pre-compacted embryo. Compaction is the formation of structural junctions between blastomeres. Once compaction occurs, the zona pellucida is no longer essential (Hammadeh 2011). Zona hardening occurs naturally after fertilisation to ensure this threefold function. A combination of lysins produced by the cleaving embryo or the uterus and physical expansion then reduces the zona thickness in preparation for hatching. Zona hardening, although not readily quantifiable, may also be induced by in vitro culture and by in vivo aging (De Vos 2000).

After fertilisation, the zona maintains the three-dimensional integrity of the uncompacted embryo for up to six days in early development, facilitates free passage of the compacted embryo through the fallopian tube into the uterus, and protects the embryo from micro-organisms and immune cells (Bronson 1970). During embryonic development, fluid starts to accumulate between cells at the morulae stage. As the volume of fluid increases, a cavity gradually appears, forming the blastocoele. This normally happens between Days 4 and 5 in human embryos in vitro and is known as the blastocyst stage. Concurrently with the increase in fluid inside the blastocyst, the number of cells increases. This combination causes progressive enlargement of the blastocyst and its cavity, leading to progressive thinning of the ZP. Finally, the blastocyst breaks free of the ZP through a process called hatching (Hardarson 2012). This process occurs before implantation.

Human embryos resulting from controlled ovarian hyperstimulation develop more slowly in vitro compared to embryos in vivo. These embryos manifest a relatively high degree of cytogenetic abnormalities and cellular fragmentation and a reduced rate of blastocyst development, although maternal age and treatment protocols may influence rates (Hsu 1999). Cultured embryos also hatch and implant at lower rates than occurs naturally (Harlow 1982; Mercader 2001). It is unclear whether this is due to 'hardening' of the zona pellucida as a result of cross-linking of its constituent ZP glycoproteins in an in vitro environment (Cohen 1991). With IVF and ICSI treatment, the possible combination of delayed embryo hatching and advanced endometrial development may present an unfavourable environment for implantation (Check 1999; Hsu 1999).

Description of the intervention

Artificial disruption of the zona pellucida is known as assisted hatching (AH) and was first suggested in the 1980s. It was subsequently observed in women undergoing embryo biopsy for pre-implantation genetic diagnosis (Fehilly 1985). AH is a complementary technique to conventional ART and involves thinning or completely diminishing a small fraction of the outer coat of a fertilised egg or early embryo to encourage hatching and implantation in the receptive endometrium.

A variety of techniques have since been employed to assist embryo hatching, including chemical drilling of the ZP with acidic Tyrode's medium, ZP thinning using acidic Tyrode's, mechanical piercing of the ZP with a microneedle, known also as partial zona dissection (PZD), and mechanical expansion of the ZP via injected hydrostatic pressure, carving a hole in ZP via piezoelectric pulses and laser-assisted zona drilling (Avella 2019). Regardless of the AH technique employed, it is important to distinguish whether the zona has remained unbreached (as in thinning), has been fully breached (when a hole is made chemically), or has been completely removed. This distinction may have implications for whether an embryo is able to undergo normal zona expansion and escape following AH (Blake 2001), as well as for subsequent monozygotic twinning (da Costa 2001; Menezo 2003; Schieve 2000). At present, laser AH is the most popular complementary ART technique (Hammadeh 2011)

How the intervention might work

Zona thickness appears to be influenced by a variety of factors including the woman's age (Balakier 2012), hormone profile, smoking, and cause of infertility. Increased ZP thickness correlates

negatively with embryo implantation rates (Avella 2019, Loret de Mola 1997). Implantation of the embryo into the endometrium is initiated by hatching of the embryo from the surrounding ZP (Hardarson 2012). Failure of the embryonic zona pellucida to rupture following blastocyst expansion has been suggested as a possible contributing factor to failure of embryo implantation. To help embryos escape from their zona during blastocyst expansion, different types of assisted hatching have been proposed (De Vos 2000).

Many mechanisms by which AH could improve embryo implantation have been postulated. One of these mechanisms is AH overcoming the zona pellucida hardening caused by IVF and cell culture or cryopreservation. Additionally, some evidence indicates that embryos that have undergone zona manipulation for AH tend to implant one day earlier than unhatched embryos (Rink 1995). Finally, as suggested by Cohen 1992, artificial opening could enhance hormonal and metabolite exchange, as well as messaging between the embryo and the endometrium.

Why it is important to do this review

Zona manipulation of some form has been offered to women of advanced age and to those with high follicle-stimulating hormone (FSH) levels, with high risk of zona hardening (as with in vitro oocyte maturation), and status post repeated implantation failure (Al-Nuaim 2002). However, considerable uncertainty remains over whether AH significantly improves IVF and ICSI clinical outcomes and embryo implantation rates, or whether it is associated with negative consequences for embryo development and viability. Previous updates showed that AH results in an increase in clinical pregnancy rates when compared with no AH, but AH has failed to result in a statistically significant increase in live birth rates. However, few trials have reported on live birth rates. We hope that updating this review and incorporating more studies will provide more conclusive evidence of effects of AH on live birth rate particularly rather than only on clinical pregnancy rates, as well as its effects on other outcomes such as miscarriage and multiple pregnancy rates.

OBJECTIVES

To determine effects of assisted hatching (AH) of embryos derived from assisted conception on live birth and multiple pregnancy rates.

METHODS

Criteria for considering studies for this review

Types of studies

Published and unpublished randomised controlled trials (RCTs) were eligible for inclusion. We excluded non-randomised studies (e.g. studies with evidence of inadequate sequence generation such as alternate days and patient numbers), as they are associated with high risk of bias. Trials were eligible for inclusion only if data could be extracted per woman and not per cycle. We excluded trials that presented results as per cycle rather than as per woman (unless it was clear in the text that per cycle and per woman were used interchangeably). We excluded cross-over trials, as the design is not valid in this context.

Types of participants

Eligible participants were women of all nationalities and reproductive ages undergoing assisted conception by IVF or ICSI, using their own gametes and consenting to participation in a trial of AH after fertilisation.

In the subgroup analysis, poor prognosis referred to women with increased age, previous IVF failure, high FSH, or use of frozen embryos, or it was used when the primary study protocol referred to women with a poor prognosis.

Types of interventions

Trials that investigated any known method of AH after fertilisation were included. Techniques used to disrupt the zona pellucida before embryo replacement were of the following forms.

- Mechanical.
- Chemical.
- Laser.

Trials in which assisted hatching took place to the following extent were eligible.

- Breaching the zona pellucida by a hole (by laser, chemical, or mechanical means).
- Thinning the zona pellucida (with no actual hole created).
- Removing the entire zona pellucida.

Trials were eligible when AH was performed on fresh embryos and cryopreserved embryos following thawing and before embryo transfer, as well as on vitrified-warmed embryos that were transferred at the cleavage stage. The effects of these interventions were compared to those of a control by which AH was not performed.

Trials directly comparing different AH methods (without including a control group with no assisted hatching performed) were excluded because the objective of this review was to determine the overall effectiveness of the technique of AH.

We excluded biopsied embryos for purposes of pre-genetic screening (PGS)/pre-genetic diagnosis (PGD) during assisted reproduction because this approach aims towards embryo selection - not towards increased possibility of implantation per se. As assisted hatching has been proposed to improve implantation, leading to improved clinical pregnancy and live birth rates, biopsied embryos for PGS and PGD were excluded.

Types of outcome measures

Primary outcomes

- Live birth rate per woman
- Multiple pregnancy rate per woman

Secondary outcomes

- Clinical pregnancy rate per woman (defined as pregnancy diagnosed by ultrasonographic visualisation of one or more gestational sacs, or definitive clinical signs of pregnancy)
- Miscarriage rate per woman (loss of pregnancy up to 20 weeks' gestation per woman)
- Monozygotic twinning per woman

- Ectopic pregnancy rate per woman
- Congenital or chromosomal abnormalities per woman

Only trials that reported at least clinical pregnancy rate per woman were included. The first version of the review included trials with implantation as an outcome; however for this update, we have removed implantation rate as an outcome. It is not possible to pool implantation, as the data are reported per embryo. We recorded live birth as an event per woman and not by the number of infants delivered because of the large number of multiple births.

Search methods for identification of studies

We searched for all published and unpublished RCTs of AH versus no AH, without language restrictions, from inception of the databases until 27 May 2020, in consultation with the Gynaecology and Fertility Group Information Specialist.

Electronic searches

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

- Cochrane Gynaecology and Fertility (CGF) Group Specialised Register of Controlled Trials, ProCite platform, searched 27 May 2020, (Appendix 1);
- CENTRAL, via the Cochrane Register of Studies Online (CRSO), Web platform, searched 27 May 2020, (Appendix 2);
- MEDLINE, Ovid platform, searched from 1946 to 27 May 2020, (Appendix 3);
- Embase, Ovid platform, searched from 1980 to 27 May 2020, (Appendix 4);
- PsycINFO, Ovid platform, searched from 1806 to 27 May 2020, (Appendix 5).

Searching other resources

- International trial registers: the ClinicalTrials database, a service of the US National Institutes of Health (clinicaltrials.gov/ct2/home) and the World Health Organization International Trials Registry Platform search portal (www.who.int/trialsearch/Default.aspx);
- We also handsearched the reference lists of relevant articles retrieved by the search.

Data collection and analysis

We conducted data collection and analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019).

Selection of studies

Two review authors (LL and SH) scanned titles and abstracts from the first searches; the same methods were adopted by another review author for the second searches. Trials that appeared relevant were selected and formally assessed for inclusion independently by three review authors using an inclusion and exclusion form. We resolved disagreements through discussion. Multiple reports of the same study were collated under a single reference. We corresponded with study investigators as required to clarify study eligibility. Trials excluded at this stage are detailed in the table Characteristics of excluded studies table. We have documented the selection process with a PRISMA flow chart (Figure 1).

Figure 1. Study flow diagram.

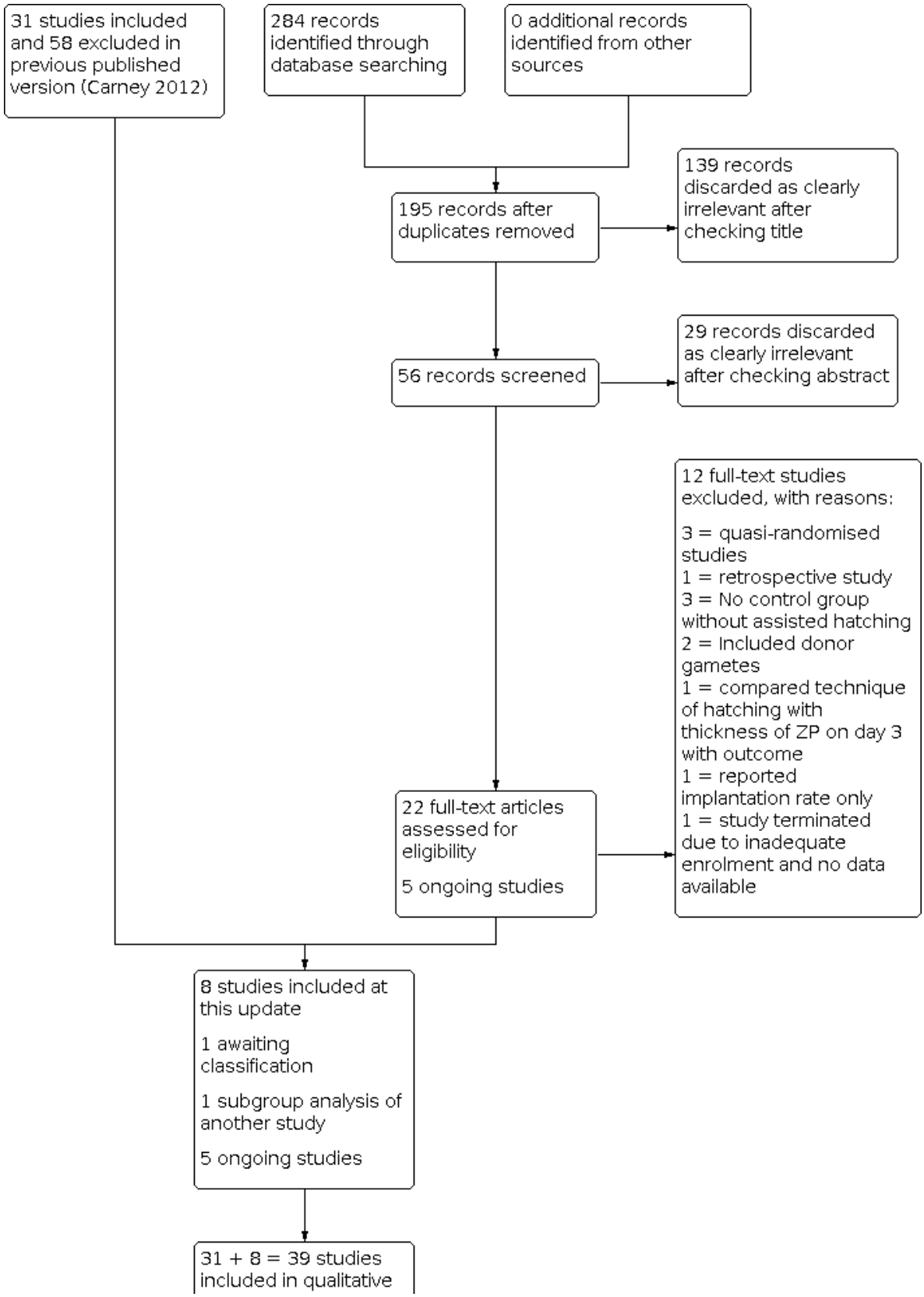
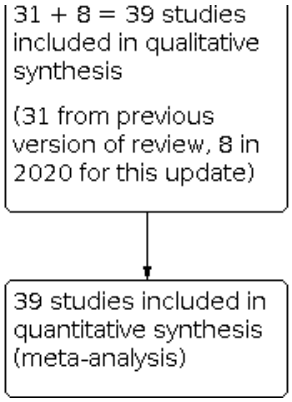


Figure 1. (Continued)



Data extraction and management

Two review authors (LL and MAA) independently extracted data from eligible studies using the Cochrane data collection form for Intervention reviews (RCTs only) (Figure 2; Figure 3). Discrepancies in data extraction were resolved by consensus during discussions with another review author (MWS or SF).

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

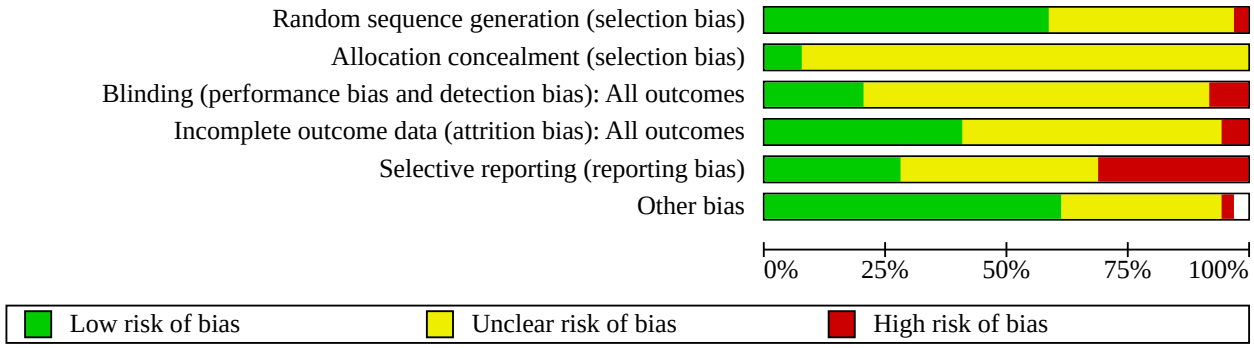


Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.

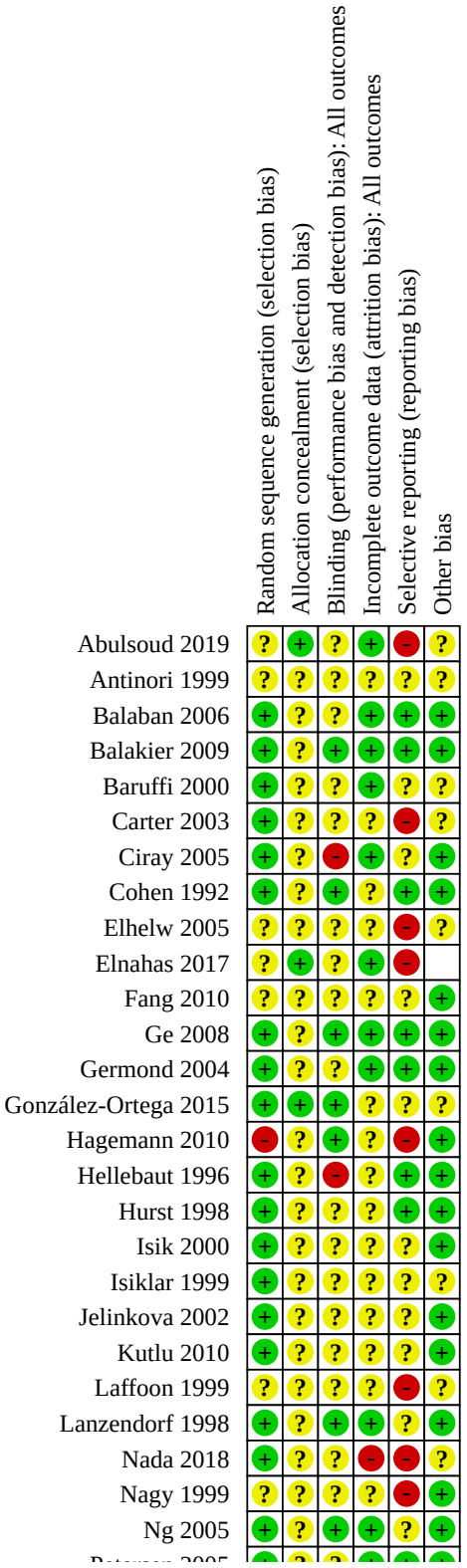
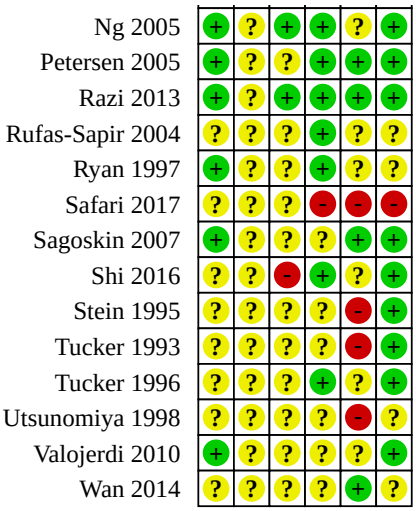


Figure 3. (Continued)



Assessment of risk of bias in included studies

Two review authors (LL and MAA) independently assessed the included studies for risk of bias using the Cochrane risk of bias assessment tool to assess allocation (random sequence generation and allocation concealment), blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective reporting, and other bias (http://handbook.cochrane.org). Disagreements were resolved by discussion or by a third review author (MWS or SF).

For each trial, it was determined whether adequate allocation concealment was described, and the trial was classed as being at low risk of bias if this was the case. If it was not the case, or if it was unclear how allocation concealment was achieved, the trial was classed as being at high risk or unclear risk, respectively. For each trial, we determined whether an acceptable method of sequence generation was described within the text (e.g. by stating that a computer-generated randomisation list had been used). If this was the case, again the trial was classed as being at low risk in this respect. Similarly, if this was unclear, the trial was classed as having unclear risk of bias. We determined who was blinded in each trial. If participants and medical staff in the trial were blinded to allocation, the trial was at low risk. If this was not stated or if it was clear that this was not the case, the trial was again classed as having unclear risk or high risk of bias, respectively. Finally, selective reporting is an important issue in this review and is an important contributor to reporting bias, with only a minority of trials reporting on the primary outcome of live birth. Each trial that reported live birth was classed as low risk unless it did not report clinical pregnancy; in that case, it was reported as high risk. Each trial that did not report live birth was classed as high risk. Study authors were contacted if risks of bias were unclear to support the assessment

Measures of treatment effect

All outcomes were dichotomous, and results were expressed for each trial as an odds ratio (OR) with 95% confidence interval (CI); P values were calculated.

Unit of analysis issues

The primary analysis was per woman randomised. Data that did not allow valid analysis (e.g. 'per cycle' data) were not pooled. Multiple live births (e.g. twins, triplets) were counted as one live birth event.

Dealing with missing data

Attempts were made to obtain additional information on trial methods, actual original trial data, or both, by contacting the principal authors of the trials. Reminders were sent (when necessary) to study authors if no reply had been received four weeks after the initial request. Only available data were analysed, and no imputation of data was undertaken.

Assessment of heterogeneity

Consideration of the clinical and methodological characteristics of included studies was undertaken to ascertain if they were sufficiently similar for meta-analysis to provide a clinically meaningful result. Heterogeneity between the results of different trials was examined using the I^2 statistic. Statistical heterogeneity was deemed significant if the P value was ≤ 0.1, that is, an indication of greater variation than would be expected by chance. I^2 values were also examined, and higher values (> 40%) were taken to indicate high heterogeneity (Higgins 2019).

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, review authors aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies, and by staying alert for duplication of data. If 10 or more studies were included in an analysis, we planned to use a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies). Asymmetry can be found in funnel plots, especially when high heterogeneity is noted. Asymmetry detected in a funnel plot would probably be due to true heterogeneity (Stuck 1998).

Data synthesis

Studies were combined via meta-analysis using a fixed-effect model for AH versus no AH with RevMan 5.4 software (RevMan 2020). An increase in the odds of a particular outcome was displayed graphically in the meta-analyses to the right of the centre-line, and a decrease in the odds of an outcome was displayed to the left of the centre-line.

Subgroup analysis and investigation of heterogeneity

The following subgroup analyses were undertaken for the 2012 and 2020 updates.

- Number of attempts: first or repeat attempt at assisted conception.
- Mode of assisted conception: IVF or ICSI.
- Method of assisted hatching: chemical, laser, or mechanical.
- Prognosis of woman: good or poor.
- Extent of AH: thinning, breaching, complete removal of zona pellucida.
- Type of embryo: fresh or frozen embryo transfer (frozen embryo transfer included thawed cryopreserved and vitrified-warm embryo).

Sensitivity analysis

We performed sensitivity analysis to examine the stability of results for our primary outcomes and for clinical pregnancy, in relation to:

- adequacy of allocation concealment, by removing trials with unclear or inadequate allocation concealment; and
- adequacy of the randomisation process, by removing trials for which the method of randomisation was unclear.

Summary of findings and assessment of the certainty of the evidence

We prepared a Summary of findings table using GRADEpro and Cochrane methods (Higgins 2011; GRADEpro GDT 2015).This table evaluated the overall quality of the body of evidence for the main review outcomes (live birth, multiple pregnancy, clinical pregnancy and miscarriage) for the main review comparison (assisted hatching versus no assisted hatching). We assessed the quality of the evidence using GRADE criteria: risk of bias, consistency of effect, imprecision, indirectness and publication bias). Judgements about evidence quality (high, moderate, low or very low) were be made by two review authors working independently (LL and MAA), with disagreements resolved by discussion. Judgements were justified, documented, and incorporated into reporting of results for each outcome. We planned to extract study data, format our comparisons in data tables and prepare a summary of findings table before writing the results and conclusions of our review.

RESULTS

Description of studies

Results of the search

In our updated search in 2020, we identified 284 discrete records, of which 22 were examined in full text as they were potentially eligible. Twelve were excluded in 2020 as they did not meet our inclusion criteria, one was identified as a subgroup analysis of an included study (Desai 2013; Hagemann 2010), and one is awaiting classification (Elnahas A 2018); therefore eight were eligible for inclusion. The previous version of the review included 31 studies and excluded 58 studies, so altogether there are now 39 included studies and 70 excluded studies (Figure 1). Five ongoing trials are registered at http://www.clinicaltrials.gov (a service of the US National Institutes of Health) and at http://www.who.int/trialsearch/Default.aspx (The World Health Organization International Trials Registry Platform search portal) (see Characteristics of ongoing studies).

All included trials were described in published reports (full papers or abstracts). One included study was published in Spanish (González-Ortega 2015), and one excluded study was published in Chinese (Lu 2016). In total, the studies included in our review recruited a total of 7249 women undergoing IVF or ICSI: 3688 women in the assisted hatching groups and 3561 women in the control groups.

Included studies

Study design and setting

We included a total of 39 studies, including eight new studies for this update (Abulsoud 2019; Elnahas 2017; González-Ortega 2015; Nada 2018; Razi 2013; Safari 2017; Shi 2016; Wan 2014) (Figure 1).

The trials were carried out in 17 different countries: USA (Carter 2003; Cohen 1992; Hagemann 2010; Hurst 1998; Laffoon 1999; Lanzendorf 1998; Sagoskin 2007; Tucker 1993; Tucker 1996), Italy (Antinori 1999; Nagy 1999), Belgium (Hellebaut 1996), Turkey (Balaban 2006; Ciray 2005; Isik 2000; Isiklar 1999; Kutlu 2010), Brazil (Baruffi 2000; Petersen 2005), Australia (Ryan 1997), Germany (Jelinkova 2002), China (Fang 2010; Ge 2008; Ng 2005; Shi 2016; Wan 2014), Japan (Utsunomiya 1998), Israel (Rufas-Sapir 2004; Stein 1995), Iran (Razi 2013; Safari 2017; Valojerdi 2010), Canada (Balakier 2009), Egypt (Abulsoud 2019; Elhelw 2005; Elnahas 2017; Nada 2018), and Mexico (González-Ortega 2015). One European multi-centre study involved women at IVF centres in Switzerland, France, Germany, and Spain (Germond 2004).

Participants

The age of participants ranged from 27 to 42 years (when reported). Some trials had subgroup data within them (e.g. Elnahas 2017; Ge 2008; Germond 2004; Kutlu 2010; Rufas-Sapir 2004; Shi 2016; Stein 1995; and Tucker 1996 presented pregnancy for different age groups), whilst other studies included only women 35 years of age and older (e.g. González-Ortega 2015; Lanzendorf 1998) or younger than 35 years old (Antinori 1999; Hurst 1998). Other studies included women of other specific age groups, for example, 38 years of age or younger (Balakier 2009; Hagemann 2010). Subgroup analysis based on age of the women has not been achievable, as studies did not categorise age groups in a universal way.

Fourteen trials included women with a poor prognosis (Abulsoud 2019; Antinori 1999; Cohen 1992; Elhelw 2005; Ge 2008; Germond 2004; González-Ortega 2015; Jelinkova 2002; Kutlu 2010; Lanzendorf 1998; Petersen 2005; Rufas-Sapir 2004; Stein 1995; Utsunomiya 1998). Fourteen trials included women with a good prognosis (Antinori 1999; Balakier 2009; Carter 2003; Ciray 2005; Cohen 1992; Elnahas 2017; Ge 2008; Hellebaut 1996; Hurst 1998; Kutlu 2010; Laffoon 1999; Nada 2018; Sagoskin 2007; Tucker 1993), and the remaining studies did not provide information.

Interventions

Twelve trials were repeat cycles, and eight included women undergoing their first assisted reproductive technology (ART) cycle; 19 trials did not report whether the treatment cycle was a first or repeat cycle or were mixed cycles. A total of 12 trials included women undergoing ICSI alone, 15 were IVF only, and the rest were unstated or included mixed ICSI and IVF cycles. Twenty-eight trials involved transfer of fresh embryos exclusively, nine involved frozen or vitrified-warmed embryos only, two used fresh and frozen embryos (Germond 2004; Ge 2008), and one study used a combination of fresh and frozen embryos (Ryan 1997).

Eleven trials employed chemical means for assisted hatching, five employed mechanical means, and 23 employed laser.

Seventeen trials utilised a breach of the zona pellucida with a hole (Antinori 1999; Cohen 1992; Germond 2004; Hagemann 2010; Hellebaut 1996; Hurst 1998; Isiklar 1999; Laffoon 1999; Lanzendorf 1998; Nagy 1999; Razi 2013; Rufas-Sapir 2004; Ryan 1997; Sagoskin 2007; Stein 1995; Tucker 1996; Wan 2014), a further 17 utilised non-breach thinning (Abulsoud 2019; Balaban 2006; Balakier 2009; Baruffi 2000; Ciray 2005; Elhelw 2005; Elnahas 2017; Ge 2008; Kutlu 2010; Nada 2018; Ng 2005; Petersen 2005; Safari 2017; Shi 2016; Tucker 1996; Utsunomiya 1998; Valojerdi 2010), and two performed complete zona removal (Isik 2000; Jelinkova 2002). For two studies, this was unknown (Carter 2003; González-Ortega 2015), whilst another study used a new method of AH whereby the zona pellucida was expanding mechanically (Fang 2010). Three trials reported the thickness of the zona pellucida (in each case, choosing zona thickness > 12 µm as an inclusion criterion).

Twenty-five trials reported the interval between AH and embryo transfer (20 trials reported less than four hours; three trials, four to eight hours; and two, longer than eight hours).

Blastocyst transfer occurred in four trials (Isik 2000; Isiklar 1999; Laffoon 1999; Wan 2014), one of which involved complete zona removal (Isik 2000).

Outcomes

Outcome measures utilised for this review were reported by varying numbers of trials.

- 14 trials reported live birth rate.
- 18 trials reported multiple pregnancy rate.
- 39 trials reported clinical pregnancy rate.
- 17 trials reported miscarriages.
- 6 trials reported monozygotic twinning.
- 5 trials reported ectopic pregnancy.
- 3 trials reported congenital or chromosomal abnormalities, or both.
- 3 trials reported embryo damage.
- No trials reported in vitro blastocyst development post AH.

Further details about the included trials are provided in the Characteristics of included studies table and in Table 1 and Table 2.

Excluded studies

We excluded 72 studies from the review (see Characteristics of excluded studies). Reasons for exclusion included inadequate method of randomisation, no per woman data, inadequate reporting of clinical pregnancy, no control group, inclusion of women with donor gametes, and, in the remainder, studies were not randomised and two studies were found to be retrospective studies on close examination of the text. Conference abstracts were excluded only when further details were asked from study authors and no response was provided, and we could not utilise available data.

Risk of bias in included studies

The overall methodological quality of the included trials was considered sub-optimal, largely due to risk of bias in the included studies. Further details of the trials' risk of bias can be found in the Characteristics of included studies table. Summaries of risk of bias for all included studies are presented in Figure 2 and Figure 3.

Allocation

Random sequence generation

All 39 trials stated that randomisation had occurred. Regarding random sequence generation, 23 studies were at low risk of this bias, 15 had unclear risk, and one was at high risk (Hagemann 2010).

Allocation concealment

Three studies were at low risk of selection bias related to allocation concealment (Abulsoud 2019; Elnahas 2017; González-Ortega 2015), and 36 studies had unclear risk.

Blinding

Although blinding was unlikely to influence findings for the primary review outcome (live birth), eight trials employed double blinding with both the woman and the outcome assessor unaware of the allocation (Balakier 2009; Cohen 1992; Ge 2008; González-Ortega 2015; Hagemann 2010; Lanzendorf 1998; Ng 2005; Razi 2013). In 28 studies, it is unclear if blinding was used or who was blinded (participant or assessor), and in the remaining three studies, no blinding was reported (Ciray 2005; Hellebaut 1996; Shi 2016).

Incomplete outcome data

Ideally, studies should randomise women on the day of assessment of embryos for suitability for embryo transfer. Two studies we assessed as high risk related to incomplete outcome data (Nada 2018; Safari 2017). One excluded participants after randomisation due to cycle cancellation but gave no reason for why cycles were cancelled, and one randomised 32 participants into each trial arm but reported outcomes for only 30 participants. A total of 16 studies were at low risk of bias related to incomplete outcome data, and 21 studies had unclear risk.

Selective reporting

All pre-specified outcomes were reported within the outcomes of all studies. Many studies did not report live birth, multiple pregnancy, or miscarriage outcomes.

Other potential sources of bias

Age groups were matched in trials with similar means in the AH and control groups.

Thirty-two trials were reported as full published papers. Seven trials were published in conference abstract form only (Antinori

1999; Carter 2003; Elhelw 2005; Laffoon 1999; Rufas-Sapir 2004; Ryan 1997; Utsunomiya 1998).

No funding bias or any other conflicts of interests were noted in the included studies.

Effects of interventions

See: **Summary of findings 1** Assisted hatching compared to no assisted hatching for women undergoing assisted conception

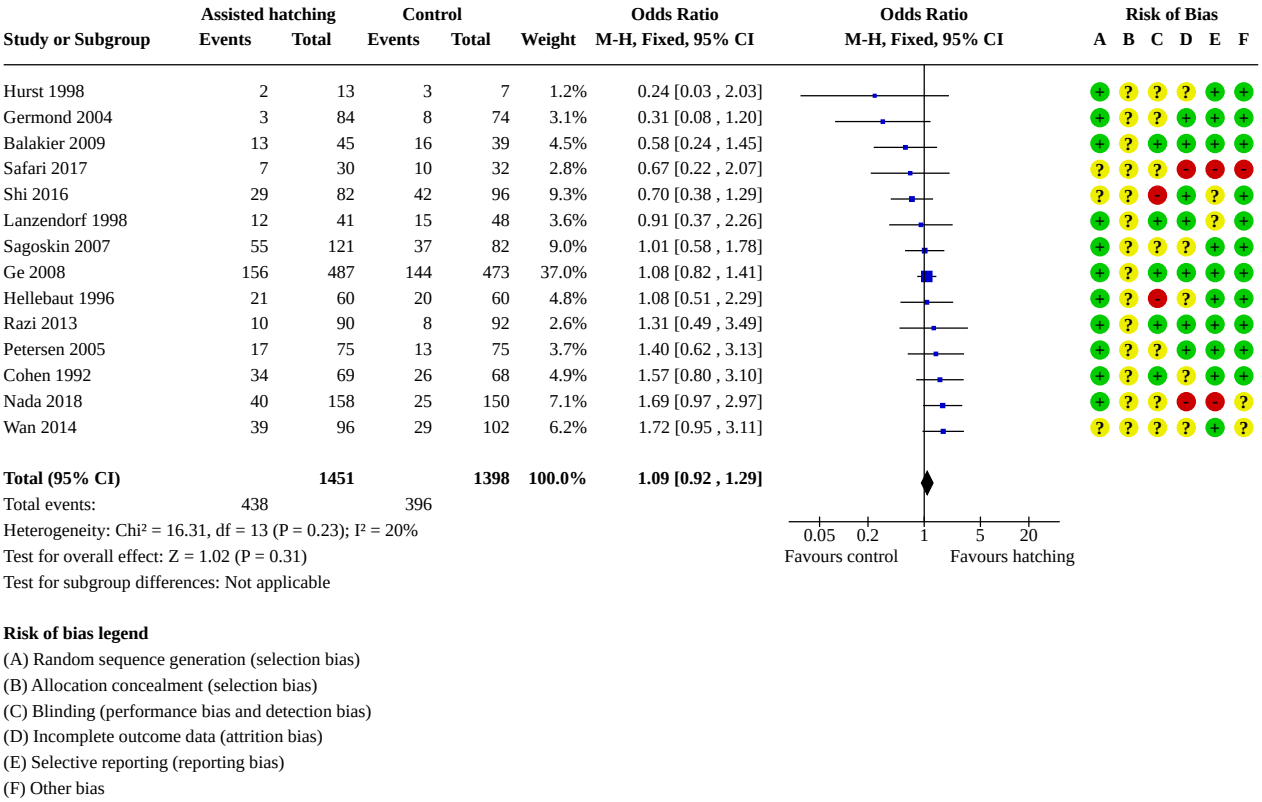
Assisted hatching compared to no assisted hatching

Primary outcomes

Live birth per woman

Only 14 of the 39 trials reported live birth rate. We are uncertain of the effect of AH on live birth rate when compared to no AH (odds ratio (OR) 1.09, 95% confidence interval (CI) 0.92 to 1.29; 14 RCTs, N = 2849; I^2 = 20%; low-quality evidence; Analysis 1.1; Figure 4).

Figure 4. Forest plot of comparison: 1 Live birth rate, outcome: 1.1 Live birth per woman randomised.



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Incomplete outcome data (attrition bias)
(E) Selective reporting (reporting bias)
(F) Other bias

Subgroup analysis

- Number of attempts - first or repeat attempt at ART: no evidence indicates that the effect of assisted hatching differed between those undergoing their first or subsequent attempts (P = 0.23). We are uncertain of the effect of AH on the live birth rate in women in their first attempt at ART (OR 0.78, 95% CI 0.48 to 1.28; 3 RCTs, n = 380; I^2 = 15%) or in subsequent attempts at ART (OR 1.40, 95% CI 0.62 to 3.13; 1 RCT only) when compared to no AH (Analysis 1.2)
- Mode of conception - assisted conception procedure (IVF/ICSI): no evidence shows that the effect of assisted hatching differed between those undergoing IVF and those undergoing ICSI (P = 0.20). For couples undergoing ICSI, AH may improve slightly the live birth rate when compared to no AH (OR 1.54, 95% CI 1.02 to 2.33; 3 RCTs, n = 640; I^2 = 0%). For couples undergoing IVF, there may be little to no difference in live birth rate with AH compared to no AH (OR 1.00, 95% CI 0.60 to 1.68; 3 RCTs, n = 241; I^2 = 58%) (Analysis 1.3)
- Method of assisted hatching: no evidence suggests that the effect of assisted hatching differed between chemical and laser methods (P = 0.80). We are uncertain of the effect of chemical AH or laser AH on live birth rate when compared to no AH (OR 1.13, 95% CI 0.74 to 1.74; 4 RCTs, n = 366; I^2 = 5%; and OR 1.07, 95% CI 0.89 to 1.28; 10 RCTs, n = 2473; I^2 = 24%, respectively). None of the trials that employed mechanical forms of AH reported on live births (Analysis 1.4)
- Prognosis: no evidence shows that the effect of assisted hatching differed between women in poor prognosis groups and women in good prognosis groups (P = 0.12). We are uncertain of the effect of AH on live birth rate in women with a good prognosis compared with no AH (OR 1.03, 95% CI 0.83 to 1.28; 6 RCTs, n = 1495; I^2 = 23%) and in women with a poor prognosis who underwent AH compared to no AH (OR 1.46, 95% CI 0.99 to 2.15; 4 RCTs, n = 576; I^2 = 0%) (Analysis 1.5)
- Extent of zona manipulation: no evidence indicates that the effect of assisted hatching differed between thinning of zona and breaching the zona with a hole (P = 0.64). We are uncertain of the

effect of AH with thinning of the zona pellucida on live birth rate compared with no AH and AH with breech of the zona pellucida on live birth rate compared with no AH (OR 1.06, 95% CI 0.86 to 1.30; 6 RCTs; I^2 = 31%; and OR 1.15, 95% CI 0.87 to 1.51; 8 RCTs, n = 1107; I^2 = 21%, respectively) (Analysis 1.6)

- Type of embryo - fresh or frozen embryo: no evidence shows that the effect of assisted hatching differed between fresh and frozen embryos (P = 0.35). We are uncertain of the effect of AH on live birth rate in women who had fresh embryo transfer compared with the no AH group (OR 1.16, 95% CI 0.94 to 1.44; 11 RCTs, N = 1669; I^2 = 16%). Only one study reported use of frozen embryos (Safari 2017), and two studies reported use of fresh and frozen embryos (Ge 2008; Germond 2004)

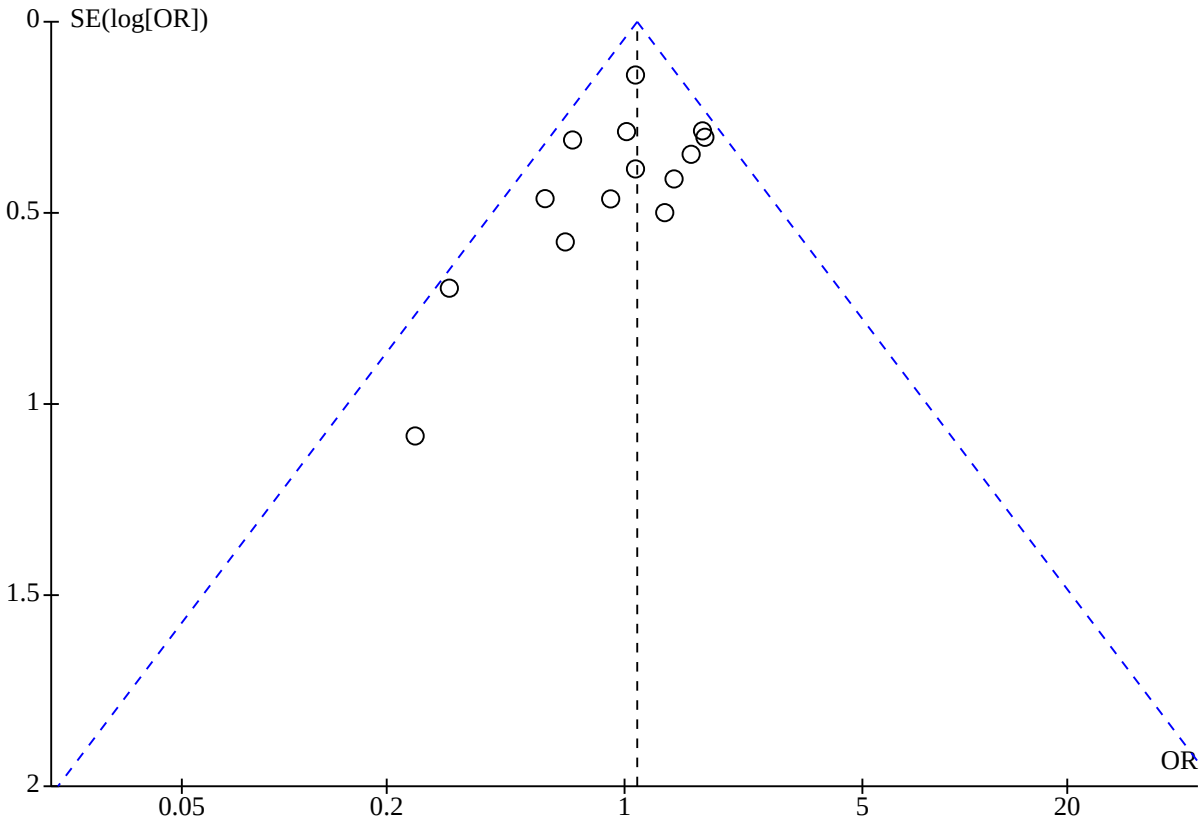
Sensitivity analysis

- Allocation concealment: no trials were assessed as low risk
- Method of randomisation: 11 trials stated the method of randomisation (Balakier 2009; Cohen 1992; Ge 2008; Germond 2004; Hellebaut 1996; Hurst 1998; Lanzendorf 1998; Nada 2018; Petersen 2005; Razi 2013; Sagoskin 2007). Analysis of the data from these trials suggests that there was no improvement in live birth rate between AH groups and control groups (OR 1.10, 95% CI 0.92 to 1.32; n = 2411; I^2 = 11%)

Other analyses

We used a funnel plot to assess publication bias and small-study effects. The funnel plot shows only some mild asymmetry (Figure 5). The funnel plot is likely to be detecting small-study effects.

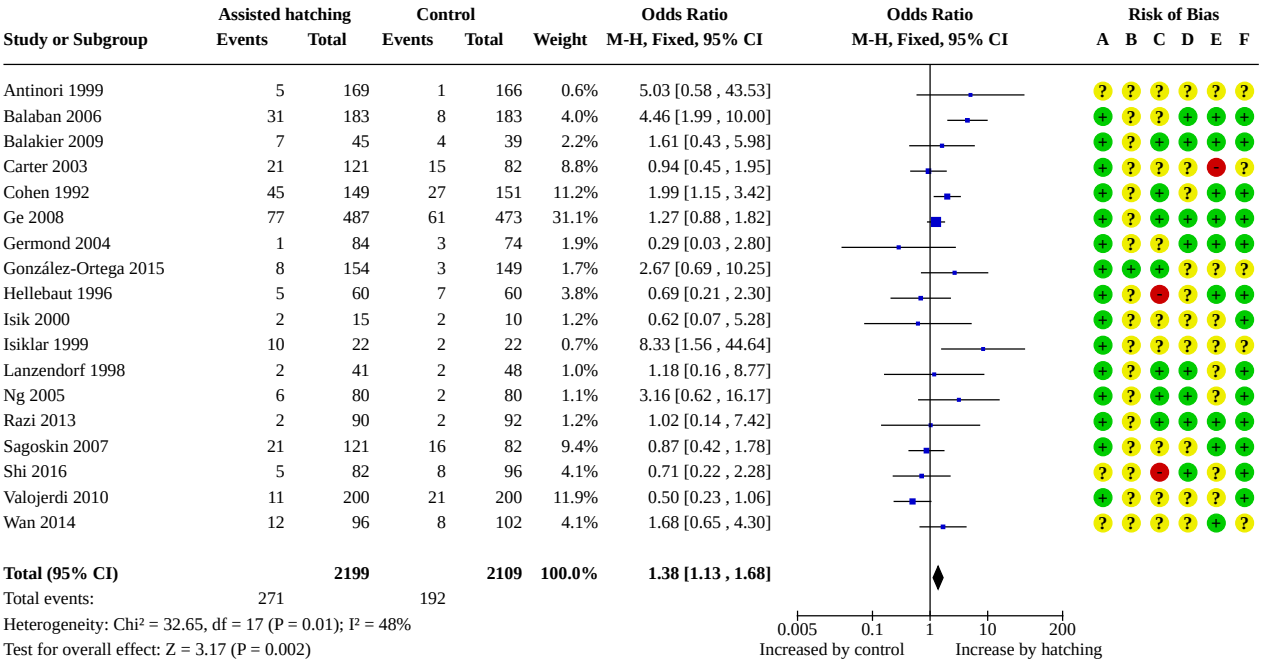
Figure 5. Funnel plot of comparison: 1 Live birth: assisted hatching compared with no assisted hatching, outcome: 1.1 Live birth per woman randomised.



Multiple pregnancy per woman

Eighteen of the 39 trials reported on multiple pregnancy. AH may lead to a higher multiple pregnancy rate compared to no AH (OR 1.38, 95% CI 1.13 to 1.68; 18 RCTs, n = 4308; I^2 = 48%; low-quality evidence; Analysis 2.1; Figure 6).

Figure 6. Forest plot of comparison: 4 Multiple pregnancy rate, outcome: 4.1 Multiple pregnancy rate per woman randomised.



Risk of bias legend

(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Incomplete outcome data (attrition bias)
(E) Selective reporting (reporting bias)
(F) Other bias

Subgroup analysis

- Number of attempts - first attempt or repeat attempt at ART: no evidence indicates that the effect of AH differed between those undergoing their first attempt and those undergoing subsequent attempts (P = 0.28). We are uncertain of the effect of AH on the multiple pregnancy rate in women who underwent AH compared with no AH in their first attempt at ART (OR 0.73, 95% CI 0.31 to 1.72; 4 RCTs, n = 654; I^2 = 0%) or in subsequent attempts at ART (OR 1.25, 95% CI 0.80 to 1.94; 5 RCTs, n = 1068; I^2 = 25%) (Analysis 2.2)
- Mode of conception- assisted conception procedure (IVF/ICSI): no evidence shows that the effect of AH differed between those undergoing IVF and those undergoing ICSI treatment (P = 0.21). For couples undergoing ICSI, AH may lead to slightly higher multiple pregnancy rates when compared to no AH (OR 3.09, 95% CI 1.57 to 6.08; 3 RCTs, n = 573; I^2 = 52%). For couples undergoing IVF, AH may lead to slightly higher multiple pregnancy rates when compared to no AH (OR 1.87, 95% CI 1.28 to 2.72; 6 RCTs, n = 1126; I^2 = 36%) (Analysis 2.3)
- Method of assisted hatching: evidence suggests that the effect of AH differed between chemical, laser, and mechanical methods (P = 0.08). We are uncertain of the effect of chemical AH on the multiple pregnancy rate when compared to no AH (OR 1.55, 95% CI 0.98 to 2.47; 4 RCTs, n = 534; I^2 = 10%). Both laser and mechanical AH may lead to a slightly higher multiple pregnancy rate when compared to no AH (OR 1.29, 95% CI 1.03 to 1.61; 4 RCTs, n = 534; I^2 = 50%; and OR 8.33, 95% CI 1.56-44.64; 1 RCT only, n = 44, respectively) (Analysis 2.4)
- Prognosis: some evidence suggests that the effect of AH differed between women in poor prognosis groups and women in good prognosis groups (P = 0.02). We are uncertain of the effect of AH on the multiple pregnancy rate in women with a good prognosis compared with no AH (OR 1.08, 95% CI 0.81 to 1.44; 6 RCTs, n = 1569; I^2 = 0%). In women with a poor prognosis, AH may lead to a slightly higher multiple pregnancy rate when compared to no AH (OR 1.95, 95% CI 1.27 to 3.00; 6 RCTs, n = 1186; I^2 = 0%) (Analysis 2.5)
- Extent of zona manipulation: no evidence indicates that the effect of AH differed between thinning of zona and breaching of zona with a hole (P = 0.65). AH may lead to a slightly higher multiple pregnancy rate in women who had assisted hatching with thinning of the zona pellucida compared with no AH (OR 1.34, 95% CI 1.02 to 1.76; 6 RCTs, n = 2148; I^2 = 71%) and assisted hatching with breech of the zona pellucida by a hole only compared with no AH (OR 1.51, 95% CI 1.08 to 2.11; 9 RCTs, n = 1629; I^2 = 35%). Only one study reported complete removal of the zona pellucida (Isik 2000) (Analysis 2.6)
- Type of embryo - fresh or frozen embryo: no evidence suggests that the effect of assisted hatching differed between fresh and frozen embryos (P = 0.46). We are uncertain of the affect of AH

on the multiple pregnancy rate in women who had fresh embryo transfer compared with no AH (OR 1.30, 95% CI 0.98 to 1.73; 13 RCTs, n = 2264; I^2 = 0%) and in women who had frozen embryo transfer compared with no AH (OR 1.60, 95% CI 1.00 to 2.55; 3 RCTs, n = 926; I^2 = 88%) (Analysis 2.7)

- Multiple pregnancy per pregnancy: overall, AH may lead to a higher multiple pregnancy rate compared to control (OR 1.37, 95% CI 1.09 to 1.72; 17 trials, n = 1598; I^2 = 25%) (Analysis 2.8)

Sensitivity analysis

- Allocation concealment: only one trial was assessed as low risk (González-Ortega 2015)
- Method of randomisation: 15 trials stated the method of randomisation (Balaban 2006; Balakier 2009; Carter 2003; Cohen 1992; Ge 2008; Germond 2004; González-Ortega 2015; Hellebaut 1996; Isik 2000; Isiklar 1999; Lanzendorf 1998; Ng 2005; Razi 2013; Sagoskin 2007; Valojerdi 2010). Analysis of the data from these trials suggests that AH may lead to a high multiple pregnancy rate when compared to no AH (OR 1.37, 95% CI 1.11 to 1.69; n = 3597; I^2 = 53%)

Secondary outcomes

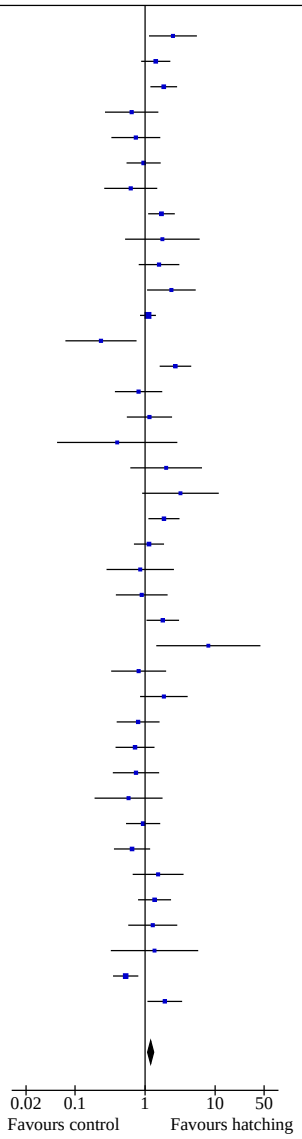
Clinical pregnancy rate per woman

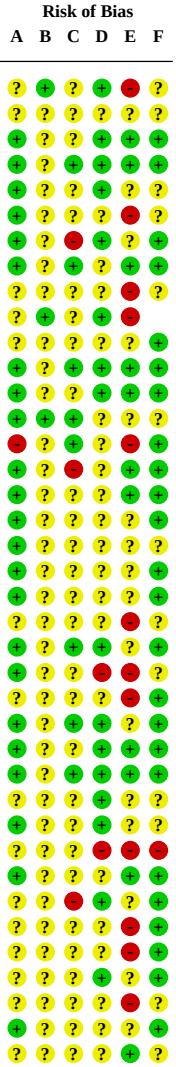
Thirty-nine trials reported clinical pregnancy data. AH may improve slightly the clinical pregnancy rate compared to no AH (OR 1.20, 95% CI 1.09 to 1.33; I^2 = 55%; low-quality evidence; Analysis 3.1; Figure 7). Furthemore, the forest plots show high heterogeneity. When a random-effects model is used, there may be little to no difference in clinical pregnancy rate among women who underwent AH compared with those given control (P = 0.04).

Figure 7. Forest plot of comparison: 2 Clinical pregnancy, outcome: 2.1 Clinical pregnancy rate per woman randomised.

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Abulsoud 2019	25	65	13	65	1.1%	2.50 [1.14 , 5.49]
Antinori 1999	52	169	41	172	4.0%	1.42 [0.88 , 2.29]
Balaban 2006	75	183	50	183	4.2%	1.85 [1.19 , 2.86]
Balakier 2009	16	45	18	39	1.8%	0.64 [0.27 , 1.55]
Baruffi 2000	17	51	21	52	2.0%	0.74 [0.33 , 1.65]
Carter 2003	62	121	43	82	3.5%	0.95 [0.54 , 1.67]
Ciray 2005	17	76	12	38	1.7%	0.62 [0.26 , 1.49]
Cohen 1992	85	164	64	166	4.3%	1.71 [1.11 , 2.66]
Elhelw 2005	8	37	5	37	0.6%	1.77 [0.52 , 6.01]
Elnahas 2017	30	80	22	80	1.9%	1.58 [0.81 , 3.08]
Fang 2010	23	61	13	64	1.1%	2.37 [1.07 , 5.28]
Ge 2008	189	487	173	473	15.1%	1.10 [0.85 , 1.43]
Germond 2004	4	84	13	74	1.9%	0.23 [0.07 , 0.76]
González-Ortega 2015	61	154	29	149	2.5%	2.71 [1.62 , 4.56]
Hagemann 2010	21	49	26	54	2.0%	0.81 [0.37 , 1.76]
Hellebaut 1996	23	60	21	60	1.8%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	0.4%	0.40 [0.06 , 2.89]
Isik 2000	15	24	10	22	0.6%	2.00 [0.62 , 6.49]
Isiklar 1999	16	22	10	22	0.4%	3.20 [0.91 , 11.27]
Jelinkova 2002	59	128	40	127	3.0%	1.86 [1.12 , 3.10]
Kutlu 2010	67	131	58	121	4.2%	1.14 [0.69 , 1.86]
Laffoon 1999	9	28	10	28	1.0%	0.85 [0.28 , 2.58]
Lanzendorf 1998	16	41	20	48	1.6%	0.90 [0.38 , 2.10]
Nada 2018	46	158	28	150	2.9%	1.79 [1.05 , 3.06]
Nagy 1999	10	20	2	18	0.1%	8.00 [1.44 , 44.30]
Ng 2005	10	80	12	80	1.5%	0.81 [0.33 , 2.00]
Petersen 2005	21	75	13	75	1.3%	1.85 [0.85 , 4.05]
Razi 2013	18	90	22	92	2.5%	0.80 [0.39 , 1.61]
Rufas-Sapir 2004	22	104	28	103	3.1%	0.72 [0.38 , 1.36]
Ryan 1997	14	100	18	100	2.2%	0.74 [0.35 , 1.59]
Safari 2017	7	30	11	32	1.1%	0.58 [0.19 , 1.78]
Sagoskin 2007	63	121	44	82	3.5%	0.94 [0.53 , 1.65]
Shi 2016	40	82	57	96	3.8%	0.65 [0.36 , 1.18]
Stein 1995	15	72	12	82	1.3%	1.54 [0.67 , 3.54]
Tucker 1993	49	110	40	108	3.2%	1.37 [0.79 , 2.35]
Tucker 1996	21	50	18	50	1.5%	1.29 [0.58 , 2.88]
Utsunomiya 1998	5	27	4	28	0.5%	1.36 [0.32 , 5.73]
Valojerdi 2010	57	200	86	200	8.7%	0.53 [0.35 , 0.80]
Wan 2014	49	96	36	102	2.4%	1.91 [1.08 , 3.38]
Total (95% CI)		**3688**		**3561**	**100.0%**	**1.20 [1.09 , 1.33]**
Total events:	1340		1146			

Heterogeneity: Chi² = 85.10, df = 38 (P < 0.0001); I² = 55%
Test for overall effect: Z = 3.61 (P = 0.0003)
Test for subgroup differences: Not applicable





Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Incomplete outcome data (attrition bias)
(E) Selective reporting (reporting bias)
(F) Other bias

Among the 14 trials reporting both clinical pregnancy and live births, analysis demonstrated that we are uncertain of the effect of AH on clinical pregnancy rate in women who underwent AH compared with those given the control (OR 1.07, 95% CI 0.92 to 1.25; I^2 = 45%) (Analysis 4.1).

Subgroup analysis

- Number of attempts - first or repeat attempt at ART: some evidence suggests that the effect of AH differed between those undergoing their first attempt and those undergoing subsequent attempts (P < 0.00001; fixed model). When the random-effects model is used, the effect is less pronounced (P = 0.0002). With the fixed-effect model, there may be a slight

decrease in the clinical pregnancy rate among women who underwent AH compared with no AH in their first attempt at ART (OR 0.74, 95% CI 0.56 to 0.98; 8 RCTs, n = 1010 women; I^2 = 9%). There may be slight improvement in clinical pregnancy rate among women who underwent AH compared to no AH in women undergoing repeat attempts at ART (OR 1.65, 95% CI 1.34 to 2.04; 11 RCTs, n = 1798; I^2 = 38%) (Analysis 3.2)

- Mode of conception - assisted conception procedure (IVF/ICSI): no evidence shows that the effect of AH differed between those undergoing IVF and those undergoing ICSI treatment (P = 0.62). For couples undergoing ICSI, AH may improve slightly the clinical pregnancy rate when compared to no AH (OR 1.40, 95% CI 1.14 to 1.71; 11 RCTs; n = 1825; I^2 = 30%). For couples undergoing IVF, AH may improve slightly the clinical pregnancy rate when compared to no AH (OR 1.31, 95% CI 1.10 to 1.55; 15 RCTs, n = 2460; I^2 = 28%) (Analysis 3.3)
- Method of assisted hatching: no evidence indicates that the effect of AH differed between laser, chemical, and mechanical methods (P = 0.48) (Analysis 3.4). Chemical AH may improve slightly the clinical pregnancy rate when compared to no AH (OR 1.33, 95% CI 1.08 to 1.64; 11 RCTs, n = 1536; I^2 = 0%). Laser AH may improve slightly the clinical pregnancy rate when compared to no AH (OR 1.15, 95% CI 1.03 to 1.30; 23 RCTs, n = 5127; I^2 = 67%). We are uncertain of the effect of mechanical AH on the clinical pregnancy rate when compared to control (OR 1.30, 95% CI 0.89 to 1.88; 5 RCTs, n = 586; I^2 = 51%)
- Prognosis: some evidence suggests that the effect of AH differed between women in poor prognosis groups and women in good prognosis groups (P = 0.0009; fixed-effect model). When a random-effects model is used, the effect is less pronounced (P = 0.005) than with the fixed-effect model. In women with a good prognosis, we are uncertain of the effect of AH on the clinical pregnancy rate when compared with no AH (OR 1.10, 95% CI 0.94 to 1.29; 14 RCTs, n = 2721; I^2 = 0%). In women with a poor prognosis, AH may improve slightly the clinical pregnancy rate when compared with no AH (OR 1.68, 95% CI 1.38 to 2.04; 14 RCTs, n = 2108; I^2 = 25%) (Analysis 3.5)
- Extent of zona manipulation: some evidence shows that the effect of AH differed depending upon the extent of assisted hatching (P = 0.04). In women who had AH with thinning of the zona pellucida, we are uncertain of the effect on the clinical pregnancy rate when compared to no AH (OR 1.10, 95% CI 0.96 to 1.26; 17 RCTs, n = 3774; I^2 = 57%). In women who had AH with breech of the zona pellucida by a hole only, we are uncertain of the effect of AH on clinical pregnancy rate when compared with control (OR 1.17, 95% CI 0.98 to 1.39; 17 RCTs, n = 2543; I^2 = 46%). Only two studies used AH with complete removal of the zona pellucida (Isik 2000; Jelinkova 2002), and only one study used AH with expansion of the zona pellucida (Fang 2010) (Analysis 3.6)
- Type of embryos - fresh or frozen embryo: in fresh embryo groups: no evidence indicates that the effect of AH differed between fresh and frozen embryos (P = 0.58). AH may improve slightly the clinical pregnancy rate in women who had fresh embryo transfer when compared with no AH (OR 1.23, 95% CI 1.10 to 1.38; 30 RCTs, n = 5349; I^2 = 41%). We are uncertain of the effect of AH on the clinical pregnancy rate in women who had frozen embryo transfer when compared to no AH (OR 1.15, 95% CI 0.93 to 1.42; 10 RCTs, n = 1700; I^2 = 76%) (Analysis 3.7)

Sensitivity analysis

- Allocation concealment: limiting the analysis to trials that reported allocation concealment left only three trials (Abulsoud 2019; Elnahas 2017; González-Ortega 2015). Analysis of the data from these trials suggests that there was improvement in the clinical pregnancy rate in the AH group when compared to the no AH group (OR 2.28, 95% CI 1.59 to 3.27; n = 593; I^2 = 0%)
- Method of randomisation: 23 trials stated an acceptable method of randomisation. Analysis of the data from these trials suggests that there was improvement in the clinical pregnancy rate in the AH group compared to the no AH group (OR 1.16, 95% CI 1.03 to 1.30; n = 5050; I^2 = 63%)

Other analyses

We used a funnel plot to assess publication bias and small-study effects. The funnel plots are symmetrical (Figure 8 Figure 9).

Figure 8. Funnel plot of comparison: 3 Clinical pregnancy: assisted hatching compared with no assisted hatching, outcome: 3.1 Clinical pregnancy rate per woman randomised.

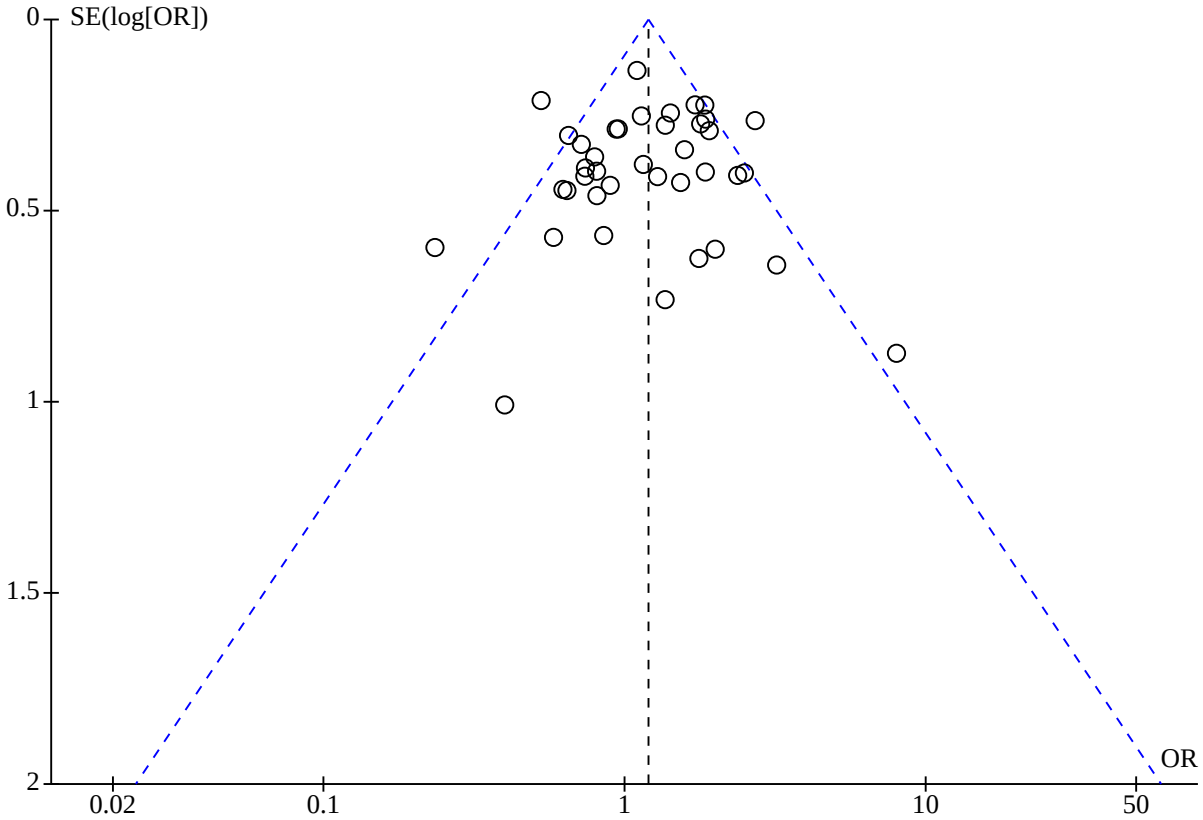
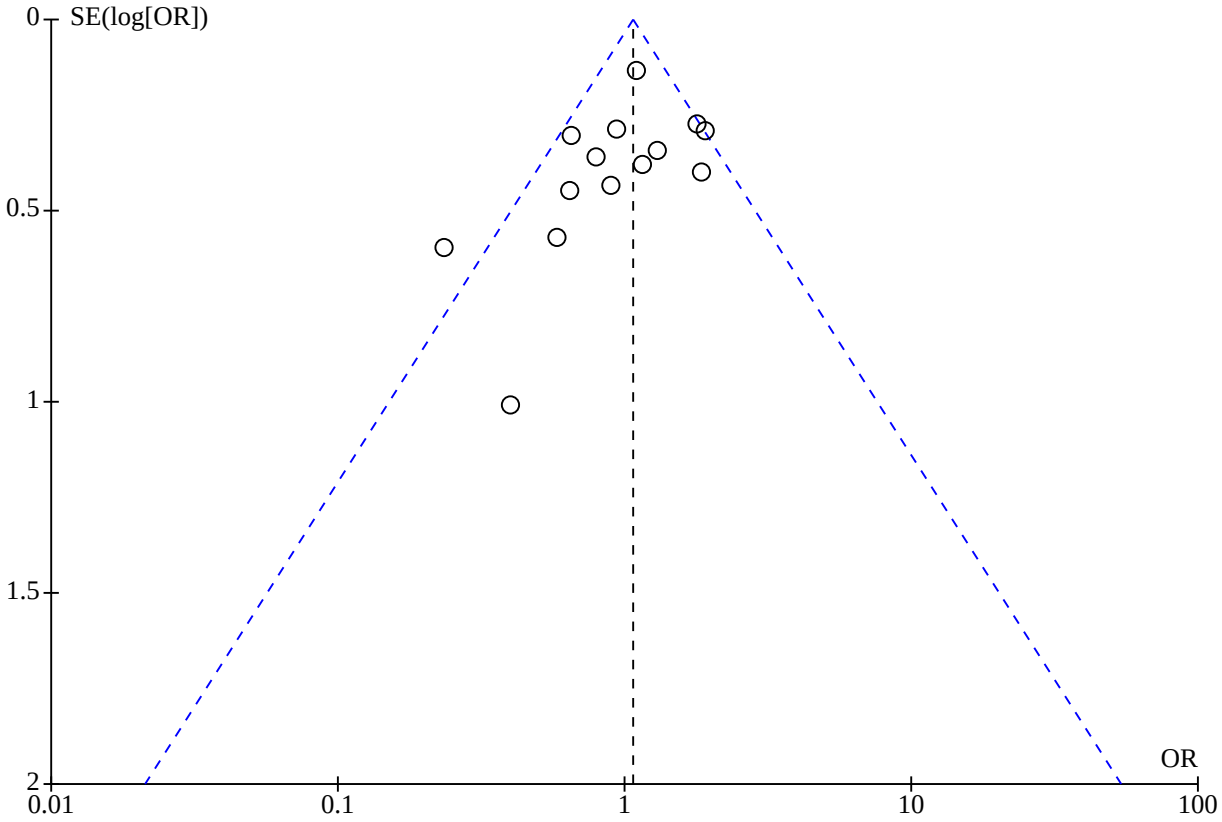


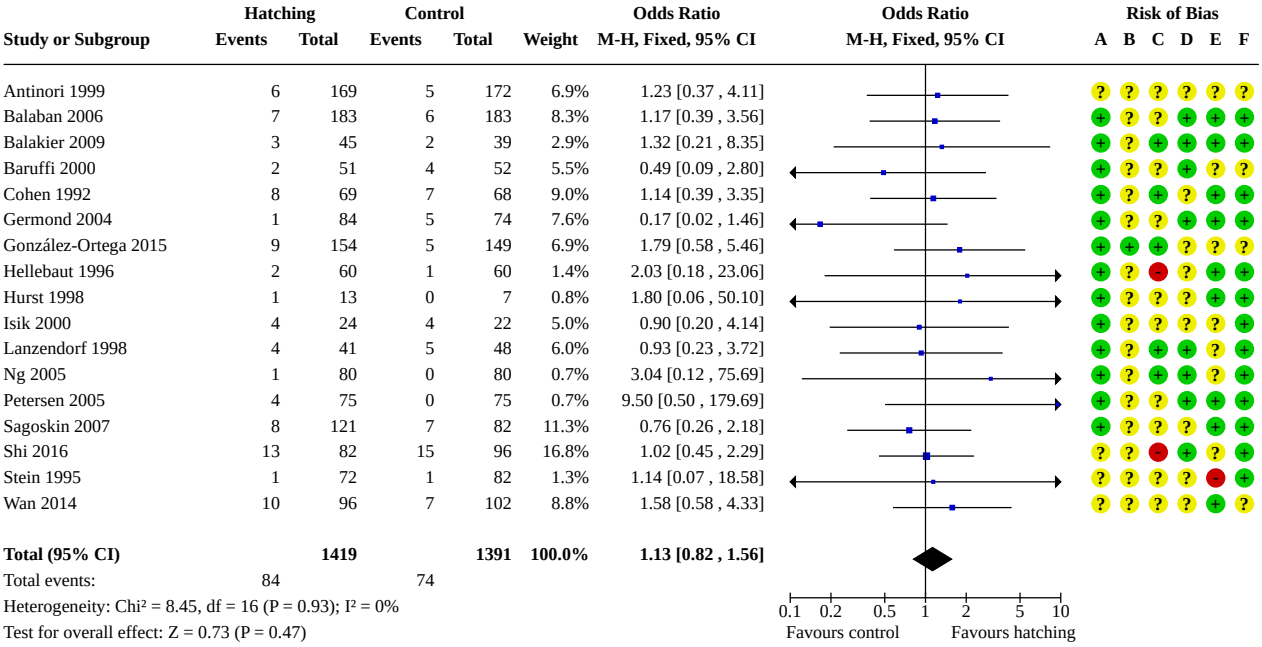
Figure 9. Funnel plot of comparison: 4 Clinical pregnancies in trials that reported live births: assisted hatching compared with no assisted hatching, outcome: 4.1 Clinical pregnancies in trials reporting live births.



Miscarriage per woman

Seventeen (17) trials reported miscarriage rates, accounting for 2810 women. Due to the quality of the evidence, we are uncertain about the difference in miscarriage rate among women who underwent AH compared with those who underwent no AH (OR 1.13, 95% CI 0.82 to 1.56; 17 RCTs, n = 2810; I^2 = 0%; very low-quality evidence; Figure 10; Analysis 5.1).

Figure 10. Forest plot of comparison: 3 Miscarriage rate, outcome: 3.1 Miscarriage per woman randomised.



Risk of bias legend

(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Incomplete outcome data (attrition bias)
(E) Selective reporting (reporting bias)
(F) Other bias

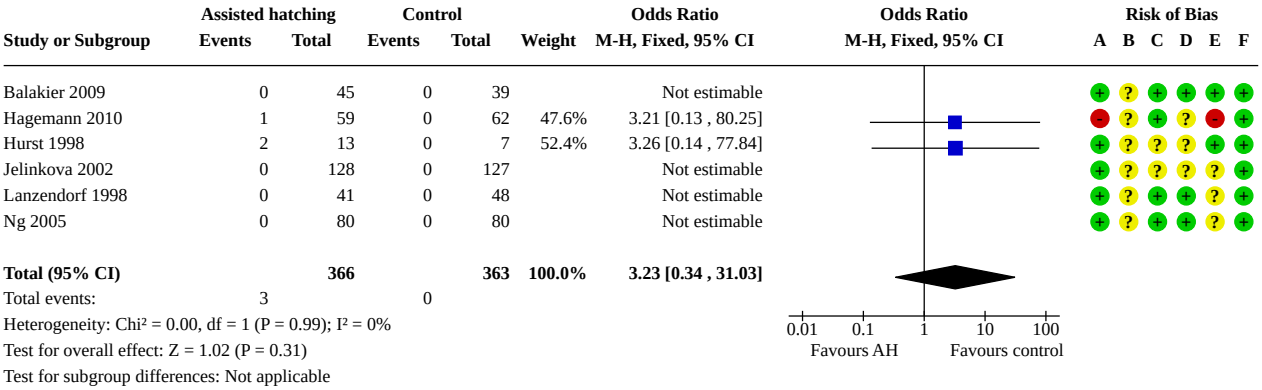
Subgroup analysis

- Number of attempts - first or repeat attempt at ART: no evidence indicates that the effect of AH differed between those undergoing their first or subsequent attempts (P = 0.18) (Analysis 5.2)
- Mode of conception - assisted conception procedure (IVF/ICSI): no evidence shows that the effect of AH differed between those undergoing IVF and those undergoing ICSI treatment (P = 0.90) (Analysis 5.3)
- Method of assisted hatching: no evidence suggests that the effect of AH differed between methods of AH (P = 1.00) (Analysis 5.4)
- Prognosis: no evidence indicates that the effect of AH differed between women in poor prognosis groups and women in good prognosis groups (P = 0.73) (Analysis 5.5)

Monozygotic twinning

Six trials reported data on monozygotic twinning (Figure 11). Hurst 1998 reported two monozygotic twins from three pregnancies in the AH group and none in the control group (0 from three pregnancies). Hagemann 2010 reported one case of monozygotic twins in the AH group also. Balakier 2009, Isik 2000, Jelinkova 2002, Lanzendorf 1998, and Ng 2005 reported absence of monozygotic twins in either group. There was an overall rate of 0.8% for the AH group and 0% for the control group (Analysis 6.1).

Figure 11. Forest plot of comparison: 5 Monozygotic twinning rate, outcome: 5.1 Monozygotic twinning per woman randomised.



Risk of bias legend
(A) Random sequence generation (selection bias)
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(F) Other bias

Ectopic pregnancy

Five trials reported ectopic pregnancy data. Lanzendorf 1998 reported one ectopic pregnancy in the control group and none in the AH group. Hagemann 2010, Hellebaut 1996, and Hurst 1998 reported absence of ectopic pregnancies. Shi 2016 reported one ectopic pregnancy each in the AH and control groups.

Congenital or chromosomal abnormalities

Two trials reported absence of congenital or chromosomal abnormalities (Hurst 1998; Lanzendorf 1998), and two trials reported fetal abnormalities in both AH and control groups (Hagemann 2010; Razi 2013).

Failure to transfer any embryos per woman

No trials reported data on this outcome.

Embryo damage

Three trials reported absence of embryo damage (Hurst 1998; Lanzendorf 1998; Stein 1995).

In vitro blastocyst development

No trials reported data on in vitro blastocyst development.

No further analyses were performed because of the paucity of data on these secondary outcomes.

Assessment of reporting bias

We produced funnel plots for the outcomes of live birth (Figure 5) and clinical pregnancy (Figure 8; Figure 9), and we did not find any strong suggestion of publication bias.

DISCUSSION

Summary of main results

Live birth

For this update, the primary outcome remained live birth rate. However only 14 of the 39 studies reported this outcome, representing 39% of all women randomised in the studies.

We are uncertain of the effects of assisted hatching (AH) on live birth rates when compared to no AH (Summary of findings 1). It is disappointing that the conclusions of this review are still limited by the paucity of available data since publication of the first Cochrane Review on this topic. Researchers still are not publishing the most important and sought after statistic on the impact of AH on assisted conception, namely, the 'live birth (take home baby rate)'. This reflects the gap that continues to exist between the practice of assisted conception and clinical obstetrics, with the absence of a central database of patient records that would facilitate follow-up of these women by authorised agencies. Moving forward, we hope that with publication of the modified CONSORT statement to improve reporting of fertility trials, the primary outcome measure of all fertility trials in the future will be live birth (Harbin Consensus Conference Workshop Group 2014). Reported live birth data in one-third of all studies suggest haste on the part of study authors to disseminate data limited to short-term outcomes, and for all intents and purposes, these data are incomplete.

Multiple pregnancy

AH may lead to a higher multiple pregnancy rate compared to no AH; furthermore, an increase in multiple pregnancies per clinical pregnancy has been noted (37% increase in odds ratio (OR)). Given this significance in combination with uncertainty about any evidence of an increase in success at achieving live birth with AH, we may need to consider the overall risks versus benefits of this technique.

Only half of the studies that report multiple pregnancy rates also report live birth data. This is unfortunate as it limits interpretation of results, given this high multiple pregnancy rate, because as many as 5% of multiple pregnancies are lost at between 20 and 40 weeks' gestation. In addition, many studies were transferring two to four embryos, although the numbers transferred were balanced between groups. The increase in multiple pregnancies can be attributed to an increase in implantation rates resulting in higher pregnancy rates or monozygotic twinning, or both, with AH. This must be taken into consideration in planning this procedure.

It is likely that reducing to one the number of embryos transferred will not completely eliminate monozygotic twinning. Implantation rate was not considered as an outcome in this update for two reasons. Pooling of embryo implantation data for meta-analysis is statistically problematic. Implantation is traditionally expressed 'per embryo transferred', without regard for the number of women. However, more than one embryo is normally transferred per woman, resulting in an embryo clustering effect and necessitating more advanced analysis to render the results meaningful. A statistically valid and easier approach is to express implantation 'per woman randomised'. This also confers the advantage of being more useful in aiding understanding of resulting live births. This approach requires, however, that the number of women with at least one gestation sac is reported, which is not the case in practice.

Clinical pregnancy

All 39 included trials reported on clinical pregnancy. Similar to the previous update (in 2012), this update suggests that overall, AH may slightly improve the chance of achieving a clinical pregnancy, but these results are less reliable because of high risk of bias and dependence upon the statistical model. Subgroup analysis supports these results.

When analysis of clinical pregnancy rate was restricted to those trials that went on to report live birth, the clinical pregnancy result showed little to no difference in effect between AH and control groups.

Miscarriage

We are uncertain of the effect of AH on miscarriage rates when compared to controls.

Other outcomes

The impact of AH on ectopic pregnancy, congenital and chromosomal abnormalities, blastocyst formation, and embryo damage could unfortunately not be determined by this review because of the paucity of available data. This is disappointing as it leaves many unanswered questions about perceived risks of the procedure, from embryo damage to chromosomal and congenital abnormalities.

Overall completeness and applicability of evidence

A large number of trials were incorporated into this review, involving a large sample size. The results of 7249 women in 39 trials are included in this review, leading to a low to very low level of evidence. Failure of many trials to report on primary outcomes (live birth, multiple pregnancy) and variable levels of reporting on other outcomes allow potential bias to be introduced into the analysis. This calls for standardised outcome reporting for future assisted conception trials as discussed.

Quality of the evidence

The quality of the evidence is low to very low. The main limitations are serious risk of bias associated with poor reporting of study methods, inconsistency, imprecision, and publication bias (Summary of findings 1).

Potential biases in the review process

Three review authors (LL, SH, and MAA) with varying levels of expertise undertook the search process several times to minimise the risk of bias introduced by review authors; they had no conflicts of interest.

We were unable to get responses from authors of various studies when abstracts were published and we had requested relevant or additional data. These studies could potentially have been included in the review.

Agreements and disagreements with other studies or reviews

Overall, the addition of eight new trials to this update has not changed the findings regarding live birth that were reported in previous reviews, namely, that no current evidence suggests that AH increases the chances of a live birth.

Clinical pregnancy rate may improve slightly in women undergoing AH, but these results are not reliable and robust.

Three recent non-Cochrane systematic reviews have been published (He 2018; Li 2016; Zeng 2018). Li 2016 used random-effects models for their meta-analysis. They suggested that there is an increase in clinical pregnancy and multiple pregnancy rates with AH when compared to control. We agree with these findings generally, but Li 2016 did not present clear assessment of the quality and robustness of evidence related to these outcome improvements. Review authors suggested there was no improvement in live birth rates and no difference in miscarriage rates between AH and control groups. Li 2016 reported clinical pregnancy in 36 RCTs; however, this present Cochrane Review included 39 RCTs reporting clinical pregnancy (some different from the studies included by Li 2016), One RCT - Urman 2002 - which is reported in Li 2016 - was excluded from the previous published Cochrane Review due to inadequate methods of allocation. He 2018 published a systematic review about AH that focused on a population of women older than 35 years of age. These review authors similarly demonstrated no increase in live birth rate or miscarriage rate with AH compared to control. Conversely, they demonstrated no increase in multiple pregnancy and no improvement in clinical pregnancy rate with AH compared to control. Our Cochrane Review did not specifically look at this subgroup, but this population was incorporated into our poor prognosis subgroup. Our data for the poor prognosis subgroup suggest that AH may improve slightly clinical pregnancy and may increase multiple pregnancy when compared to control. Zeng 2018 examined laser AH only in cryopreserved embryos. Their analysis led to the conclusions that AH improved clinical pregnancy rates and increased multiple pregnancy rates when compared to control but led to no difference in live birth rate and miscarriage rate. Our subgroup analysis of cryopreserved embryos includes all methods of AH. Our results suggest that AH makes little to no difference in live birth, clinical pregnancy, or multiple pregnancy when compared to control in this subgroup. Zeng 2018 included studies that reported

data outcomes per embryo transfer rather than per woman; this could explain in part the differences in results.

AUTHORS' CONCLUSIONS

Implications for practice

This update suggests that we are uncertain of the effect of assisted hatching (AH) on improving live birth rate, but it may slightly improve the chance of achieving a clinical pregnancy. However this result is not robust. The increase in clinical pregnancy rate is slightly higher in women with poor prognosis including those with previously failed in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI). Most trials still fail to report on live birth rates. Low-quality evidence suggests increased risk of multiple pregnancy with assisted hatching, and very low-quality evidence suggests that AH does not increase the miscarriage rate.

Implications for research

This review once again highlights a wide range of currently unresolved issues that provide potential avenues for future research, including the need for high-quality trials that report live births, clinical pregnancies, and adverse events (including multiple pregnancies, miscarriages, and long-term adverse outcomes) and are powered to investigate effects in clinical subgroups.

The potential of assisted hatching in assisted conception makes it imperative that studies of high methodological quality (preferably multi-centre trials of appropriate design, adequate power, and appropriate duration of follow-up) are undertaken to provide these urgently needed answers; such studies should be funded only if they report the important primary outcome measures of live birth and multiple pregnancy.

ACKNOWLEDGEMENTS

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We wish to thank Dr. Demián Glujovsky for translating González-Ortega et al., 2015 (published in Spanish) and for extracting data from the paper for the purposes of this review.

We wish to thank Dr. Jasmine Lee for translating Lu et al., 2016 (published in Chinese) and for extracting data for the purposes of this review.

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We acknowledge the editorial board of the Cochrane Gynaecology and Fertility Group for support and advice provided.

We acknowledge the significant contributions of others to the original review and the updates that followed, including previous review authors, S Dias and Prof C Farquhar.

Edmond Edi-Osagie contributed to the designing and worked on the original review.

Lee Hooper developed the second search strategy, undertook the February 2002 searches, and screened these search results, the late Phil McGinlay contributed to designing the original review, and we acknowledge the contributions of Sarah-Kate Carney and Linsey Nelson.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Abulsoud 2019

Study characteristics	
Methods	Randomised into 2 groups using sealed envelopes but no further details
	No information on random sequence generation
	Single centre
	Unclear if participants were blinded
	Unclear if outcome assessor was blinded
	No power calculation was documented
	Published full paper
Participants	130 patients attending a private IVF centre in Cairo, Egypt, undergoing fresh ICSI using Day 3 embryos. Age ≥ 38 years, requiring ≥ 375 IU of gonadotrophin per day, with previously failed ICSI
	Mean age: control group 39.5, AH group 39.2
Interventions	LAH 3 hours before embryo transfer, thinning of zona pellucida until 25% irradiated
	Control – 65 women
	LAH – 65 women
Outcomes	Chemical pregnancy (defined as bhCG > 25 on blood 14 days after ET)
	Clinical pregnancy (defined as presence of FH 7 weeks after ET)
Notes	Mean number of embryos transferred: control 2.5, LAH 2.7
Risk of bias	

Bias	**Authors' judgement**	**Support for judgement**

Abulsoud 2019 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Sealed envelopes used
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No information given about whether participants or outcome assessors were blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients had outcomes reported
Selective reporting (reporting bias)	High risk	No reporting of miscarriage, multiple pregnancy, or live birth
Other bias	Unclear risk	No information

Antinori 1999

Study characteristics	
Methods	Randomisation stated but method unclear Allocation concealment unclear Unclear if single centre/multi-centre Unclear whether participants and assessors were blinded Unclear whether power calculation performed ITT analysis unclear Published as abstract
Participants	341 women from Italy undergoing IVF. Subgrouped by previous IVF experience: (a) without previous IVF experience (n = 199) or (b) with more than 6 previous IVF failures (n = 142) Mean age: control group 27.0, AH group 27.5 years
Interventions	AH (laser; complete zona breach; unclear how long from egg retrieval to AH; unclear how long from AH to transfer) - 169 women randomised, 221 embryos transferred (estimated) vs Control - no AH - 172 women randomised, 247 embryos transferred (estimated)
Outcomes	Clinical pregnancy, miscarriage, multiple pregnancy
Notes	No reply No. of embryos transferred: AH 2.3, control 2.4

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomisation stated but method unclear

Antinori 1999 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Unclear; no details provided
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear whether participants and assessors were blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No evidence of post-randomisation exclusions but timing of allocation unclear
Selective reporting (reporting bias)	Unclear risk	Protocol not viewed. Not sure if these are all planned outcomes
Other bias	Unclear risk	Conference abstract

Balaban 2006

Study characteristics	
Methods	Randomisation by computer-generated numbers
Participants	366 women from Turkey undergoing ICSI treatment only Exclusion: women undergoing IVF
Interventions	AH (laser thinning) (n = 183) vs No AH (laser thinning) (n = 183) Unclear on how long before transfer, frozen-thawed embryos only
Outcomes	Primary: implantation rate Secondary: clinical pregnancy, miscarriage, multiple pregnancy rate
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation by computer-generated numbers
Allocation concealment (selection bias)	Unclear risk	No details in text
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details on blinding
Incomplete outcome data (attrition bias)	Low risk	No losses to follow-up and all women analysed

Balaban 2006 *(Continued)*

All outcomes		
Selective reporting (reporting bias)	Low risk	All outcomes listed in the methods section reported
Other bias	Low risk	None identified

Balakier 2009

Study characteristics		
Methods	Single centre Unclear whether power calculation performed ITT analysis unclear Published as full paper	
Participants	84 women from Canada with no more than 1 unsuccessful previous IVF attempt, ≤ 37 years of age, Day 3 FSH ≤ 10 mIU/mL Mean age: control: 33.8 ± 3.2; AH: 32.5 ± 3.8 54 women underwent their first IVF cycle; the other 30 (13 AH) their second cycle	
Interventions	Laser-assisted thinning (n = 45): total length of laser cut was approximately 30 to 40 µm, and about 60% to 80% of the outer layer of the zona pellucida was thinned without complete breaching, applying 2 ms laser beams vs Control (n = 39)	
Outcomes	Clinical pregnancy; multiple pregnancies; spontaneous miscarriage; live birth	
Notes		
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation list
Allocation concealment (selection bias)	Unclear risk	No details in text
Blinding (performance bias and detection bias) All outcomes	Low risk	Study was double-blinded to patients and medical personnel
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up and all women analysed
Selective reporting (reporting bias)	Low risk	Live birth reported
Other bias	Low risk	None identified

Baruffi 2000

Study characteristics	
Methods	Single centre Unclear whether power calculation performed ITT analysis unclear Published as full paper
Participants	103 women from Brazil aged 37 years or younger, undergoing ICSI for the first time. Mean zona thickness: control group 17.1 µm (SD 1.7); AH 16.6 µm (SD 2.2). Mean age: control group 31.4 (3.6); AH group 31.8 (3.6)
Interventions	AH (laser; thinning partial; 48 hour egg retrieval to AH; 0 hour AH to transfer), 51 women randomised, 141 embryos transferred vs No AH, 52 women randomised, 149 embryos transferred
Outcomes	Implantation, clinical pregnancy, miscarriage
Notes	No reply No. of embryos transferred: AH 2.76; control 2.87

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Women were selected at random via a randomisation table
Allocation concealment (selection bias)	Unclear risk	No information in the text
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No information in the text
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up and all women analysed
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth not reported, so not sure it was planned
Other bias	Unclear risk	No reply from authors - see notes

Carter 2003

Study characteristics	
Methods	Single centre Unclear whether power calculation performed Published as abstract; study authors provided additional information

Carter 2003 *(Continued)*

Participants	203 women from fertility clinic in USA Age < 40 years FSH < 10, ovulatory menstrual cycles, day 3 ET with good embryo quality Women with more than 1 failed IVF cycle excluded
Interventions	Laser hatching (n = 121) vs No hatching (n = 82)
Outcomes	Clinical pregnancy rate, multiple pregnancy rate
Notes	Additional information provided by study authors Dropouts included for the denominator in this review No. of embryos: AH 2.2; control 2.1

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised by computer generation on Day 3
Allocation concealment (selection bias)	Unclear risk	Unclear; no details provided
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not stated but included dropouts
Selective reporting (reporting bias)	High risk	This was a conference abstract only and was not published as a full paper, although study authors did provide additional information. Live birth was not reported
Other bias	Unclear risk	Conference abstract

Ciray 2005

Study characteristics

Methods	Single centre Power calculation not reported ITT analysis not stated Published as full paper
Participants	114 women from Turkey undergoing ART for ASRM grade 3 to 4 endometriosis only (poor prognosis) Age < 40 years; AH group 33.1 (4.2); control group 34.0 (3.7) Basal FSH: AH group 7.4 (3.5); control group 9.0 (5.1)
Interventions	Laser hatching (thinning to a quarter), 76 women randomised, 146 embryos transferred (16 cancelled)

Ciray 2005 *(Continued)*

	vs No hatching, only fresh embryo transfer cycles, 38 women randomised, 72 embryos transferred (8 cancelled)
Outcomes	Clinical pregnancy rate, implantation rate
Notes	No. of embryos: AH 2.4; control 2.4

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised method stated 2:1 date, with the aid of computer programme
Allocation concealment (selection bias)	Unclear risk	Unclear; no details
Blinding (performance bias and detection bias) All outcomes	High risk	No evidence of blinding of participants or assessors
Incomplete outcome data (attrition bias) All outcomes	Low risk	All women appear to have been analysed
Selective reporting (reporting bias)	Unclear risk	All outcomes reported but original protocol not viewed; live birth not reported
Other bias	Low risk	None identified

Cohen 1992

Study characteristics

Methods	Single centre Unclear whether power calculation performed ITT analysis unclear Published as full paper
Participants	330 women from North America undergoing IVF Mean age: control group 36.7 (3.7); AH group 36.5 (3.3)
Interventions	AH by acid Tyrode's (chemical; complete zona breach hole; 68 to 72 hour egg retrieval to AH; 4 to 8 hours AH to transfer), 69 women with FSH < 15 (trial 1), 80 women with poor prognosis (trial 2, thick zona pellucida, low developmental rate, excessive fragmentation), 15 women with FSH > 15 (trial 3) No AH, 68 women with FSH < 15 (trial 1), 83 women with poor prognosis (trial 2, thick zona pellucida, low developmental rate, excessive fragmentation), 15 women with FSH > 15 (trial 3)
Outcomes	Implantation, clinical pregnancy (rates given for trials 1, 2, and 3), live births (rates given for women in trial 1 only), multiple pregnancy (rates given for women in trials 1 and 2 only)
Notes	Attempted to contact author about this study. Reply received, but no additional information offered

Cohen 1992 *(Continued)*

	No. of embryos: AH 3.5; control 3.4

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Pre-printed randomisation list
Allocation concealment (selection bias)	Unclear risk	Unclear
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants blinded Assessor blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No evidence of post-randomisation exclusions, but timing of allocation unclear
Selective reporting (reporting bias)	Low risk	Original protocol not viewed. Live birth reported
Other bias	Low risk	None identified

Elhelw 2005

Study characteristics

Methods	Power calculation not reported ITT not stated Published as abstract only
Participants	74 women from Egypt undergoing ICSI only Poor prognosis Previous 2 implantation failures Cryo-thaw cycles only
Interventions	Laser hatching (thinning to quarter) vs no hatching. AH done 1 hour before embryo transfer AH: 37 women randomised, 121 embryos transferred Control: 37 women randomised, 130 embryos transferred
Outcomes	Implantation rate, clinical pregnancy rate
Notes	No author contact as all details in the article No data on no. of embryos transferred

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not stated; no details

Elhelw 2005 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Not clear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participant blinding unclear Assessor blinding unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No details
Selective reporting (reporting bias)	High risk	Conference abstract only. No evidence of a full paper. Live birth not reported
Other bias	Unclear risk	Conference abstract

Elnahas 2017

Study characteristics	
Methods	Randomised into 2 groups on day of ET using sealed envelope but no further details No information on random sequence generation No information about centres Unclear whether participants were blinded Unclear whether outcome assessor blinded No power calculation documented Published full paper
Participants	160 patients undergoing frozen ET after IVF using Day 3 embryos. Age 18 to 40 years, following first IVF pregnancy, following 1 implantation failure, or following postponement of transfer to avoid sequelae of OHSS Only included excellent (≥ 8 cells and fragmentation < 10%) or good (≥ 8 cells and fragmentation between 10% and 20%) quality Day 3 embryos Mean age: control group 31.7, AH group 31.0
Interventions	LAH 1 hour before embryo transfer, thinning of zona pellucida at only one-eighth of its surface (no breaching) Control – 80 women LAH – 80 women
Outcomes	Clinical pregnancy (defined by transvaginal ultrasound scan on fourth and sixth weeks to detect IU GS and fetal pulsations)
Notes	Mean age: LAH group 31.02, non-LAH group 31.71

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Elnahas 2017 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	No information given
Allocation concealment (selection bias)	Low risk	Sealed envelopes used
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No information about clinician or participant blinding nor blinding of outcome assessors
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all patients
Selective reporting (reporting bias)	High risk	No multiple pregnancy, miscarriage, or live birth data given. Implantation rates given as percentages only

Fang 2010

Study characteristics	
Methods	Single-centre randomised controlled trial
Participants	125 women in China who had their first IVF/ICSI cycles between 2006 and 2008, with fresh IVF-ET failures or without fresh embryo transfers Mean age: 32.3 in AH group, 32.1 in control group Setting: fertility centre, China (2006 to 2008)
Interventions	Mechanical assisted hatching: expanding/stretching zona pellucida via injected hydrostatic pressure AH: 61 women, 178 embryos Control: 64 women, 190 embryos
Outcomes	Clinical pregnancy, implantation rates
Notes	Unclear whether power calculation performed ITT analysis unclear Published as full paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear whether participants were blinded

Fang 2010 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth not reported, so not sure it was planned
Other bias	Low risk	None identified

Ge 2008

Study characteristics	
Methods	Randomised controlled trial
Participants	760 women from China having IVF with fewer than 5 failed cycles of ART with normal baseline FSH concentration. Those participants with uterine abnormality or low fertilisation capacity (rate of fertilisation < 20% and late ICSI following fertilisation failure of IVF) were excluded Mean age: fresh, 31.08 AH, 30.44 control; frozen, 31.84 AH, 30.66 control
Interventions	Laser thinning to about 50% of initial ZP thickness AH: 387 women with fresh embryos, 100 women with frozen-thawed embryos Control: 373 women with fresh embryos, 100 women with frozen-thawed embryos
Outcomes	Implantation rate, pregnancy rate, live birth
Notes	Unclear whether power calculation performed ITT not stated Published as full paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Women were randomised according to a randomisation list based on sequential numbers in sealed envelopes
Allocation concealment (selection bias)	Unclear risk	Unclear allocation
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinded both clinicians and patient
Incomplete outcome data (attrition bias) All outcomes	Low risk	Fresh embryo transfer cycles: total of 831 IVF/ICSI cycles were performed during the study period. Of these, 772 met the inclusion criteria, but 12 participants abandoned embryo transfer for various reasons such as avoiding potential risks of ovarian hyperstimulation syndrome Frozen-thawed embryo transfer: total of 245 frozen-thawed cycles were also performed, of which 45 were excluded because they did not meet the criteria of the study or because embryo transfer was abandoned

Ge 2008 *(Continued)*

Selective reporting (reporting bias)	Low risk	Original protocol not viewed. Live birth reported
Other bias	Low risk	None identified

Germond 2004

Study characteristics	
Methods	Multi-centre RCT
Participants	153 women in 4 European IVF centres between 20 and 45 years old, with ≥ 1 functional ovary, normal FSH and prolactin levels, no clinically significant findings within 6 months before starting treatment, and normal uterine cavity
Interventions	Laser assisted hatching using diode laser AH: 56 women undergoing first cycle of frozen-thawed embryos, 23 women who had a poor prognosis using fresh embryos Control: 53 women undergoing first cycle of frozen-thawed embryos, 21 women who had a poor prognosis using fresh embryos
Outcomes	Clinical pregnancies, live births, miscarriages, multiple pregnancies
Notes	Power calculation performed ITT not stated Published as full paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Women were randomised according to a randomisation list
Allocation concealment (selection bias)	Unclear risk	Not stated in the text
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated in the text
Incomplete outcome data (attrition bias) All outcomes	Low risk	No evidence of attrition bias
Selective reporting (reporting bias)	Low risk	Live birth reported
Other bias	Low risk	None identified

González-Ortega 2015

Study characteristics	
Methods	Prospective randomised study IVF and ICSI Repeat cycle January 2008 till June 2010
Participants	Inclusion criteria: ≥ 38 years old, basal FSH ≥ 12.0 mUI/mL, ≥ 2 failed IVF-ICSI cycles with ≥ 6 good quality embryos already transferred, with adequate endometrial receptiveness, with atraumatic embryo transfers Exclusion criteria: frozen-thawed embryo transfers, egg donation cycles, fewer than 2 growing follicles, bad quality embryos, non-receptive endometrium, traumatic embryo transfer
Interventions	Timing of assisted hatching: ≥ 1 hour before embryo transfer (on Days 2 and 3) Method of assisted hatching: laser
Outcomes	Clinical pregnancy rate, multiple pregnancy rate, miscarriage rate
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Correspondence from study author - computer-generated randomisation
Allocation concealment (selection bias)	Low risk	Correspondence from study author - sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Correspondence from study author - clinicians and patients blinded to allocation
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No losses reported
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth not reported, so not sure it was planned
Other bias	Unclear risk	Not enough information provided

Hagemann 2010

Study characteristics	
Methods	Randomised single-centre cross-over trial
Participants	103 women in the United States younger than 38 years of age with any embryo with zona pellucida thickness > 13 µm and more than 2 previously failed IVF cycles

Hagemann 2010 *(Continued)*

	Mean age: 32.1 years in hatched group, 31.2 in unhatched group
Interventions	AH performed by acidic Tyrode's solution AH: 49 women Control: 54 women
Outcomes	Clinical intrauterine pregnancy rate, implantation rate, spontaneous pregnancy loss, live birth rate
Notes	Power calculation: study states it has inadequate power. Study as ultimately performed had sufficient statistical power to identify only a 30% absolute effect size with alpha = 0.05 and beta = 0.80 ITT analysis unclear Published as full paper

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Randomisation was performed by IVF lab staff by drawing 1 of 200 opaque envelopes from a box
Allocation concealment (selection bias)	Unclear risk	Opaque envelopes drawn but not numbered
Blinding (performance bias and detection bias) All outcomes	Low risk	Study arm to which participants belonged was blinded to caregivers, with the exception of IVF embryologists, as well as to participants
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear
Selective reporting (reporting bias)	High risk	Live birth reported (but results not included in this study, as results were given only for both cycles combined, not for just the first cycle; these are the data we are using). No other evidence of reporting bias
Other bias	Low risk	None identified

Hellebaut 1996

Study characteristics

Methods	Randomised single-centre trial
Participants	120 women from Belgium undergoing IVF or ICSI Mean age: control group 30.8 (3.9); AH group 30.9 (4.3) years
Interventions	AH (mechanical; complete zona breach hole; 48 hour egg retrieval to AH; 0.2 hour AH to transfer) vs no AH AH: 60 women randomised, 168 embryos transferred Control: 60 women randomised, 162 embryos transferred
Outcomes	Implantation, clinical pregnancy, live birth, miscarriage, ectopic pregnancy

Hellebaut 1996 *(Continued)*

Notes	Attempted to contact author about this study. A reply including much useful additional information was received No. of embryos transferred: AH 2.8 (0.6); control 2.7 (0.6) Unclear whether power calculation performed ITT analysis unclear Published as full paper

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	By computer on day of transfer
Allocation concealment (selection bias)	Unclear risk	Allocation concealment unclear
Blinding (performance bias and detection bias) All outcomes	High risk	Participants not blinded Assessor not blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	Low risk	Live birth reported. Study authors responded to requests for details. No other evidence of bias; all outcomes stated were reported
Other bias	Low risk	None identified

Hurst 1998

Study characteristics

Methods	Single-centre randomised trial
Participants	20 women from North America undergoing IVF with no prior IVF (30 years or less, FSH < 10 IU/L, normal endometrium and sperm) or with prior IVF (35 years or less, 6 embryos, 50% fertilisation, normal endometrium). Mean age: control group 30 (0.8); AH group 30 (0.9)
Interventions	AH by acid Tyrode's (chemical; complete zona breach hole; ? hour egg retrieval to AH; ? hour AH to transfer) vs no AH AH: 13 women randomised, 52 embryos transferred Control: 7 women randomised, 28 embryos transferred
Outcomes	Implantation, clinical pregnancy, live births
Notes	Attempted to contact author about this study. A reply including much useful additional information was received No of embryos transferred: AH 4.0; control 4.0 Unclear whether power calculation performed ITT analysis unclear Published as full paper

Hurst 1998 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-randomised
Allocation concealment (selection bias)	Unclear risk	Unclear; no details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	Low risk	Protocol not viewed but outcomes reported including live birth
Other bias	Low risk	None identified

Isik 2000

Study characteristics	
Methods	Single-centre randomised trial
Participants	46 women from Turkey with > 5 Day 3 cleavage stage embryos (FSH at Day 3: control 6.1 (3.0); AH 5.5 (1.4) IU/L) undergoing ICSI Mean duration of infertility: 6.7 years Mean age: control group 29.1 (3.6); AH group 30.5 (5.2) years
Interventions	AH enzymatic (chemical; complete and total zona breach; 120 to 144 hour egg retrieval to AH; 0.5 to 1 hour AH to transfer) vs no AH AH: 24 women randomised, 71 embryos transferred Control: 22 women randomised, 63 embryos transferred
Outcomes	Implantation
Notes	Study author response No. of embryos transferred, blastocyst transfer: AH 2.95 (0.9); control 2.86 (0.8) Unclear whether power calculation performed ITT analysis unclear Published as full paper

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random number table used on Day 3

Isik 2000 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Allocation unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not stated
Selective reporting (reporting bias)	Unclear risk	Protocol not viewed; however live birth not reported
Other bias	Low risk	None identified

Isiklar 1999

Study characteristics	
Methods	Single-centre randomised trial
Participants	44 women from Turkey undergoing IVF Mean age not stated
Interventions	AH (mechanical; complete zona breach; ? hour egg retrieval to AH; ? hour AH to transfer) vs no AH AH: 22 women randomised, 83 embryos transferred Control: 22 women randomised, 78 embryos transferred
Outcomes	Implantation, clinical pregnancy, multiple pregnancy
Notes	Attempted to contact author about this study No. of embryos transferred: AH 3.7; control 3.5 Unclear whether power calculation performed ITT analysis unclear Published as abstract

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomised
Allocation concealment (selection bias)	Unclear risk	Allocation concealment unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear whether participants and assessor were blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear

Isiklar 1999 *(Continued)*

Selective reporting (reporting bias)	Unclear risk	This publication was in abstract form only, no full paper publication was identified. Study authors do not report on live birth
Other bias	Unclear risk	Conference abstract

Jelinkova 2002

Study characteristics	
Methods	Single-centre randomised trial
Participants	255 IVF participants only; at least 2 previous failures Age: AH 32.3 (4.24), control 32.1 (3.16) Germany
Interventions	AH (chemical removal by acid; complete zona breach) AH: 128 women Control: 127 women
Outcomes	Clinical pregnancy rate, implantation rate
Notes	Attempted to contact author about this study No. of embryos transferred: AH 2.2; control 2.2

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation stated
Allocation concealment (selection bias)	Unclear risk	Unclear; no details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear whether participants were blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not stated
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth not reported, so not sure if it was planned
Other bias	Low risk	None identified

Kutlu 2010

Study characteristics	

Kutlu 2010 *(Continued)*

Methods	Single-centre randomised trial
Participants	252 infertile couples receiving ART treatment at Medicana Camlica Hospital, Istanbul, Turkey. Sub-grouped by prognosis: poor (n = 113) or good (n = 139)
Interventions	AH was performed by laser method AH: 73 women younger than 35 years, 58 women aged 35 or over Control: 66 women younger than 35 years, 55 women aged 35 or over
Outcomes	Clinical pregnancy rate, implantation rate
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed in a computerised manner
Allocation concealment (selection bias)	Unclear risk	Not stated within the text
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated within the text
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth not reported, so not sure if it was planned
Other bias	Low risk	None identified

Laffoon 1999

Study characteristics

Methods	Single-centre randomised trial
Participants	56 women from North America younger than 40 years undergoing IVF. Mean age not stated
Interventions	AH (mechanical; complete zona breach; ? hour egg retrieval to AH; ? hour AH to transfer) vs no AH AH: 28 women randomised, embryos transferred not stated Control: 28 women randomised, embryos transferred not stated
Outcomes	Clinical pregnancy
Notes	Attempted to contact author about this study No. of embryos transferred not stated

Laffoon 1999 *(Continued)*

	Unclear whether power calculation performed ITT analysis unclear Published as abstract

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Timing and method not stated
Allocation concealment (selection bias)	Unclear risk	Allocation concealment unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	High risk	Published as a conference abstract. Unable to find a full paper publication. Live birth not reported
Other bias	Unclear risk	Conference abstract

Lanzendorf 1998

Study characteristics

Methods	Single-centre randomised trial
Participants	94 women from North America 36 years of age or older (mean basal FSH: control 7.6 IU/L (SD 2.0); AH 7.9 IU/L (SD 2.5)), undergoing IVF (some with ICSI), half previously treated with IVF Mean age: control 38.5 (0.26); AH 38.3 (0.31)
Interventions	AH by acid Tyrode's (chemical; complete zona breach; 55 hour egg retrieval to AH; ? hour AH to transfer) vs no AH AH: 42 women randomised, 180 embryos transferred Control: 52 women randomised, 212 embryos transferred
Outcomes	Implantation, clinical pregnancy, multiple pregnancy, live births
Notes	Attempted to contact author about this study. A reply including much useful additional information was received No. of embryos stated: AH 4.4; control 4.4 Unclear whether power calculation performed ITT analysis performed

Risk of bias

Bias	Authors' judgement	Support for judgement

Lanzendorf 1998 *(Continued)*

Random sequence generation (selection bias)	Low risk	Randomised; method stated
Allocation concealment (selection bias)	Unclear risk	Allocation concealment via sealed envelopes on day of aspiration
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants blinded Assessor blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	ITT analysis performed
Selective reporting (reporting bias)	Unclear risk	No evidence of selective reporting, although original protocol not viewed. Study authors did report on live birth
Other bias	Low risk	None identified

Nada 2018

Study characteristics	
Methods	Randomisation – computer-generated list of random numbers but block randomisation with block size of 4 – allocation concealment unclear Participant blinding unclear Conducted at 2 private centres in Cairo and Beni Suif Participants not blinded or unclear Full article
Participants	326 women Only high-quality Day 3 embryos were used (defined as 8 to 10 cells on Day 3, < 15% fragmentation, absence of multi-nucleation, symmetrical blastomeres, absence of ZP dysmorphism, absence of perivitelline space granularity, colourless cytoplasm with moderate granulation, no inclusions)
Interventions	LAH to dissolve 25% to 30% of ZP with 3 adjacent pulses of the laser LAH – 163 patients (5 with cycle cancellation) – 158 remaining No LAH – 163 patients (13 with cycle cancellation) remaining LAH just before transfer (no further information given)
Outcomes	Live birth rate Clinical pregnancy (defined as serum hCG > 20 IU/L and TVS confirming GS with pulsating fetal pole 4 weeks post transfer or 6 weeks post menstrual) Implantation rate (per embryo transferred, defined as number of GS present on TV USS 4 weeks after transfer/number of embryos transferred)
Notes	2 to 3 embryos transferred per cycle Sample size calculation performed (presumed pregnancy rate of 57% and 40% in control)

Nada 2018 *(Continued)*

	ITT analysis Mean age: LAH 31.27, control 32.64 Study registered on Pan African Clinical Trials Registry: PACTR201602001467322

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated list of random numbers
Allocation concealment (selection bias)	Unclear risk	Block randomisation with block size of 4 with 1:1 ratio of LAH vs control but no further information
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Assessor blinded to allocation No information about participants or other personnel
Incomplete outcome data (attrition bias) All outcomes	High risk	18 participants excluded after randomisation due to cycle cancellation – no reasons for cancellation given
Selective reporting (reporting bias)	High risk	Multiple pregnancy rate given with denominator per embryo transferred
Other bias	Unclear risk	No information Although registered on Pan African Clinical Trials Registry, no primary or secondary outcomes stated in trial information

Nagy 1999

Study characteristics

Methods	Single-centre randomised trial
Participants	38 women from Italy with cryopreserved embryos undergoing IVF and ICSI Mean age: control group 31.4 (3.7); AH group 32.0 (4.0)
Interventions	AH (laser; complete zona breach; ? hour egg retrieval to AH; ? hour AH to transfer) with concomitant removal of damaged blastomeres vs no AH and no damaged blastomere removal AH: 20 women randomised, 65 embryos transferred Control: 18 women randomised, 52 embryos transferred
Outcomes	Clinical pregnancy
Notes	Attempted to contact author about this study. Reply received No. of embryos: AH: 2.9, control: 3.2 Unclear whether power calculation performed ITT analysis unclear

Risk of bias

Nagy 1999 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation stated but method unclear or incorrect
Allocation concealment (selection bias)	Unclear risk	Unclear; no details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	High risk	Published as a conference abstract only. No evidence of a full paper publication. Study authors did not report on live birth
Other bias	Low risk	None identified

Ng 2005

Study characteristics	
Methods	Randomised trial
Participants	160 women from Hong Kong with frozen embryo transfer Mean age: 34.0 years
Interventions	Laser-assisted thinning 1/4 with frozen embryos compared to frozen embryos AH: 80 women Control: 80 women
Outcomes	Clinical pregnancy, miscarriage, multiple pregnancy rates
Notes	No study author contact as all details clearly stated in article No. of embryos stated: AH, transferred 2 in 52.5% and 3 in 41.3%; control, transferred 2 in 36.2% and 3 in 61.3%

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation
Allocation concealment (selection bias)	Unclear risk	'Sealed envelopes' used but unclear if these were opaque and how they were numbered
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blinding until completion of the study

Ng 2005 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Low risk	No evidence of attrition bias
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Study authors did not report on live birth
Other bias	Low risk	None identified

Petersen 2005

Study characteristics	
Methods	Randomised trial
Participants	150 women from Brazil undergoing ART cycles All participants had 1 failed treatment cycle Mean age: 34 years
Interventions	ICSI cycles only AH quarter-laser thinning vs control AH: 35 women with 1 previous implantation failure, 40 women with repeated implantation failures Control: 35 women with 1 previous implantation failure, 40 women with repeated implantation failures
Outcomes	Live birth, clinical pregnancy, miscarriage, multiple pregnancy
Notes	Study author response No. of ET: mean 2.7

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random numbers table
Allocation concealment (selection bias)	Unclear risk	Unclear - code ID to mask identity of participants but not clear how or who generated this
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated
Incomplete outcome data (attrition bias) All outcomes	Low risk	All women randomised appear to have been analysed
Selective reporting (reporting bias)	Low risk	Original protocol not viewed but study authors did report on live birth
Other bias	Low risk	None identified

Razi 2013

Study characteristics	
Methods	Prospective randomised First ICSI cycle March 2009 to April 2010 Fresh ET on Day 2
Participants	182 infertile couples with male factor
Interventions	LAH on the morning of fresh ET (Day 2 embryos) Experimental group had LAH (n = 90) Control group had no LAH (n = 92)
Outcomes	Live birth rate, multiple pregnancy rate, clinical pregnancy rate, congenital anomaly rate
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated number
Allocation concealment (selection bias)	Unclear risk	No information provided in article or by correspondence
Blinding (performance bias and detection bias) All outcomes	Low risk	Correspondence with study author - both clinicians and patients blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	Appears complete
Selective reporting (reporting bias)	Low risk	Appears complete
Other bias	Low risk	None identified

Rufas-Sapir 2004

Study characteristics	
Methods	Unknown randomisation method and allocation concealment. Occurred on day of embryo transfer
Participants	207 women 3 consecutive failed IVF cycles All ages

Rufas-Sapir 2004 *(Continued)*

	Undergoing IVF only
Interventions	Mechanical partial zonal dissection: complete breach technique vs control AH - 104 women Control - 103 women
Outcomes	Clinical pregnancy, miscarriage
Notes	Study author response AH 3.4; ET control 3.7

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Unknown randomisation method
Allocation concealment (selection bias)	Unclear risk	Unknown allocation concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data appear complete
Selective reporting (reporting bias)	Unclear risk	Unclear
Other bias	Unclear risk	Conference abstract

Ryan 1997

Study characteristics

Methods	Single-centre randomised trial
Participants	200 women from Sydney, Australia, undergoing ART cycles
Interventions	AH: Tyrode's complete breach - hole chemical means on both fresh and frozen-thawed embryos: 100 women Control: 100 women
Outcomes	Clinical pregnancy
Notes	Additional information received from first author regarding definition of pregnancy. No further publication planned Mean: ET 2.17

Ryan 1997 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random numbers table
Allocation concealment (selection bias)	Unclear risk	Unclear whether allocation concealed
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear whether blinding took place
Incomplete outcome data (attrition bias) All outcomes	Low risk	All women randomised appear to have been analysed
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Study authors did not report on live birth
Other bias	Unclear risk	Conference abstract

Safari 2017

Study characteristics	
Methods	Randomised study – block randomisation – selection of blocks conducted by simple random method but no further information about allocation sequence generation of blocks Allocation concealment – block randomisation used with block size of 6 but unclear about whether there was allocation concealment from the methods described Participant blinding unclear Assessor blinding unclear No power calculation Full article Conducted at single centre in Yazd, Iran
Participants	96 patients Previously underwent IVF or ICSI with embryo cryopreservation Day 2 or 3 vitrified-warmed embryos at 4 to 8 cells with grade B or C (B defined as a little inequality in the size of blastomeres, < 10% cytoplasmic fragments; C defined as unequal blastomeres with < 50% fragmentation)
Interventions	Randomised into 3 arms 32 randomised to cosmetic micromanipulation and LAH (excluded from this review) 32 randomised to sham/LAH 32 randomised to control (no LAH or CM)

Safari 2017 *(Continued)*

Outcomes	Live birth rate (unclear about what difference is in delivery rate) Delivery rate Clinical pregnancy Chemical pregnancy
Notes	Mean female age: sham/LAH 30.6, control/no LAH 29.23 Mean no. of embryos transferred: sham/LAH 2, control/no LAH 2

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated simple random method used for block randomisation but no details
Allocation concealment (selection bias)	Unclear risk	Block randomisation used with block size of 6 but unclear about whether there was allocation concealment from the methods described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No information about whether participants or outcome assessors were blinded
Incomplete outcome data (attrition bias) All outcomes	High risk	32 randomised in Sham/LAH group; results reported only for 30 participants with no explanation given
Selective reporting (reporting bias)	High risk	Separate data not given for IVF and ICSI. Another cosmetic micromanipulation group also involved, which we have not used in our data
Other bias	High risk	Clinical pregnancy in LAH group: 7/30; live birth: 8/30; delivery rate: 7/30

Sagoskin 2007

Study characteristics

Methods	Randomised trial
Participants	199 women from USA undergoing IVF or ICSI Good prognosis group with only 1 previous implantation failure Fresh embryo transfer cycles only
Interventions	Laser hatching (breach with hole) AH: 121 randomised, 118 analysed, 254 embryos; control: 82 randomised, 81 analysed, 170 embryos
Outcomes	Live birth, clinical pregnancy, miscarriage, multiple pregnancy rates
Notes	No study author contacted as all details clearly stated in article ET: AH 2.2 (0.4); control 2.1 (0.3) Power calculation not reported Published as a full paper

Sagoskin 2007 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Treatment assignments were determined by a computer-generated randomised series at a 3:2 ratio of treatments to controls
Allocation concealment (selection bias)	Unclear risk	Not stated within text
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated within text
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT unclear
Selective reporting (reporting bias)	Low risk	Live birth reported
Other bias	Low risk	None identified

Shi 2016

Study characteristics	
Methods	Prospective randomised study in China IVF and ICSI Time period of study not known Fresh ET on Day 3
Participants	178 patients aged 35 to < 42 years
Interventions	LAH on Day 3 embryos Laser thinning of zona Experimental group had LAH (n = 82) (53 IVF and 29 ICSI) Control group had no LAH (n = 96) (70 IVF and 26 ICSI)
Outcomes	Live birth, clinical pregnancy, miscarriage, multiple pregnancy rates
Notes	No loss to follow-up in both groups Study registered on clinical trial registry at clinicaltrial.gov: NCT01765322

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	States only that patients were randomly allocated to AH group and control group but provides no details

Shi 2016 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	No information
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label study
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up; outcomes reported for all
Selective reporting (reporting bias)	Unclear risk	No information about monozygotic twins provided even though multiple pregnancy rates provided. Results for IVF/ICSI not provided separately
Other bias	Low risk	None identified Study reported primary and secondary outcomes as stated on clinical trials registry

Stein 1995

Study characteristics	
Methods	Single-centre randomised trial
Participants	154 women from Israel with repeated implantation failure (> 3 attempts) undergoing IVF Mean age not stated
Interventions	AH (mechanical; complete zona breach; ? hour egg retrieval to AH; 1.5 hour AH to transfer) vs no AH AH: 72 women randomised, 230 embryos transferred Control: 82 women randomised, 295 embryos transferred
Outcomes	Clinical pregnancy, miscarriage
Notes	Attempted to contact author about this study; no reply received Unclear whether power calculation performed ITT analysis unclear Published as full paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomisation stated but method unclear or incorrect
Allocation concealment (selection bias)	Unclear risk	Unclear; no details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias)	Unclear risk	ITT analysis unclear

Stein 1995 *(Continued)*

All outcomes		
Selective reporting (reporting bias)	High risk	Original protocol not viewed but study authors did not report on live birth
Other bias	Low risk	None identified

Tucker 1993

Study characteristics		
Methods	Single-centre randomised trial	
Participants	218 women from North America undergoing IVF (mean basal FSH: control group 9.0 (5.3); AH group 8.8 (3.7) IU/L) Mean age: control group 34.2 (4.1); AH group 34.1 (4.8)	
Interventions	AH with acid Tyrode's thinning to 1/4; 72 hour egg retrieval to AH; 1 to 3 hour AH to transfer) vs no AH AH: 110 women randomised, 333 embryos transferred Control: 108 women randomised, 312 embryos transferred	
Outcomes	Implantation, clinical pregnancy	
Notes	Attempted to contact author about this study; no reply received ET: AH 2.9, control 3.0 Unclear whether power calculation performed ITT analysis unclear Published as full paper	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomisation stated but method unclear or incorrect
Allocation concealment (selection bias)	Unclear risk	Unclear; no details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	High risk	Original protocol not viewed but study authors did not report on live birth
Other bias	Low risk	None identified

Tucker 1996

Study characteristics	
Methods	Single-centre randomised trial
Participants	100 women from North America undergoing ICSI Mean age: control group 33.5 (4.3); AH group 35.3 (4.2)
Interventions	AH with acid Tyrode's (chemical; complete zona breach; 72 hour egg retrieval to AH; 4 hour AH to transfer) vs no AH AH: 50 women randomised, 189 embryos transferred Control: 50 women randomised, 184 embryos transferred
Outcomes	Implantation, clinical pregnancy
Notes	Attempted to contact author about this study; no reply received ET: AH 3.7, control 3.8 Unclear whether power calculation performed ITT analysis unclear

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomisation stated but method unclear or incorrect
Allocation concealment (selection bias)	Unclear risk	Allocation concealment unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	Women randomised appear to be analysed
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed but study authors did not report on live birth
Other bias	Low risk	None identified

Utsunomiya 1998

Study characteristics	
Methods	Single-centre randomised trial
Participants	55 women from Japan undergoing either ICSI or IVF No data provided on age
Interventions	AH with acid (chemical): 27 women No other details about the day of treatment provided

Utsunomiya 1998 *(Continued)*

	Control: 28 women
Outcomes	Clinical pregnancy rate only (gestation sac on ultrasound)
Notes	No attempt to contact study author No. of ETs not stated Unclear whether power calculation performed ITT analysis unclear Published as abstract only

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation stated but method unclear or incorrect
Allocation concealment (selection bias)	Unclear risk	Allocation concealment unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	High risk	Published as a conference abstract only and did not report on live births
Other bias	Unclear risk	Conference abstract

Valojerdi 2010

Study characteristics

Methods	Single-centre randomised trial
Participants	400 women in Iran undergoing first treatment cycle and women with previous failed cycles Mean age: control group 29.85 (5.14); AH group 30.86 (5.82)
Interventions	Partially thinned by laser AH: 200 women randomised Control: 200 women randomised
Outcomes	Clinical pregnancy, implantation rates
Notes	Power calculation not reported ITT analysis unclear

Valojerdi 2010 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation list participants
Allocation concealment (selection bias)	Unclear risk	Methodological or linguistic confusion in description of allocation in the study. Sequential numbers in sealed envelopes (200 participants in each group)
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of clinician but not patient
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not stated
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed but study authors did not report on live birth
Other bias	Low risk	None identified

Wan 2014

Study characteristics	
Methods	Prospective randomised Not known whether participant had first or previous treatment IVF or ICSI June 2010 to August 2011 Fresh ET done with cleavage stage - no result provided Blastocysts vitrified and randomised in 2 groups, then FET after warming
Participants	203 infertile couples
Interventions	Control group - no LAH (n = 102) Experimental group - had LAH (n = 101) (2 blastocysts did not survive after warming, 3 were lost to follow-up), so total results n = 96 2 embryos (blastocysts) transferred in both groups after warming
Outcomes	Live birth rate, multiple pregnancy rate, clinical pregnancy rate, miscarriage rate
Notes	Study author contacted but no response received Figure 2 and tables are contradictory due to number of participants in each group as stated in the paper; we accepted the data as described in the text and presumed that there was an error in Figure 2
Risk of bias	

Wan 2014 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "during the study period, 203 patients who met the inclusion criteria were enrolled and randomly divided into two groups"
Allocation concealment (selection bias)	Unclear risk	Paper states patients "were randomly divided into two groups" but no further information provided
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No information provided
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	5 patients in the QLAH group who met the exclusion criteria were excluded from analyses (2 patients had no surviving blastocysts for transfer after warming) but 3 patients were lost to follow-up. No patients in the control group were lost to follow-up
Selective reporting (reporting bias)	Low risk	No response from study author on further details. Live birth rate reported
Other bias	Unclear risk	Description of population - unclear whether they had first or previous treatment

AH: assisted hatching.
ART: assisted reproductive technology.
ASRM: American Society for Reproductive Medicine.
CM: cosmetic micromanipulation
ET: embryo transfer.
FET: fresh embryo transfer.
FSH: follicle-stimulating hormone.
GS: gestation sac
hCG: human chorionic gonadotropin.
ICSI: intracytoplasmic sperm injection.
ITT: intention-to-treat.
IVF: in vitro fertilisation.
LAH: laser-assisted hatching.
QLAH: quarter laser-assisted hatching.
TV: transvaginal.
TVS: transvaginal sonography.
USS: ultrasound scan.
ZP: zona pellucida.
Mean age given in years (standard deviation).
Note: only arms where all or no embryos transferred and were treated with AH were accepted for data extraction.

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

Study	Reason for exclusion
Abdelmassih 2002	Pooled oocytes, then randomised; no per woman data provided
Amorocho 2012	Compares technique of hatching with thickness of ZP on Day 3 of embryo development. Further details asked for but no response from study authors
Antinori 1996a	Not a randomised controlled trial Mentions randomly selected, not randomly allocated

Study	Reason for exclusion
Antinori 1996b	No randomised comparison between control and assisted hatching groups
Balaban 2002	Not randomised No appropriate controls
Bider 1997	Not randomised
Blake 2001	Not randomised No embryo transfer occurred, so no review outcomes could be measured
Carter 2003a	No per woman data
Chao 1997	Assessment of pregnancy was by hCG only 14 days after embryo transfer
Check 1996	Not randomised Benefits of AH confounded by concurrent assessment of 2 different culture media
Chen 1999	Not randomised Benefits of assisted hatching confounded by concurrent assessment of 2 different culture media
Chimote 2013	Compared techniques of hatching. No information regarding randomisation method; does not suggest it is a randomised study
Cieslak 1999	Comparison of 2 types of assisted hatching; no 'no assisted hatching' control group was used More than 1 cycle per woman
Cohen 1990	Not randomised
Debrock 2011	Primary outcome was implantation; results per embryo transfer, not per woman
De Croo 2013	Implantation rate per woman reported in the abstract. Contacted for full data; no response from study authors, so excluded
Demirol 2003	No pregnancy data provided
Dirnfeld 2003	No hatching
Dokras 1994	No appropriate outcome measure
Domitrz 2000	No clear information about randomisation and allocation
Ebner 2002	No per woman data
Edirisinghe 1999	Not randomised
Feng 2009	Not a prospective study - a retrospective study
Figueira 2012	Implantation and pregnancy rates provided in percentages. Date per embryo transfer. Not enough data to utilise study. Study population egg donation cryobank programme, which was not the reason for exclusion
Frydman 2006	No per woman data
Gabrielsen 2004	Pseudo-randomised (alternate days)

Study	Reason for exclusion
Grace 2007	No control. Comparing assisted hatching in good embryos with assisted hatching in poor embryos
Hershlag 1999	Not randomised Control group from the period 1990 to 1993; assisted hatching group from 1994 to 1996 (historical controls)
Hiraoka 2009	No control. Comparing half thinning vs quarter thinning
Hur 2011	Not clear whether randomised; results appear to be per embryo transfer rather than per woman
Huttelova 1999	Not randomised Benefits of AH confounded by concurrent assessment of 2 different culture media
Kanyo 2016	Quasi-randomisation. Randomised based on last number/digit of registration number given by administrator at the reception desk at first visit to the centre; study authors contacted but no further details obtained; therefore decision to exclude
Kirienko 2019	Included cycles with donor oocytes; contacted to see whether could provide separate data for donor/autologous cycles – no reply
Komarovsky 2002	No per woman data
Komarovsky 2003	No per woman data
Le 2018	No control arm; this is an RCT of thinning assisted hatching vs drilling assisted hatching of ZP before FET
Lee 1999	Not randomised
Levron 2003	No per woman data
Lu 2016	Quasi-randomised study
Lu 2019	Full article demonstrated this was a retrospective study - not an RCT
Ma 2007	No per woman data
Magli 1998	No per woman data
Mahadevan 1998	Not randomised No concurrent controls
Mansour 2000	Randomisation by alternate day - inadequate allocation
Meldrum 1998	Not randomised No concurrent controls
Montag 1999	Not randomised No concurrent controls
Nadir 2005	Alternate randomisation.
Nagy 2003	No per woman data
Nakayama 1998	No appropriate outcome measure

Study	Reason for exclusion
Nakayama 1999	No per woman data
NCT02124291	Study terminated for insufficient enrolment, (only 18 patients enrolled); no data available
Ng 2008	No control. Compared 2 methods of laser
Obradors 2012	Vitrified embryos from oocyte donation programme
Obruca 1994	Not randomised No concurrent controls
Olivennes 1997	No per woman data
Peterson 2006	Results per embryo transfer only No per woman data
Ren 2013	Study looked at effects of the site of assisted hatching on embryo comparing performing AH at the site near inner cell mass (ICM) vs performing AH at the site opposite to the ICM; there was no control group with no AH
Rienzi 2002	Assisted hatching was part of the ICSI method
Ringler 1999	Not clear how many women were included in the study or for how many cycles (only cycles were mentioned); mixture of participants and donated eggs was used for the study
Schoolcraft 1994	Not randomised Control and intervention groups recruited at different times
Shahin 2003	No per woman data
Sifer 2005	Per cycle data only No per woman data
Szell 1998	Not randomised Benefits of assisted hatching confounded by concurrent assessment of 2 different culture media
Tao 1997	Not randomised Some of the women in the assisted hatching group were randomised but most were allocated to assisted hatching routinely, with no control option
Tucker 1991	Not randomised
Urman 2002	Inadequate method of allocation
Valojerdi 2008	Inadequate method of allocation
Yano 2007	No per woman data, only per cycle data
Zech 1998	Numbers in tables do not add up correctly and text and tables are contradictory on age groups used in the prospective part of the study. Asked for clarification from authors - no response
Zhang 2009	Not a prospective study - retrospective study

AH: assisted hatching.
FET: fresh embryo transfer.

hCG: human chorionic gonadotropin.
ICM: inner cell mass.
ICSI: intracytoplasmic sperm injection.
RCT: randomised controlled trial.
ZP: zona pellucida.

Characteristics of studies awaiting classification *[ordered by study ID]*

Elnahas A 2018

Methods	Prospective randomised study
Participants	120 women undergoing fresh embryo transfer and 120 women undergoing frozen embryo transfer
Interventions	Laser-assisted hatching with infrared diode laser to induce zonal microdissection 60 women from fresh ET group randomised to LAH; unclear number of women in frozen ET group randomised to LAH
Outcomes	Clinical pregnancy rate Implantation rate
Notes	Study authors contacted but no reply at present

ET: embryo transfer.
LAH: laser-assisted hatching.

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

NCT02752568

Study name	Assisted hatching vs endometrial scratch in recurrent Implantation failure
Methods	Allocation: randomised Intervention model: factorial assignment Masking: open-label Primary purpose: treatment
Participants	18 to 40 years Inclusion criteria: recurrent implantation failure, normal uterine cavity by transvaginal ultrasound
Interventions	Laser-assisted hatching vs endometrial scratch vs no intervention Group 1 consists of 100 patients who will undergo endometrial scratch followed by controlled ovarian hyperstimulation; Group 2 consists of 100 patients who will undergo controlled ovarian hyperstimulation and assisted hatching; Group 3 consists of 100 patients who will undergo controlled ovarian hyperstimulation
Outcomes	Primary outcome measure: number of patients with positive pregnancy test
Starting date	April 2016
Contact information	Suzy Abdelaziz; mailto:suzyabdelaziz92%40gmail.com?subject=NCT02752568, ivfobgyn, Assisted Hatching Versus Endometrial Scratch in Recurrent Implantation Failure
Notes	http://apps.who.int/trialsearch/Trial2.aspx?TrialID=NCT02752568 Not yet recruiting

NCT02752568 *(Continued)*

	Date first received 27 April 2016

NCT03623659

Study name	Does partial zona pellucida removal from vitrified-warmed human blastocysts improve delivery rate in IVF? A multicentric RCT on laser assisted hatching
Methods	Randomised Parallel assignment Masking - triple (participant, care provider, and outcome assessor)
Participants	700 participants 18 to 40 years old Female Inclusion criteria: single-embryo transfer of vitrified/warmed blastocyst (SET); first or second frozen IVF (with or without intracytoplasmic sperm injection) cycle of blastocysts; first or second oocyte retrieval Exclusion criteria: pre-implantation genetic testing (PGT) cycle; BMI > 35 kg/m^2; severe male factor; abnormal uterine cavity
Interventions	Active comparator: AH group Subjects whose vitrified/warmed blastocysts will be subjected to treatment of laser-assisted hatching Procedure: laser-assisted hatching After warming, blastocysts are subjected to laser-assisted hatching (LAH) following standard procedure. LAH procedure lasts 1 minute per blastocyst No intervention: control group Subjects whose vitrified/warmed blastocysts will be subjected to the same procedures except for treatment of laser-assisted hatching
Outcomes	Primary outcome measures Delivery rate [time frame: 38 weeks after embryo transfer] Number of deliveries that result in a live birth per transferred blastocyst Secondary outcome measures Implantation rate [time frame: 6 to 7 weeks after transfer] • Number of gestational sacs observed at echographic screening at 6 weeks of pregnancy divided by the number of transferred embryos Clinical pregnancy rate [time frame: 4 weeks after transfer] • Ultrasonographic demonstration of an intrauterine gestational sac divided by the number of included women Biochemical pregnancy rate [time frame: 4 weeks after transfer] • Pregnancies failing to progress to the point of ultrasound confirmation divided by the number of women with a positive pregnancy test on blood

NCT03623659 *(Continued)*

	Ongoing pregnancy rate [time frame: 20 weeks after transfer] • Ultrasonographic demonstration of an intrauterine gestational sac with fetal hearth divided by the number of included women Multiple pregnancy rate [time frame: 4 weeks after transfer] • Pregnancy in which more than 1 fetus develops in the uterus at the same time divided by the number of women with a clinical pregnancy Obstetrical and neonatal complication rates [time frame: after birth; 9 to 10 months after transfer] • Condition that adversely affects women and their fetal health during delivery Congenital anomalies rate [time frame: after birth, 9 to 10 months after transfer] • Birth defects, congenital disorders, congenital malformations, and congenital abnormalities are conditions of prenatal origin that are present at birth, potentially impacting an infant's health, development, and/or survival divided by the number of live births
Starting date	5 September 2018
Contact information	Alessandra Alteri; alteri.alessandra@hsr.it Paola Vigano; vigano.paola@hsr.it
Notes	ClinicalTrials.gov Identifier: NCT03623659 https://clinicaltrials.gov/ct2/show/NCT03623659?cond=assisted+hatching&draw=2&rank=4 Date first received 9 August 2018

NCT03810157

Study name	Does laser-assisted hatching (LAH) improve the pregnancy outcomes in humans?
Methods	Randomised Parallel assignment
Participants	1200 participants 22 to 45 years Female Inclusion criteria: patients undergoing IVF/ICSI-ET cycle; zona pellucida of cleavage-stage embryo thicker than 8 µm Exclusion criteria: number of embryos transferred per cycle > 2; transferred embryos including fresh and frozen cycle in the same cycle; embryos developed from frozen-thawed oocytes
Interventions	Experimental: laser-assisted hatching system Embryos were exposed to a dose of laser energy focused outside the zona pellucida by laser-assisted hatching system Device: laser-assisted hatching system ZP was thinned or drilled with the laser-assisted hatching system. Laser pulse was 0.296 ms. Laser aperture was 8 µm No intervention: control group

NCT03810157 *(Continued)*

	Nothing is done
Outcomes	Primary outcome measure Efficacy of LAH in ART [time frame: 6 months] • Clinical pregnancy assessed Secondary outcome measure Feasibility of LAH in ART [time frame: 1 year] • Incidence of LAH adverse events assessed by miscarriage rate and multiple gestation rate
Starting date	26 December 2018
Contact information	Ming Wang; wangmingbio@snnu.edu.cn Tangdu Hospital, Xi'an, Shaanxi, China 710038
Notes	ClinicalTrials.gov Identifier: NCT038101 https://clinicaltrials.gov/ct2/show/NCT03810157?cond=assisted+hatching&draw=2&rank=8 Other study ID number: 1215 Date first received 14 January 2019

NCT03833869

Study name	The effect of assisted hatching on implantation rate in frozen blastocyst transfer - a prospective randomized controlled study
Methods	Current study aims to assess effects of assisted hatching on implantation rate of frozen blastocysts Randomised, parallel assignment, open-label
Participants	84 participants 18 to 39 years Female Inclusion criteria: in vitro fertilisation patients at investigators' institution intended to undergo transfer of frozen 5-day embryo (blastocyst); 18 to 39 years old; first to third treatment cycle; previously had maximum of 4 embryos transferred Exclusion criteria: over 40 years old; congenital or acquired uterine malformations; hydrosalpinx; chronic autoimmune disease; embryo intended to undergo pre-implantation genetic diagnosis
Interventions	Experimental: assisted hatching 5-Day frozen embryos will undergo assisted hatching before embryo transfer No intervention: control 5-Day frozen embryos will not undergo any additional procedures before embryo transfer Procedure: assisted hatching Controlled hatching of zona pellucida in the laboratory before embryo transfer

NCT03833869 *(Continued)*

Outcomes	Primary outcome measure Implantation rate [time frame: 5 to 6 weeks following embryo transfer] • Number of gestational sacs demonstrated on ultrasound divided by number of embryos transferred (expressed as percentage) Secondary outcome measures Chemical pregnancy [time frame: 5 to 6 weeks following embryo transfer] • Increase and subsequent decrease in beta hCG levels with no evidence of gestational sac on ultrasound Early spontaneous abortion [time frame: up to 15 weeks from embryo transfer] • Spontaneous abortion of pregnancy during first trimester of pregnancy
Starting date	1 March 2019
Contact information	Hadas Ganer Herman, MD; hadassganer@yahoo.com Edith Wolfson Medical Center, Holon, Israel
Notes	Clinicaltrials.gov identifier: NCT03833869 https://clinicaltrials.gov/ct2/show/NCT03833869 Other study ID number: 0020-19-WOMC Date first received 7 February 2019

NTR3387

Study name	A multicentre randomized controlled trial on the efficacy of laser assisted hatching in poor prognosis patients undergoing IVF or ICSI: the AHA trial
Methods	Randomised, double-blinded
Participants	Repeated implantation failure
Interventions	In the intervention group, embryos to be transferred will undergo laser-assisted hatching. One-eighth of the ZP will be completely breached using the laser. Laser pulse duration should not exceed 400 µs per pulse at maximum power of 100%, corresponding to 285 mW output peak power in clinical mode. If isotherm rings are used, the rings corresponding to 60°C and higher should not contact adjacent blastomeres. Preferably, part of the ZP is selected with underneath a large area of perivitelline space or in the vicinity of an area with extensive fragmentation
Outcomes	Primary outcome: live birth rate Secondary outcomes: pregnancy rate, ongoing pregnancy rate, implantation rate, multiple pregnancy rate, monozygotic twinning rate, percentage of major and minor malformations in children born (assessed at birth)
Starting date	2012
Contact information	MHJM Curfs Fertility Centre Isala, Isala Klinieken

NTR3387 *(Continued)*	
	P.O. Box 10400, Netherlands
	m.h.j.m.curfs@isala.nl
Notes	http://apps.who.int/trialsearch/Trial2.aspx?TrialID=NTR3387
	Study completed but not yet published
	Date first received 6 April 2012

AH: assisted hatching.
ART: assisted reproductive technologies.
ASRM: American Society of Reproductive Medicine.
CM: cosmetic micromanipulation
ET: embryo transfer.
FET: fresh embryo transfer.
FSH: follicle-stimulating hormone.
GS: gestation sac
hCG: human chorionic gonadotropin.
ICSI: intracytoplasmic sperm injection.
ITT: intention-to-treat.
IVF: in vitro fertilisation.
LAH: laser-assisted hatching.
QLAH: quarter laser-assisted hatching.
TV: transvaginal.
TVS: transvaginal sonography.
USS: ultrasound scan.
ZP: zona pellucida.

DATA AND ANALYSES

Comparison 1. Live birth: assisted hatching compared with no assisted hatching

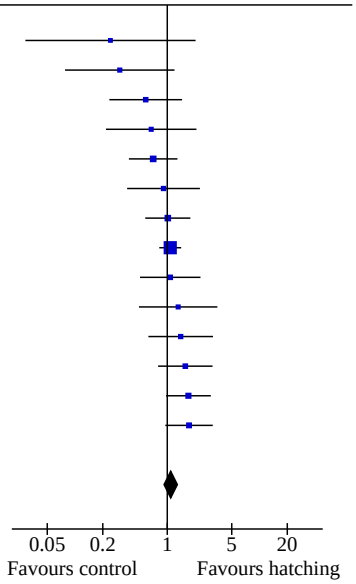
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Live birth per woman randomised	14	2849	Odds Ratio (M-H, Fixed, 95% CI)	1.09 [0.92, 1.29]
1.2 First or repeat attempt	4		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.2.1 First attempt at IVF or ICSI	3	380	Odds Ratio (M-H, Fixed, 95% CI)	0.78 [0.48, 1.28]
1.2.2 Repeat attempt at IVF or ICSI	1	150	Odds Ratio (M-H, Fixed, 95% CI)	1.40 [0.62, 3.13]
1.3 Conception mode	6		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.3.1 ICSI only	3	640	Odds Ratio (M-H, Fixed, 95% CI)	1.54 [1.02, 2.33]
1.3.2 IVF only	3	241	Odds Ratio (M-H, Fixed, 95% CI)	1.00 [0.60, 1.68]
1.4 Hatching method	14		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.4.1 Chemical	4	366	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [0.74, 1.74]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.4.2 Laser	10	2473	Odds Ratio (M-H, Fixed, 95% CI)	1.07 [0.89, 1.28]
1.5 Prognosis	9		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.5.1 Poor prognosis	4	576	Odds Ratio (M-H, Fixed, 95% CI)	1.46 [0.99, 2.15]
1.5.2 Good prognosis	6	1495	Odds Ratio (M-H, Fixed, 95% CI)	1.03 [0.83, 1.28]
1.6 Live birth rate by extent of assisted hatching	14	2849	Odds Ratio (M-H, Fixed, 95% CI)	1.09 [0.92, 1.29]
1.6.1 Thinning only	6	1742	Odds Ratio (M-H, Fixed, 95% CI)	1.06 [0.86, 1.30]
1.6.2 Breach by hole only	8	1107	Odds Ratio (M-H, Fixed, 95% CI)	1.15 [0.87, 1.51]
1.7 Fresh or frozen embryo transfer	12	1731	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.92, 1.41]
1.7.1 Fresh	11	1669	Odds Ratio (M-H, Fixed, 95% CI)	1.16 [0.94, 1.44]
1.7.2 Frozen	1	62	Odds Ratio (M-H, Fixed, 95% CI)	0.67 [0.22, 2.07]

Analysis 1.1. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 1: Live birth per woman randomised

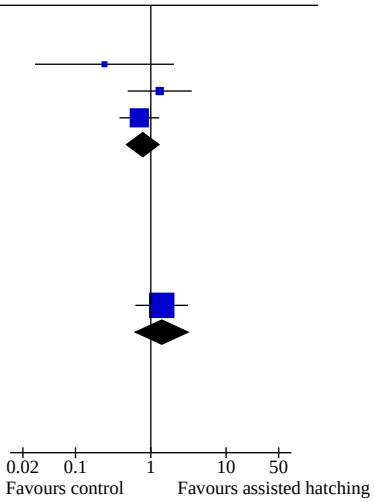
Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Hurst 1998	2	13	3	7	1.2%	0.24 [0.03 , 2.03]
Germond 2004	3	84	8	74	3.1%	0.31 [0.08 , 1.20]
Balakier 2009	13	45	16	39	4.5%	0.58 [0.24 , 1.45]
Safari 2017	7	30	10	32	2.8%	0.67 [0.22 , 2.07]
Shi 2016	29	82	42	96	9.3%	0.70 [0.38 , 1.29]
Lanzendorf 1998	12	41	15	48	3.6%	0.91 [0.37 , 2.26]
Sagoskin 2007	55	121	37	82	9.0%	1.01 [0.58 , 1.78]
Ge 2008	156	487	144	473	37.0%	1.08 [0.82 , 1.41]
Hellebaut 1996	21	60	20	60	4.8%	1.08 [0.51 , 2.29]
Razi 2013	10	90	8	92	2.6%	1.31 [0.49 , 3.49]
Petersen 2005	17	75	13	75	3.7%	1.40 [0.62 , 3.13]
Cohen 1992	34	69	26	68	4.9%	1.57 [0.80 , 3.10]
Nada 2018	40	158	25	150	7.1%	1.69 [0.97 , 2.97]
Wan 2014	39	96	29	102	6.2%	1.72 [0.95 , 3.11]
Total (95% CI)		**1451**		**1398**	**100.0%**	**1.09 [0.92 , 1.29]**
Total events:	438		396			

Heterogeneity: Chi² = 16.31, df = 13 (P = 0.23); I² = 20%
Test for overall effect: Z = 1.02 (P = 0.31)
Test for subgroup differences: Not applicable



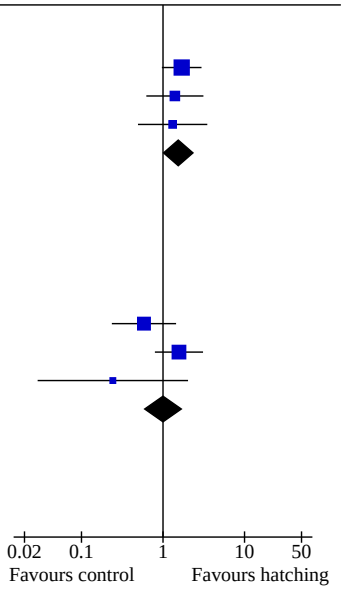
Analysis 1.2. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 2: First or repeat attempt

Study or Subgroup	Assisted hatching Events	Total	No assisted hatching Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.2.1 First attempt at IVF or ICSI						
Hurst 1998	2	13	3	7	9.3%	0.24 [0.03 , 2.03]
Razi 2013	10	90	8	92	19.9%	1.31 [0.49 , 3.49]
Shi 2016	29	82	42	96	70.8%	0.70 [0.38 , 1.29]
Subtotal (95% CI)		**185**		**195**	**100.0%**	**0.78 [0.48 , 1.28]**
Total events:	41		53			
Heterogeneity: Chi² = 2.36, df = 2 (P = 0.31); I² = 15%						
Test for overall effect: Z = 0.97 (P = 0.33)						
1.2.2 Repeat attempt at IVF or ICSI						
Petersen 2005	17	75	13	75	100.0%	1.40 [0.62 , 3.13]
Subtotal (95% CI)		**75**		**75**	**100.0%**	**1.40 [0.62 , 3.13]**
Total events:	17		13			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.81 (P = 0.42)						
Test for subgroup differences: Chi² = 1.45, df = 1 (P = 0.23), I² = 31.0%						



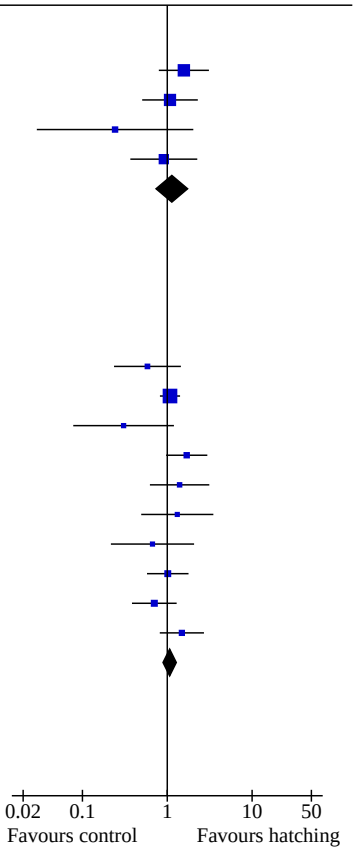
Analysis 1.3. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 3: Conception mode

Study or Subgroup	Assisted hatching Events	Total	No assisted hatching Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.3.1 ICSI only						
Nada 2018	40	158	25	150	52.9%	1.69 [0.97 , 2.97]
Petersen 2005	17	75	13	75	27.7%	1.40 [0.62 , 3.13]
Razi 2013	10	90	8	92	19.4%	1.31 [0.49 , 3.49]
Subtotal (95% CI)		**323**		**317**	**100.0%**	**1.54 [1.02 , 2.33]**
Total events:	67		46			
Heterogeneity: Chi² = 0.27, df = 2 (P = 0.87); I² = 0%						
Test for overall effect: Z = 2.03 (P = 0.04)						
1.3.2 IVF only						
Balakier 2009	13	45	16	39	42.4%	0.58 [0.24 , 1.45]
Cohen 1992	34	69	26	68	46.2%	1.57 [0.80 , 3.10]
Hurst 1998	2	13	3	7	11.5%	0.24 [0.03 , 2.03]
Subtotal (95% CI)		**127**		**114**	**100.0%**	**1.00 [0.60 , 1.68]**
Total events:	49		45			
Heterogeneity: Chi² = 4.75, df = 2 (P = 0.09); I² = 58%						
Test for overall effect: Z = 0.00 (P = 1.00)						
Test for subgroup differences: Chi² = 1.62, df = 1 (P = 0.20), I² = 38.4%						



Analysis 1.4. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 4: Hatching method

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.4.1 Chemical						
Cohen 1992	34	69	26	68	33.8%	1.57 [0.80 , 3.10]
Hellebaut 1996	21	60	20	60	33.0%	1.08 [0.51 , 2.29]
Hurst 1998 (1)	2	13	3	7	8.4%	0.24 [0.03 , 2.03]
Lanzendorf 1998	12	41	15	48	24.8%	0.91 [0.37 , 2.26]
Subtotal (95% CI)		**183**		**183**	**100.0%**	**1.13 [0.74 , 1.74]**
Total events:	69		64			
Heterogeneity: Chi² = 3.15, df = 3 (P = 0.37); I² = 5%						
Test for overall effect: Z = 0.57 (P = 0.57)						
1.4.2 Laser						
Balakier 2009	13	45	16	39	5.3%	0.58 [0.24 , 1.45]
Ge 2008	156	487	144	473	43.2%	1.08 [0.82 , 1.41]
Germond 2004	3	84	8	74	3.6%	0.31 [0.08 , 1.20]
Nada 2018	40	158	25	150	8.3%	1.69 [0.97 , 2.97]
Petersen 2005	17	75	13	75	4.4%	1.40 [0.62 , 3.13]
Razi 2013	10	90	8	92	3.1%	1.31 [0.49 , 3.49]
Safari 2017	7	30	10	32	3.2%	0.67 [0.22 , 2.07]
Sagoskin 2007	55	121	37	82	10.5%	1.01 [0.58 , 1.78]
Shi 2016	29	82	42	96	10.9%	0.70 [0.38 , 1.29]
Wan 2014	39	96	29	92	7.6%	1.49 [0.82 , 2.71]
Subtotal (95% CI)		**1268**		**1205**	**100.0%**	**1.07 [0.89 , 1.28]**
Total events:	369		332			
Heterogeneity: Chi² = 11.83, df = 9 (P = 0.22); I² = 24%						
Test for overall effect: Z = 0.71 (P = 0.48)						
Test for subgroup differences: Chi² = 0.06, df = 1 (P = 0.80), I² = 0%						

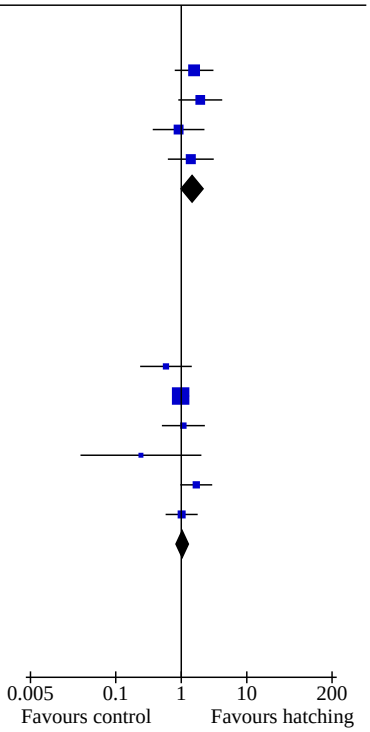


Footnotes

(1) First attempt; IVF only; participants were good prognosis women

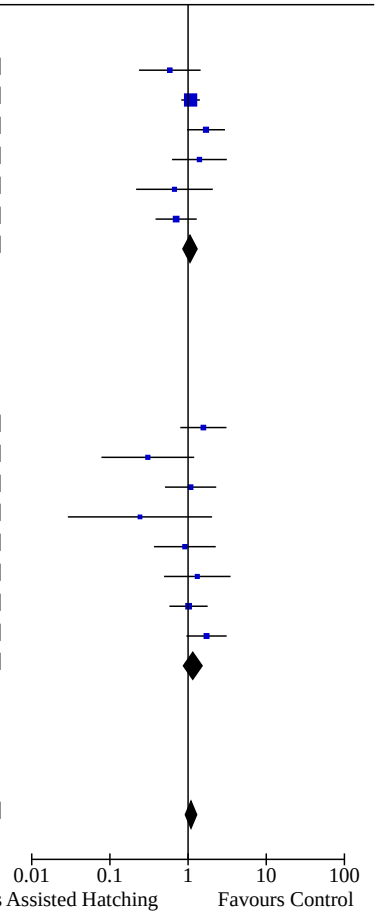
Analysis 1.5. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 5: Prognosis

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.5.1 Poor prognosis						
Cohen 1992	34	69	26	68	31.2%	1.57 [0.80 , 3.10]
Ge 2008	21	100	12	100	22.3%	1.95 [0.90 , 4.22]
Lanzendorf 1998	12	41	15	48	23.0%	0.91 [0.37 , 2.26]
Petersen 2005	17	75	13	75	23.6%	1.40 [0.62 , 3.13]
Subtotal (95% CI)		**285**		**291**	**100.0%**	**1.46 [0.99 , 2.15]**
Total events:	84		66			
Heterogeneity: Chi² = 1.63, df = 3 (P = 0.65); I² = 0%						
Test for overall effect: Z = 1.92 (P = 0.05)						
1.5.2 Good prognosis						
Balakier 2009	13	45	16	39	7.7%	0.58 [0.24 , 1.45]
Ge 2008	135	387	132	373	55.0%	0.98 [0.73 , 1.32]
Hellebaut 1996	21	60	20	60	8.2%	1.08 [0.51 , 2.29]
Hurst 1998	2	13	3	7	2.1%	0.24 [0.03 , 2.03]
Nada 2018	40	158	25	150	12.0%	1.69 [0.97 , 2.97]
Sagoskin 2007	55	121	37	82	15.1%	1.01 [0.58 , 1.78]
Subtotal (95% CI)		**784**		**711**	**100.0%**	**1.03 [0.83 , 1.28]**
Total events:	266		233			
Heterogeneity: Chi² = 6.46, df = 5 (P = 0.26); I² = 23%						
Test for overall effect: Z = 0.29 (P = 0.77)						
Test for subgroup differences: Chi² = 2.36, df = 1 (P = 0.12), I² = 57.6%						

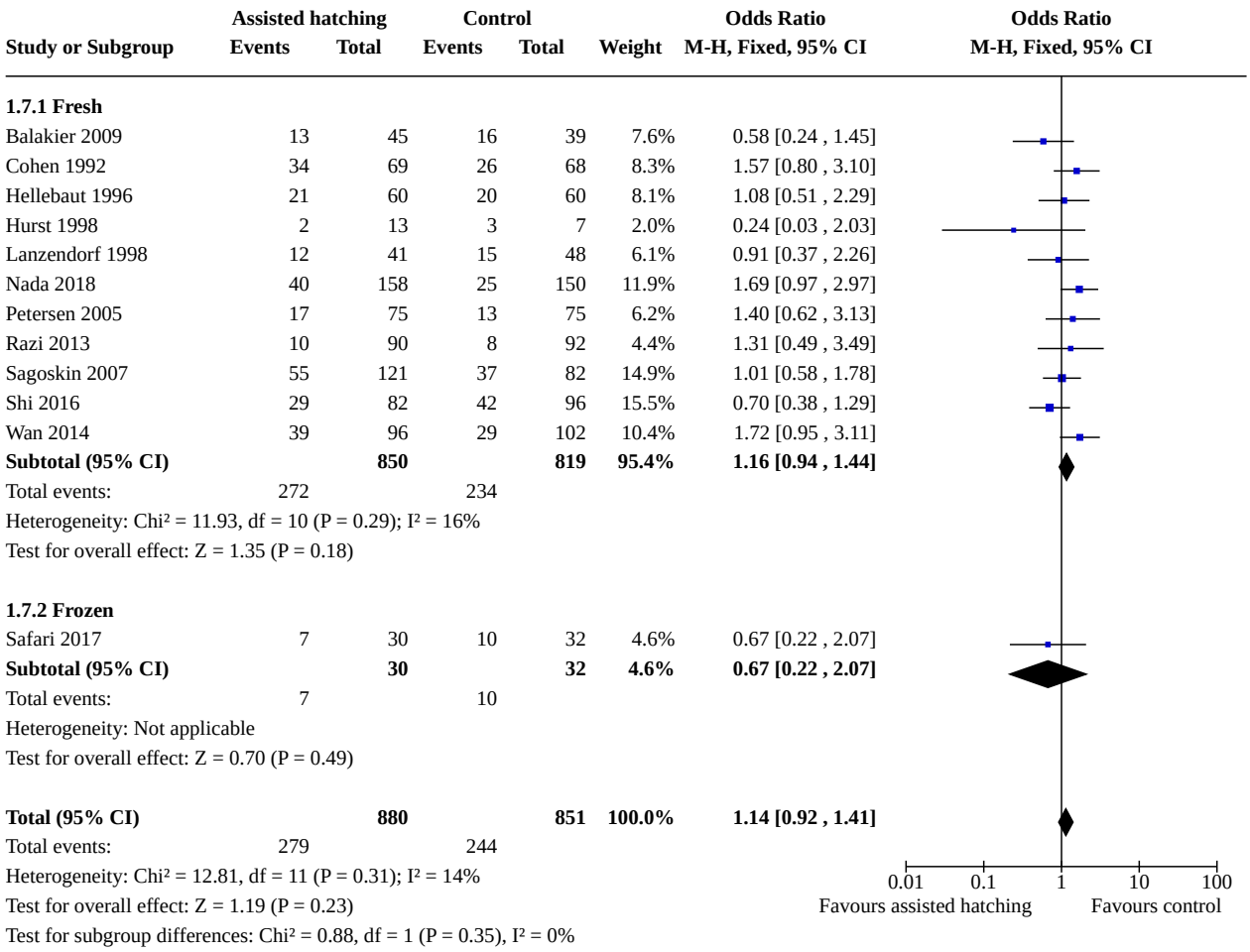


Analysis 1.6. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 6: Live birth rate by extent of assisted hatching

Study or Subgroup	Assisted Hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.6.1 Thinning only						
Balakier 2009	13	45	16	39	4.5%	0.58 [0.24 , 1.45]
Ge 2008	156	487	144	473	37.0%	1.08 [0.82 , 1.41]
Nada 2018	40	158	25	150	7.1%	1.69 [0.97 , 2.97]
Petersen 2005	17	75	13	75	3.7%	1.40 [0.62 , 3.13]
Safari 2017	7	30	10	32	2.8%	0.67 [0.22 , 2.07]
Shi 2016	29	82	42	96	9.3%	0.70 [0.38 , 1.29]
Subtotal (95% CI)		**877**		**865**	**64.5%**	**1.06 [0.86 , 1.30]**
Total events:	262		250			
Heterogeneity: Chi² = 7.22, df = 5 (P = 0.20); I² = 31%						
Test for overall effect: Z = 0.53 (P = 0.60)						
1.6.2 Breach by hole only						
Cohen 1992	34	69	26	68	4.9%	1.57 [0.80 , 3.10]
Germond 2004	3	84	8	74	3.1%	0.31 [0.08 , 1.20]
Hellebaut 1996	21	60	20	60	4.8%	1.08 [0.51 , 2.29]
Hurst 1998	2	13	3	7	1.2%	0.24 [0.03 , 2.03]
Lanzendorf 1998	12	41	15	48	3.6%	0.91 [0.37 , 2.26]
Razi 2013	10	90	8	92	2.6%	1.31 [0.49 , 3.49]
Sagoskin 2007	55	121	37	82	9.0%	1.01 [0.58 , 1.78]
Wan 2014	39	96	29	102	6.2%	1.72 [0.95 , 3.11]
Subtotal (95% CI)		**574**		**533**	**35.5%**	**1.15 [0.87 , 1.51]**
Total events:	176		146			
Heterogeneity: Chi² = 8.82, df = 7 (P = 0.27); I² = 21%						
Test for overall effect: Z = 0.98 (P = 0.33)						
Total (95% CI)		**1451**		**1398**	**100.0%**	**1.09 [0.92 , 1.29]**
Total events:	438		396			
Heterogeneity: Chi² = 16.31, df = 13 (P = 0.23); I² = 20%						
Test for overall effect: Z = 1.02 (P = 0.31)						
Test for subgroup differences: Chi² = 0.22, df = 1 (P = 0.64), I² = 0%						



Analysis 1.7. Comparison 1: Live birth: assisted hatching compared with no assisted hatching, Outcome 7: Fresh or frozen embryo transfer



Comparison 2. Multiple pregnancy: assisted hatching compared with no assisted hatching

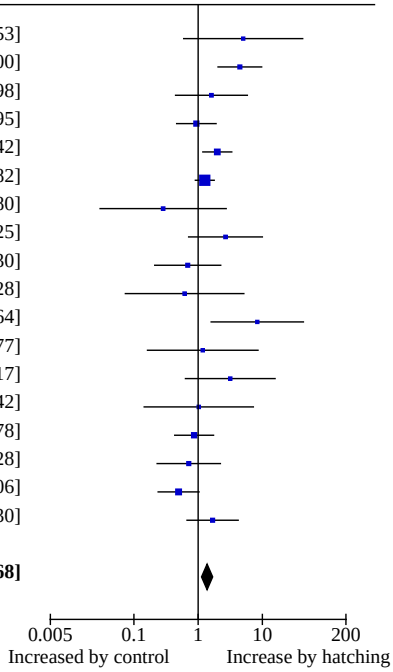
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Multiple pregnancy rate per woman randomised	18	4308	Odds Ratio (M-H, Fixed, 95% CI)	1.38 [1.13, 1.68]
2.2 First or repeat attempt	8		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.2.1 First attempt at IVF or ICSI	4	654	Odds Ratio (M-H, Fixed, 95% CI)	0.73 [0.31, 1.72]
2.2.2 Repeat attempt at IVF or ICSI	5	1068	Odds Ratio (M-H, Fixed, 95% CI)	1.25 [0.80, 1.94]
2.3 Conception mode	9		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.3.1 ICSI only	3	573	Odds Ratio (M-H, Fixed, 95% CI)	3.09 [1.57, 6.08]
2.3.2 IVF only	6	1126	Odds Ratio (M-H, Fixed, 95% CI)	1.87 [1.28, 2.72]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.4 Hatching method	18		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.4.1 Chemical	4	534	Odds Ratio (M-H, Fixed, 95% CI)	1.55 [0.98, 2.47]
2.4.2 Laser	13	3730	Odds Ratio (M-H, Fixed, 95% CI)	1.29 [1.03, 1.61]
2.4.3 Mechanical	1	44	Odds Ratio (M-H, Fixed, 95% CI)	8.33 [1.56, 44.64]
2.5 Prognosis	10		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.5.1 Poor prognosis	6	1186	Odds Ratio (M-H, Fixed, 95% CI)	1.95 [1.27, 3.00]
2.5.2 Good prognosis	6	1569	Odds Ratio (M-H, Fixed, 95% CI)	1.08 [0.81, 1.44]
2.6 Multiple pregnancy rate per woman grouped by extent of assisted hatching	16		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.6.1 Thinning only	6	2148	Odds Ratio (M-H, Fixed, 95% CI)	1.34 [1.02, 1.76]
2.6.2 Breach by hole	9	1629	Odds Ratio (M-H, Fixed, 95% CI)	1.51 [1.08, 2.11]
2.6.3 Complete removal of zona	1	25	Odds Ratio (M-H, Fixed, 95% CI)	0.62 [0.07, 5.28]
2.7 Fresh or frozen embryo transfer	16	3190	Odds Ratio (M-H, Fixed, 95% CI)	1.38 [1.08, 1.75]
2.7.1 Fresh	13	2264	Odds Ratio (M-H, Fixed, 95% CI)	1.30 [0.98, 1.73]
2.7.2 Frozen	3	926	Odds Ratio (M-H, Fixed, 95% CI)	1.60 [1.00, 2.55]
2.8 Multiple pregnancy per pregnancy	17	1598	Odds Ratio (M-H, Fixed, 95% CI)	1.37 [1.09, 1.72]

Analysis 2.1. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 1: Multiple pregnancy rate per woman randomised

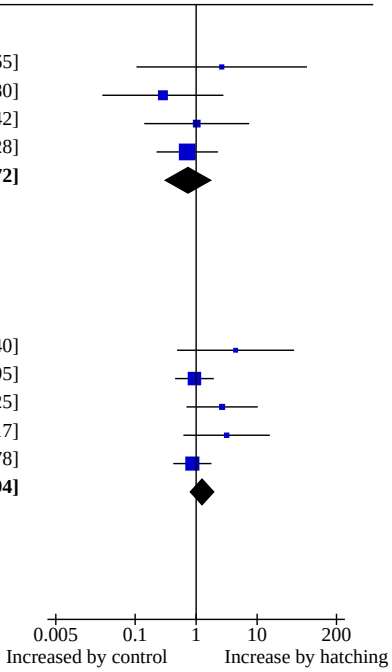
Study or Subgroup	Assisted hatching Events	Assisted hatching Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	5	169	1	166	0.6%	5.03 [0.58 , 43.53]
Balaban 2006	31	183	8	183	4.0%	4.46 [1.99 , 10.00]
Balakier 2009	7	45	4	39	2.2%	1.61 [0.43 , 5.98]
Carter 2003	21	121	15	82	8.8%	0.94 [0.45 , 1.95]
Cohen 1992	45	149	27	151	11.2%	1.99 [1.15 , 3.42]
Ge 2008	77	487	61	473	31.1%	1.27 [0.88 , 1.82]
Germond 2004	1	84	3	74	1.9%	0.29 [0.03 , 2.80]
González-Ortega 2015	8	154	3	149	1.7%	2.67 [0.69 , 10.25]
Hellebaut 1996	5	60	7	60	3.8%	0.69 [0.21 , 2.30]
Isik 2000	2	15	2	10	1.2%	0.62 [0.07 , 5.28]
Isiklar 1999	10	22	2	22	0.7%	8.33 [1.56 , 44.64]
Lanzendorf 1998	2	41	2	48	1.0%	1.18 [0.16 , 8.77]
Ng 2005	6	80	2	80	1.1%	3.16 [0.62 , 16.17]
Razi 2013	2	90	2	92	1.2%	1.02 [0.14 , 7.42]
Sagoskin 2007	21	121	16	82	9.4%	0.87 [0.42 , 1.78]
Shi 2016	5	82	8	96	4.1%	0.71 [0.22 , 2.28]
Valojerdi 2010	11	200	21	200	11.9%	0.50 [0.23 , 1.06]
Wan 2014	12	96	8	102	4.1%	1.68 [0.65 , 4.30]
Total (95% CI)		**2199**		**2109**	**100.0%**	**1.38 [1.13 , 1.68]**
Total events:	271		192			

Heterogeneity: Chi² = 32.65, df = 17 (P = 0.01); I² = 48%
Test for overall effect: Z = 3.17 (P = 0.002)
Test for subgroup differences: Not applicable



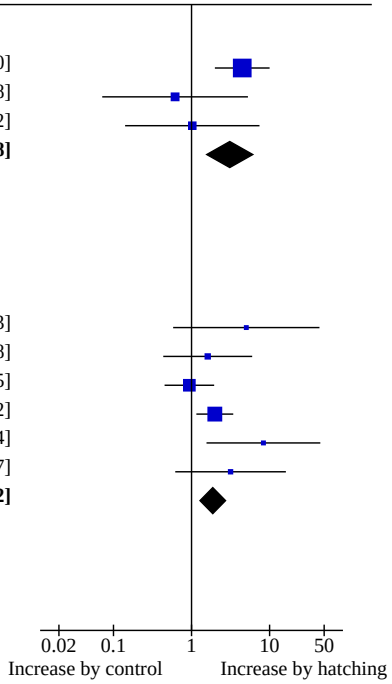
Analysis 2.2. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 2: First or repeat attempt

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.2.1 First attempt at IVF or ICSI						
Antinori 1999	1	73	0	63	4.2%	2.63 [0.11 , 65.65]
Germond 2004	1	84	3	74	25.1%	0.29 [0.03 , 2.80]
Razi 2013	2	90	2	92	15.4%	1.02 [0.14 , 7.42]
Shi 2016	5	82	8	96	55.2%	0.71 [0.22 , 2.28]
Subtotal (95% CI)		**329**		**325**	**100.0%**	**0.73 [0.31 , 1.72]**
Total events:	9		13			
Heterogeneity: Chi² = 1.37, df = 3 (P = 0.71); I² = 0%						
Test for overall effect: Z = 0.71 (P = 0.48)						
2.2.2 Repeat attempt at IVF or ICSI						
Antinori 1999	4	96	1	103	2.6%	4.43 [0.49 , 40.40]
Carter 2003	21	121	15	82	40.8%	0.94 [0.45 , 1.95]
González-Ortega 2015	8	154	3	149	8.0%	2.67 [0.69 , 10.25]
Ng 2005	6	80	2	80	5.1%	3.16 [0.62 , 16.17]
Sagoskin 2007	21	121	16	82	43.5%	0.87 [0.42 , 1.78]
Subtotal (95% CI)		**572**		**496**	**100.0%**	**1.25 [0.80 , 1.94]**
Total events:	60		37			
Heterogeneity: Chi² = 5.30, df = 4 (P = 0.26); I² = 25%						
Test for overall effect: Z = 0.99 (P = 0.32)						
Test for subgroup differences: Chi² = 1.18, df = 1 (P = 0.28), I² = 15.1%						



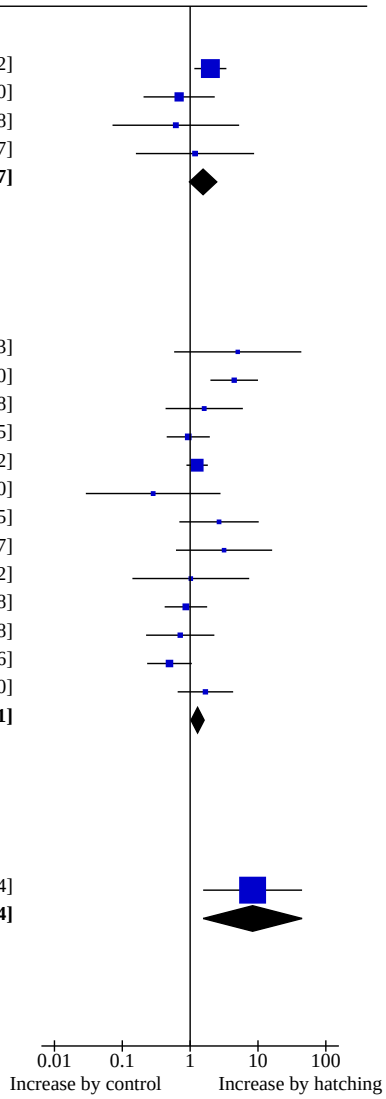
Analysis 2.3. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 3: Conception mode

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.3.1 ICSI only						
Balaban 2006	31	183	8	183	62.3%	4.46 [1.99 , 10.00]
Isik 2000	2	15	2	10	19.5%	0.62 [0.07 , 5.28]
Razi 2013	2	90	2	92	18.1%	1.02 [0.14 , 7.42]
Subtotal (95% CI)		**288**		**285**	**100.0%**	**3.09 [1.57 , 6.08]**
Total events:	35		12			
Heterogeneity: Chi² = 4.16, df = 2 (P = 0.13); I² = 52%						
Test for overall effect: Z = 3.26 (P = 0.001)						
2.3.2 IVF only						
Antinori 1999	5	169	1	166	2.4%	5.03 [0.58 , 43.53]
Balakier 2009	7	45	4	39	8.8%	1.61 [0.43 , 5.98]
Carter 2003	21	121	15	82	36.0%	0.94 [0.45 , 1.95]
Cohen 1992	45	149	27	151	45.6%	1.99 [1.15 , 3.42]
Isiklar 1999	10	22	2	22	2.7%	8.33 [1.56 , 44.64]
Ng 2005	6	80	2	80	4.5%	3.16 [0.62 , 16.17]
Subtotal (95% CI)		**586**		**540**	**100.0%**	**1.87 [1.28 , 2.72]**
Total events:	94		51			
Heterogeneity: Chi² = 7.77, df = 5 (P = 0.17); I² = 36%						
Test for overall effect: Z = 3.27 (P = 0.001)						
Test for subgroup differences: Chi² = 1.61, df = 1 (P = 0.21), I² = 37.7%						



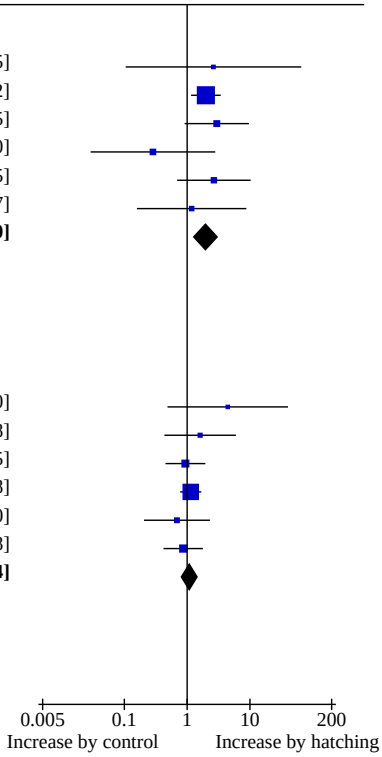
Analysis 2.4. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 4: Hatching method

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.4.1 Chemical						
Cohen 1992	45	149	27	151	64.6%	1.99 [1.15 , 3.42]
Hellebaut 1996	5	60	7	60	22.1%	0.69 [0.21 , 2.30]
Isik 2000	2	15	2	10	7.2%	0.62 [0.07 , 5.28]
Lanzendorf 1998	2	41	2	48	6.1%	1.18 [0.16 , 8.77]
Subtotal (95% CI)		**265**		**269**	**100.0%**	**1.55 [0.98 , 2.47]**
Total events:	54		38			
Heterogeneity: Chi² = 3.32, df = 3 (P = 0.35); I² = 10%						
Test for overall effect: Z = 1.86 (P = 0.06)						
2.4.2 Laser						
Antinori 1999	5	169	1	166	0.7%	5.03 [0.58 , 43.53]
Balaban 2006	31	183	8	183	4.8%	4.46 [1.99 , 10.00]
Balakier 2009	7	45	4	39	2.6%	1.61 [0.43 , 5.98]
Carter 2003	21	121	15	82	10.8%	0.94 [0.45 , 1.95]
Ge 2008	77	487	61	473	38.0%	1.27 [0.88 , 1.82]
Germond 2004	1	84	3	74	2.3%	0.29 [0.03 , 2.80]
González-Ortega 2015	8	154	3	149	2.1%	2.67 [0.69 , 10.25]
Ng 2005	6	80	2	80	1.3%	3.16 [0.62 , 16.17]
Razi 2013	2	90	2	92	1.4%	1.02 [0.14 , 7.42]
Sagoskin 2007	21	121	16	82	11.5%	0.87 [0.42 , 1.78]
Shi 2016	5	82	8	96	5.0%	0.71 [0.22 , 2.28]
Valojerdi 2010	11	200	21	200	14.5%	0.50 [0.23 , 1.06]
Wan 2014	12	96	8	102	4.9%	1.68 [0.65 , 4.30]
Subtotal (95% CI)		**1912**		**1818**	**100.0%**	**1.29 [1.03 , 1.61]**
Total events:	207		152			
Heterogeneity: Chi² = 24.04, df = 12 (P = 0.02); I² = 50%						
Test for overall effect: Z = 2.22 (P = 0.03)						
2.4.3 Mechanical						
Isiklar 1999	10	22	2	22	100.0%	8.33 [1.56 , 44.64]
Subtotal (95% CI)		**22**		**22**	**100.0%**	**8.33 [1.56 , 44.64]**
Total events:	10		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.48 (P = 0.01)						
Test for subgroup differences: Chi² = 5.03, df = 2 (P = 0.08), I² = 60.2%						



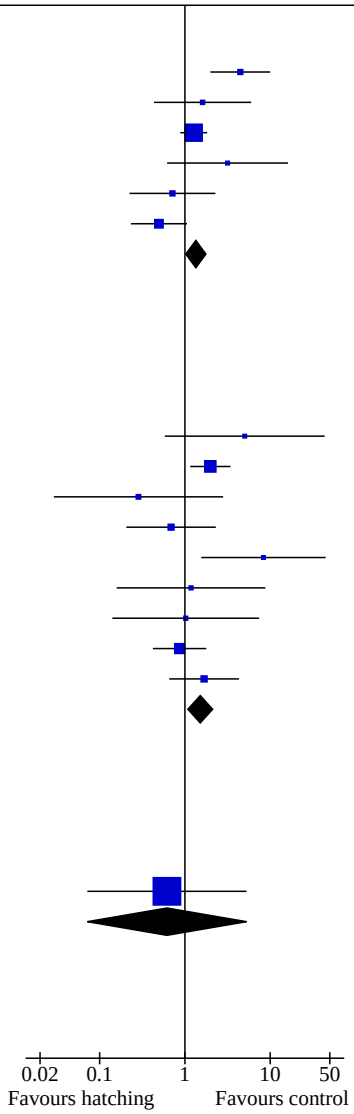
Analysis 2.5. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 5: Prognosis

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.5.1 Poor prognosis						
Antinori 1999	1	73	0	63	1.7%	2.63 [0.11 , 65.65]
Cohen 1992	45	149	27	151	61.2%	1.99 [1.15 , 3.42]
Ge 2008	11	100	4	100	11.6%	2.97 [0.91 , 9.65]
Germond 2004	1	84	3	74	10.3%	0.29 [0.03 , 2.80]
González-Ortega 2015	8	154	3	149	9.4%	2.67 [0.69 , 10.25]
Lanzendorf 1998	2	41	2	48	5.7%	1.18 [0.16 , 8.77]
Subtotal (95% CI)		**601**		**585**	**100.0%**	**1.95 [1.27 , 3.00]**
Total events:	68		39			
Heterogeneity: Chi² = 3.69, df = 5 (P = 0.59); I² = 0%						
Test for overall effect: Z = 3.06 (P = 0.002)						
2.5.2 Good prognosis						
Antinori 1999	4	96	1	103	1.0%	4.43 [0.49 , 40.40]
Balakier 2009	7	45	4	39	4.0%	1.61 [0.43 , 5.98]
Carter 2003	21	121	15	82	16.5%	0.94 [0.45 , 1.95]
Ge 2008	66	387	57	373	53.7%	1.14 [0.77 , 1.68]
Hellebaut 1996	5	60	7	60	7.2%	0.69 [0.21 , 2.30]
Sagoskin 2007	21	121	16	82	17.6%	0.87 [0.42 , 1.78]
Subtotal (95% CI)		**830**		**739**	**100.0%**	**1.08 [0.81 , 1.44]**
Total events:	124		100			
Heterogeneity: Chi² = 3.04, df = 5 (P = 0.69); I² = 0%						
Test for overall effect: Z = 0.52 (P = 0.60)						
Test for subgroup differences: Chi² = 5.08, df = 1 (P = 0.02), I² = 80.3%						



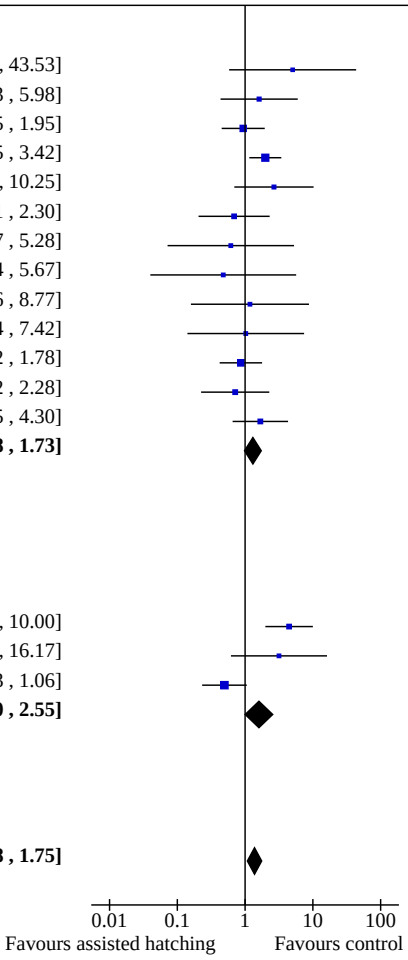
Analysis 2.6. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 6: Multiple pregnancy rate per woman grouped by extent of assisted hatching

Study or Subgroup	Hatching Events	Hatching Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.6.1 Thinning only						
Balaban 2006	31	183	8	183	7.3%	4.46 [1.99 , 10.00]
Balakier 2009	7	45	4	39	4.0%	1.61 [0.43 , 5.98]
Ge 2008	77	487	61	473	57.3%	1.27 [0.88 , 1.82]
Ng 2005	6	80	2	80	2.0%	3.16 [0.62 , 16.17]
Shi 2016	5	82	8	96	7.6%	0.71 [0.22 , 2.28]
Valojerdi 2010	11	200	21	200	21.8%	0.50 [0.23 , 1.06]
Subtotal (95% CI)		**1077**		**1071**	**100.0%**	**1.34 [1.02 , 1.76]**
Total events:	137		104			
Heterogeneity: Chi² = 17.51, df = 5 (P = 0.004); I² = 71%						
Test for overall effect: Z = 2.13 (P = 0.03)						
2.6.2 Breach by hole						
Antinori 1999	5	169	1	166	1.7%	5.03 [0.58 , 43.53]
Cohen 1992	45	149	27	151	33.1%	1.99 [1.15 , 3.42]
Germond 2004	1	84	3	74	5.6%	0.29 [0.03 , 2.80]
Hellebaut 1996	5	60	7	60	11.3%	0.69 [0.21 , 2.30]
Isiklar 1999	10	22	2	22	1.9%	8.33 [1.56 , 44.64]
Lanzendorf 1998	2	41	2	48	3.1%	1.18 [0.16 , 8.77]
Razi 2013	2	90	2	92	3.4%	1.02 [0.14 , 7.42]
Sagoskin 2007	21	121	16	82	27.9%	0.87 [0.42 , 1.78]
Wan 2014	12	96	8	102	12.0%	1.68 [0.65 , 4.30]
Subtotal (95% CI)		**832**		**797**	**100.0%**	**1.51 [1.08 , 2.11]**
Total events:	103		68			
Heterogeneity: Chi² = 12.36, df = 8 (P = 0.14); I² = 35%						
Test for overall effect: Z = 2.43 (P = 0.02)						
2.6.3 Complete removal of zona						
Isik 2000	2	15	2	10	100.0%	0.62 [0.07 , 5.28]
Subtotal (95% CI)		**15**		**10**	**100.0%**	**0.62 [0.07 , 5.28]**
Total events:	2		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.44 (P = 0.66)						
Test for subgroup differences: Chi² = 0.86, df = 2 (P = 0.65), I² = 0%						



Analysis 2.7. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 7: Fresh or frozen embryo transfer

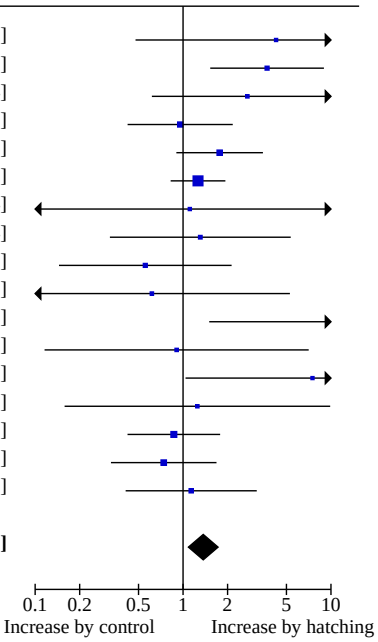
Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.7.1 Fresh						
Antinori 1999	5	169	1	166	0.9%	5.03 [0.58 , 43.53]
Balakier 2009	7	45	4	39	3.2%	1.61 [0.43 , 5.98]
Carter 2003	21	121	15	82	13.1%	0.94 [0.45 , 1.95]
Cohen 1992	45	149	27	151	16.6%	1.99 [1.15 , 3.42]
González-Ortega 2015	8	154	3	149	2.6%	2.67 [0.69 , 10.25]
Hellebaut 1996	5	60	7	60	5.7%	0.69 [0.21 , 2.30]
Isik 2000	2	15	2	10	1.8%	0.62 [0.07 , 5.28]
Isiklar 1999	1	22	2	22	1.7%	0.48 [0.04 , 5.67]
Lanzendorf 1998	2	41	2	48	1.6%	1.18 [0.16 , 8.77]
Razi 2013	2	90	2	92	1.7%	1.02 [0.14 , 7.42]
Sagoskin 2007	21	121	16	82	14.0%	0.87 [0.42 , 1.78]
Shi 2016	5	82	8	96	6.1%	0.71 [0.22 , 2.28]
Wan 2014	12	96	8	102	6.0%	1.68 [0.65 , 4.30]
Subtotal (95% CI)		**1165**		**1099**	**74.9%**	**1.30 [0.98 , 1.73]**
Total events:	136		97			
Heterogeneity: Chi² = 10.57, df = 12 (P = 0.57); I² = 0%						
Test for overall effect: Z = 1.83 (P = 0.07)						
2.7.2 Frozen						
Balaban 2006	31	183	8	183	5.9%	4.46 [1.99 , 10.00]
Ng 2005	6	80	2	80	1.6%	3.16 [0.62 , 16.17]
Valojerdi 2010	11	200	21	200	17.6%	0.50 [0.23 , 1.06]
Subtotal (95% CI)		**463**		**463**	**25.1%**	**1.60 [1.00 , 2.55]**
Total events:	48		31			
Heterogeneity: Chi² = 16.05, df = 2 (P = 0.0003); I² = 88%						
Test for overall effect: Z = 1.97 (P = 0.05)						
Total (95% CI)		**1628**		**1562**	**100.0%**	**1.38 [1.08 , 1.75]**
Total events:	184		128			
Heterogeneity: Chi² = 26.91, df = 15 (P = 0.03); I² = 44%						
Test for overall effect: Z = 2.59 (P = 0.010)						
Test for subgroup differences: Chi² = 0.54, df = 1 (P = 0.46), I² = 0%						



Analysis 2.8. Comparison 2: Multiple pregnancy: assisted hatching compared with no assisted hatching, Outcome 8: Multiple pregnancy per pregnancy

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	5	52	1	41	0.8%	4.26 [0.48 , 37.95]
Balaban 2006	31	75	8	50	4.5%	3.70 [1.53 , 8.96]
Balakier 2009	7	16	4	18	1.7%	2.72 [0.62 , 12.04]
Carter 2003	21	62	15	43	9.4%	0.96 [0.42 , 2.17]
Cohen 1992	45	78	27	62	10.2%	1.77 [0.90 , 3.47]
Ge 2008	77	189	61	173	30.4%	1.26 [0.82 , 1.93]
Germond 2004	1	4	3	13	0.9%	1.11 [0.08 , 15.04]
González-Ortega 2015	8	61	3	29	2.8%	1.31 [0.32 , 5.34]
Hellebaut 1996	5	23	7	21	4.6%	0.56 [0.14 , 2.13]
Isik 2000	2	15	2	10	1.7%	0.62 [0.07 , 5.28]
Isiklar 1999	10	16	1	10	0.4%	15.00 [1.50 , 149.70]
Lanzendorf 1998	2	23	2	21	1.5%	0.90 [0.12 , 7.07]
Ng 2005	6	10	2	12	0.6%	7.50 [1.04 , 54.12]
Razi 2013	2	18	2	22	1.3%	1.25 [0.16 , 9.88]
Sagoskin 2007	21	121	16	82	12.7%	0.87 [0.42 , 1.78]
Valojerdi 2010	11	57	21	86	10.9%	0.74 [0.33 , 1.68]
Wan 2014	12	49	8	36	5.6%	1.14 [0.41 , 3.15]
Total (95% CI)		**869**		**729**	**100.0%**	**1.37 [1.09 , 1.72]**
Total events:	266		183			

Heterogeneity: Chi² = 21.42, df = 16 (P = 0.16); I² = 25%
Test for overall effect: Z = 2.67 (P = 0.008)
Test for subgroup differences: Not applicable



Comparison 3. Clinical pregnancy: assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 Clinical pregnancy rate per woman randomised	39	7249	Odds Ratio (M-H, Fixed, 95% CI)	1.20 [1.09, 1.33]
3.2 First or repeat attempt	18		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.2.1 First attempt at IVF or ICSI	8	1010	Odds Ratio (M-H, Fixed, 95% CI)	0.74 [0.56, 0.98]
3.2.2 Repeat attempt at IVF or ICSI	11	1798	Odds Ratio (M-H, Fixed, 95% CI)	1.65 [1.34, 2.04]
3.3 Conception mode	26		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.3.1 ICSI only	11	1825	Odds Ratio (M-H, Fixed, 95% CI)	1.40 [1.14, 1.71]
3.3.2 IVF only	15	2460	Odds Ratio (M-H, Fixed, 95% CI)	1.31 [1.10, 1.55]
3.4 Hatching method	39		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.4.1 Chemical	11	1536	Odds Ratio (M-H, Fixed, 95% CI)	1.33 [1.08, 1.64]
3.4.2 Laser	23	5127	Odds Ratio (M-H, Fixed, 95% CI)	1.15 [1.03, 1.30]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.4.3 Mechanical	5	586	Odds Ratio (M-H, Fixed, 95% CI)	1.30 [0.89, 1.88]
3.5 Prognosis	24		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.5.1 Poor prognosis	14	2108	Odds Ratio (M-H, Fixed, 95% CI)	1.68 [1.38, 2.04]
3.5.2 Good prognosis	14	2721	Odds Ratio (M-H, Fixed, 95% CI)	1.10 [0.94, 1.29]
3.6 Extent of assisted hatching	37		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.6.1 Thinning only	17	3774	Odds Ratio (M-H, Fixed, 95% CI)	1.10 [0.96, 1.26]
3.6.2 Breach by hole only	17	2543	Odds Ratio (M-H, Fixed, 95% CI)	1.17 [0.98, 1.39]
3.6.3 Complete removal of zona	2	301	Odds Ratio (M-H, Fixed, 95% CI)	1.93 [1.21, 3.09]
3.6.4 Expansion of zona pellucida	1	125	Odds Ratio (M-H, Fixed, 95% CI)	2.37 [1.07, 5.28]
3.7 Fresh and frozen embryo transfer	38		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.7.1 Fresh embryo transfer	30	5349	Odds Ratio (M-H, Fixed, 95% CI)	1.23 [1.10, 1.38]
3.7.2 Frozen embryo transfer only	10	1700	Odds Ratio (M-H, Fixed, 95% CI)	1.15 [0.93, 1.42]

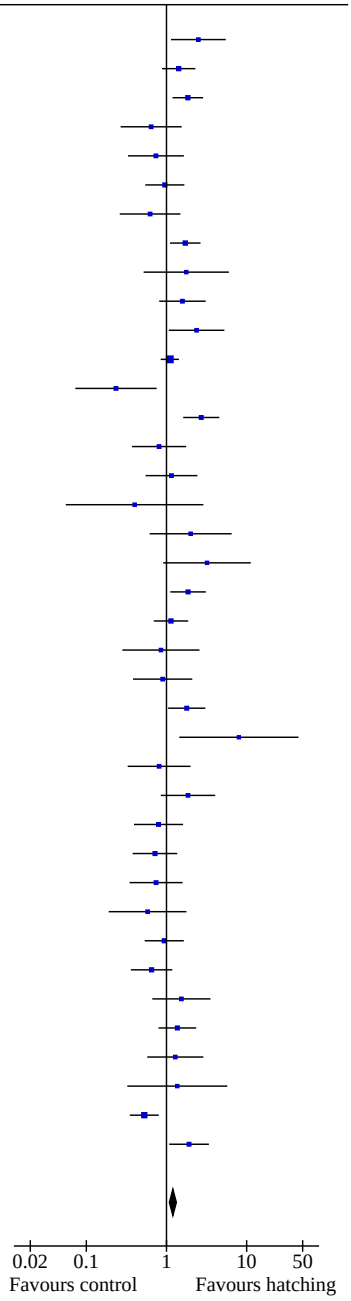
Analysis 3.1. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 1: Clinical pregnancy rate per woman randomised

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Abulsoud 2019	25	65	13	65	1.1%	2.50 [1.14 , 5.49]
Antinori 1999	52	169	41	172	4.0%	1.42 [0.88 , 2.29]
Balaban 2006	75	183	50	183	4.2%	1.85 [1.19 , 2.86]
Balakier 2009	16	45	18	39	1.8%	0.64 [0.27 , 1.55]
Baruffi 2000	17	51	21	52	2.0%	0.74 [0.33 , 1.65]
Carter 2003	62	121	43	82	3.5%	0.95 [0.54 , 1.67]
Ciray 2005	17	76	12	38	1.7%	0.62 [0.26 , 1.49]
Cohen 1992	85	164	64	166	4.3%	1.71 [1.11 , 2.66]
Elhelw 2005	8	37	5	37	0.6%	1.77 [0.52 , 6.01]
Elnahas 2017	30	80	22	80	1.9%	1.58 [0.81 , 3.08]
Fang 2010	23	61	13	64	1.1%	2.37 [1.07 , 5.28]
Ge 2008	189	487	173	473	15.1%	1.10 [0.85 , 1.43]
Germond 2004	4	84	13	74	1.9%	0.23 [0.07 , 0.76]
González-Ortega 2015	61	154	29	149	2.5%	2.71 [1.62 , 4.56]
Hagemann 2010	21	49	26	54	2.0%	0.81 [0.37 , 1.76]
Hellebaut 1996	23	60	21	60	1.8%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	0.4%	0.40 [0.06 , 2.89]
Isik 2000	15	24	10	22	0.6%	2.00 [0.62 , 6.49]
Isiklar 1999	16	22	10	22	0.4%	3.20 [0.91 , 11.27]
Jelinkova 2002	59	128	40	127	3.0%	1.86 [1.12 , 3.10]
Kutlu 2010	67	131	58	121	4.2%	1.14 [0.69 , 1.86]
Laffoon 1999	9	28	10	28	1.0%	0.85 [0.28 , 2.58]
Lanzendorf 1998	16	41	20	48	1.6%	0.90 [0.38 , 2.10]
Nada 2018	46	158	28	150	2.9%	1.79 [1.05 , 3.06]
Nagy 1999	10	20	2	18	0.1%	8.00 [1.44 , 44.30]
Ng 2005	10	80	12	80	1.5%	0.81 [0.33 , 2.00]
Petersen 2005	21	75	13	75	1.3%	1.85 [0.85 , 4.05]
Razi 2013	18	90	22	92	2.5%	0.80 [0.39 , 1.61]
Rufas-Sapir 2004	22	104	28	103	3.1%	0.72 [0.38 , 1.36]
Ryan 1997	14	100	18	100	2.2%	0.74 [0.35 , 1.59]
Safari 2017	7	30	11	32	1.1%	0.58 [0.19 , 1.78]
Sagoskin 2007	63	121	44	82	3.5%	0.94 [0.53 , 1.65]
Shi 2016	40	82	57	96	3.8%	0.65 [0.36 , 1.18]
Stein 1995	15	72	12	82	1.3%	1.54 [0.67 , 3.54]
Tucker 1993	49	110	40	108	3.2%	1.37 [0.79 , 2.35]
Tucker 1996	21	50	18	50	1.5%	1.29 [0.58 , 2.88]
Utsunomiya 1998	5	27	4	28	0.5%	1.36 [0.32 , 5.73]
Valojerdi 2010	57	200	86	200	8.7%	0.53 [0.35 , 0.80]
Wan 2014	49	96	36	102	2.4%	1.91 [1.08 , 3.38]
Total (95% CI)		**3688**		**3561**	**100.0%**	**1.20 [1.09 , 1.33]**
Total events:	1340		1146			

Heterogeneity: Chi² = 85.10, df = 38 (P < 0.0001); I² = 55%

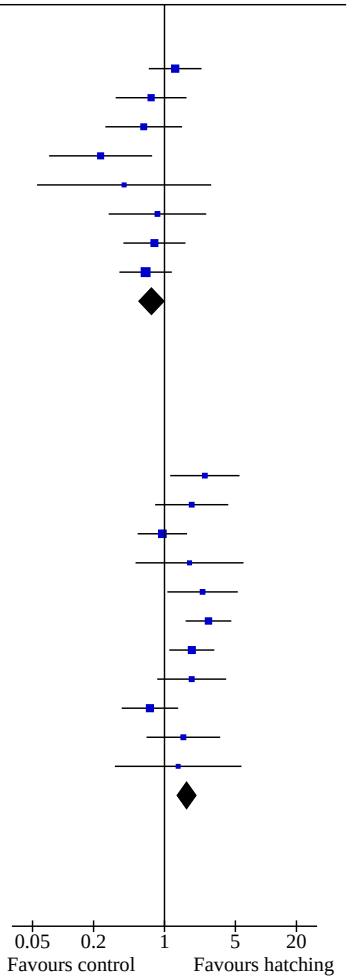
Test for overall effect: Z = 3.61 (P = 0.0003)

Test for subgroup differences: Not applicable



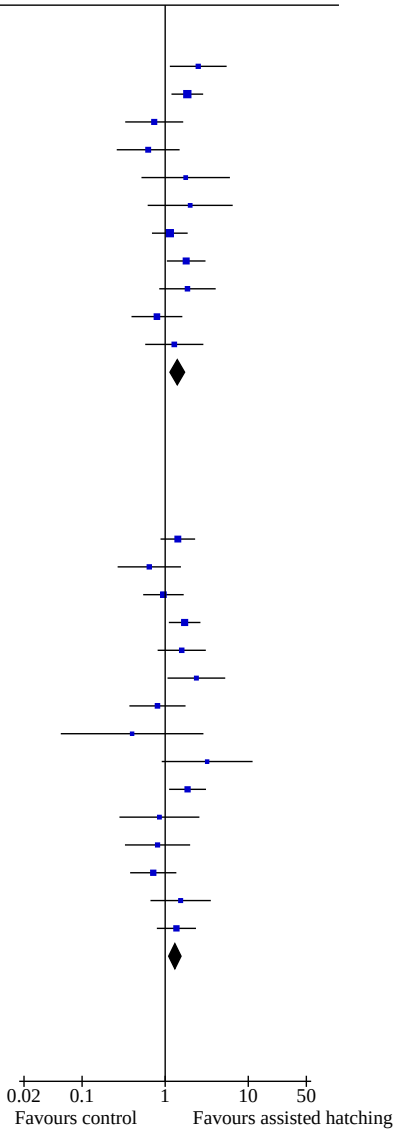
Analysis 3.2. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 2: First or repeat attempt

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.2.1 First attempt at IVF or ICSI						
Antinori 1999	33	96	30	103	16.9%	1.27 [0.70 , 2.32]
Baruffi 2000	17	51	21	52	12.3%	0.74 [0.33 , 1.65]
Ciray 2005	17	76	12	38	11.0%	0.62 [0.26 , 1.49]
Germond 2004	4	84	13	74	11.7%	0.23 [0.07 , 0.76]
Hurst 1998	3	13	3	7	2.7%	0.40 [0.06 , 2.89]
Laffoon 1999	9	28	10	28	6.0%	0.85 [0.28 , 2.58]
Razi 2013	18	90	22	92	15.5%	0.80 [0.39 , 1.61]
Shi 2016	40	82	57	96	23.9%	0.65 [0.36 , 1.18]
Subtotal (95% CI)		**520**		**490**	**100.0%**	**0.74 [0.56 , 0.98]**
Total events:	141		168			
Heterogeneity: Chi² = 7.67, df = 7 (P = 0.36); I² = 9%						
Test for overall effect: Z = 2.07 (P = 0.04)						
3.2.2 Repeat attempt at IVF or ICSI						
Abulsoud 2019	25	65	13	65	5.9%	2.50 [1.14 , 5.49]
Antinori 1999	19	73	11	69	6.1%	1.86 [0.81 , 4.25]
Carter 2003	62	121	43	82	18.3%	0.95 [0.54 , 1.67]
Elhelw 2005	8	37	5	37	2.9%	1.77 [0.52 , 6.01]
Fang 2010	23	61	13	64	5.8%	2.37 [1.07 , 5.28]
González-Ortega 2015	61	154	29	149	13.1%	2.71 [1.62 , 4.56]
Jelinkova 2002	59	128	40	127	15.9%	1.86 [1.12 , 3.10]
Petersen 2005	21	75	13	75	6.9%	1.85 [0.85 , 4.05]
Rufas-Sapir 2004	22	104	28	103	16.3%	0.72 [0.38 , 1.36]
Stein 1995	15	72	12	82	6.5%	1.54 [0.67 , 3.54]
Utsunomiya 1998	5	27	4	28	2.3%	1.36 [0.32 , 5.73]
Subtotal (95% CI)		**917**		**881**	**100.0%**	**1.65 [1.34 , 2.04]**
Total events:	320		211			
Heterogeneity: Chi² = 16.04, df = 10 (P = 0.10); I² = 38%						
Test for overall effect: Z = 4.67 (P < 0.00001)						
Test for subgroup differences: Chi² = 19.82, df = 1 (P < 0.00001), I² = 95.0%						



Analysis 3.3. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 3: Conception mode

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.3.1 ICSI only						
Abulsoud 2019	25	65	13	65	5.0%	2.50 [1.14 , 5.49]
Balaban 2006	75	183	50	183	18.6%	1.85 [1.19 , 2.86]
Baruffi 2000	17	51	21	52	8.7%	0.74 [0.33 , 1.65]
Ciray 2005	17	76	12	38	7.8%	0.62 [0.26 , 1.49]
Elhelw 2005	8	37	5	37	2.5%	1.77 [0.52 , 6.01]
Isik 2000	15	24	10	22	2.5%	2.00 [0.62 , 6.49]
Kutlu 2010	67	131	58	121	18.6%	1.14 [0.69 , 1.86]
Nada 2018	46	158	28	150	12.8%	1.79 [1.05 , 3.06]
Petersen 2005	21	75	13	75	5.9%	1.85 [0.85 , 4.05]
Razi 2013	18	90	22	92	11.0%	0.80 [0.39 , 1.61]
Tucker 1996	21	50	18	50	6.6%	1.29 [0.58 , 2.88]
Subtotal (95% CI)		**940**		**885**	**100.0%**	**1.40 [1.14 , 1.71]**
Total events:	330		250			
Heterogeneity: Chi² = 14.35, df = 10 (P = 0.16); I² = 30%						
Test for overall effect: Z = 3.24 (P = 0.001)						
3.3.2 IVF only						
Antinori 1999	52	169	41	172	12.2%	1.42 [0.88 , 2.29]
Balakier 2009	16	45	18	39	5.4%	0.64 [0.27 , 1.55]
Carter 2003	62	121	43	82	10.9%	0.95 [0.54 , 1.67]
Cohen 1992	85	164	64	166	13.3%	1.71 [1.11 , 2.66]
Elnahas 2017	30	80	22	80	6.0%	1.58 [0.81 , 3.08]
Fang 2010	23	61	13	64	3.4%	2.37 [1.07 , 5.28]
Hagemann 2010	21	49	26	54	6.1%	0.81 [0.37 , 1.76]
Hurst 1998	3	13	3	7	1.3%	0.40 [0.06 , 2.89]
Isiklar 1999	16	22	10	22	1.2%	3.20 [0.91 , 11.27]
Jelinkova 2002	59	128	40	127	9.4%	1.86 [1.12 , 3.10]
Laffoon 1999	9	28	10	28	2.9%	0.85 [0.28 , 2.58]
Ng 2005	10	80	12	80	4.6%	0.81 [0.33 , 2.00]
Rufas-Sapir 2004	22	104	28	103	9.6%	0.72 [0.38 , 1.36]
Stein 1995	15	72	12	82	3.9%	1.54 [0.67 , 3.54]
Tucker 1993	49	110	40	108	9.7%	1.37 [0.79 , 2.35]
Subtotal (95% CI)		**1246**		**1214**	**100.0%**	**1.31 [1.10 , 1.55]**
Total events:	472		382			
Heterogeneity: Chi² = 19.57, df = 14 (P = 0.14); I² = 28%						
Test for overall effect: Z = 3.07 (P = 0.002)						
Test for subgroup differences: Chi² = 0.25, df = 1 (P = 0.62), I² = 0%						

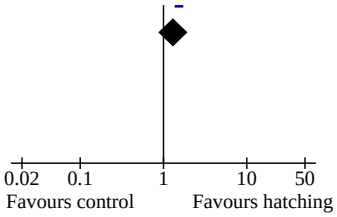


Analysis 3.4. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 4: Hatching method

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.4.1 Chemical						
Cohen 1992	85	164	64	166	20.6%	1.71 [1.11 , 2.66]
Hagemann 2010	21	49	26	54	9.5%	0.81 [0.37 , 1.76]
Hellebaut 1996	23	60	21	60	8.7%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	2.0%	0.40 [0.06 , 2.89]
Isik 2000	15	24	10	22	2.6%	2.00 [0.62 , 6.49]
Jelinkova 2002	59	128	40	127	14.5%	1.86 [1.12 , 3.10]
Lanzendorf 1998	16	41	20	48	7.5%	0.90 [0.38 , 2.10]
Ryan 1997	14	100	18	100	10.4%	0.74 [0.35 , 1.59]
Tucker 1993	49	110	40	108	15.0%	1.37 [0.79 , 2.35]
Tucker 1996	21	50	18	50	7.0%	1.29 [0.58 , 2.88]
Utsunomiya 1998	5	27	4	28	2.1%	1.36 [0.32 , 5.73]
Subtotal (95% CI)		**766**		**770**	**100.0%**	**1.33 [1.08 , 1.64]**
Total events:	311		264			
Heterogeneity: Chi² = 9.66, df = 10 (P = 0.47); I² = 0%						
Test for overall effect: Z = 2.64 (P = 0.008)						
3.4.2 Laser						
Abulsoud 2019	25	65	13	65	1.6%	2.50 [1.14 , 5.49]
Antinori 1999	52	169	41	172	5.5%	1.42 [0.88 , 2.29]
Balaban 2006	75	183	50	183	5.8%	1.85 [1.19 , 2.86]
Balakier 2009	16	45	18	39	2.4%	0.64 [0.27 , 1.55]
Baruffi 2000	17	51	21	52	2.7%	0.74 [0.33 , 1.65]
Carter 2003	62	121	43	82	4.9%	0.95 [0.54 , 1.67]
Ciray 2005	17	76	12	38	2.4%	0.62 [0.26 , 1.49]
Elhelw 2005	8	37	5	37	0.8%	1.77 [0.52 , 6.01]
Elnahas 2017	30	80	22	80	2.7%	1.58 [0.81 , 3.08]
Ge 2008	189	487	173	473	21.0%	1.10 [0.85 , 1.43]
Germond 2004	4	84	13	74	2.6%	0.23 [0.07 , 0.76]
González-Ortega 2015	61	154	29	149	3.5%	2.71 [1.62 , 4.56]
Kutlu 2010	67	131	58	121	5.8%	1.14 [0.69 , 1.86]
Nada 2018	46	158	28	150	4.0%	1.79 [1.05 , 3.06]
Nagy 1999	10	20	2	18	0.2%	8.00 [1.44 , 44.30]
Ng 2005	10	80	12	80	2.0%	0.81 [0.33 , 2.00]
Petersen 2005	21	75	13	75	1.8%	1.85 [0.85 , 4.05]
Razi 2013	18	90	22	92	3.4%	0.80 [0.39 , 1.61]
Safari 2017	7	30	11	32	1.6%	0.58 [0.19 , 1.78]
Sagoskin 2007	63	121	44	82	4.9%	0.94 [0.53 , 1.65]
Shi 2016	40	82	57	96	5.3%	0.65 [0.36 , 1.18]
Valojerdi 2010	57	200	86	200	12.0%	0.53 [0.35 , 0.80]
Wan 2014	49	96	36	102	3.3%	1.91 [1.08 , 3.38]
Subtotal (95% CI)		**2635**		**2492**	**100.0%**	**1.15 [1.03 , 1.30]**
Total events:	944		809			
Heterogeneity: Chi² = 65.82, df = 22 (P < 0.00001); I² = 67%						
Test for overall effect: Z = 2.38 (P = 0.02)						
3.4.3 Mechanical						
Fang 2010	23	61	13	64	16.3%	2.37 [1.07 , 5.28]
Isiklar 1999	16	22	10	22	5.6%	3.20 [0.91 , 11.27]
Laffoon 1999	9	28	10	28	14.0%	0.85 [0.28 , 2.58]
Rufas-Sapir 2004	22	104	28	103	45.8%	0.72 [0.38 , 1.36]
Stein 1995	15	72	12	82	18.3%	1.54 [0.67 , 3.54]
Subtotal (95% CI)		**287**		**299**	**100.0%**	**1.30 [0.89 , 1.88]**
Total events:	85		73			

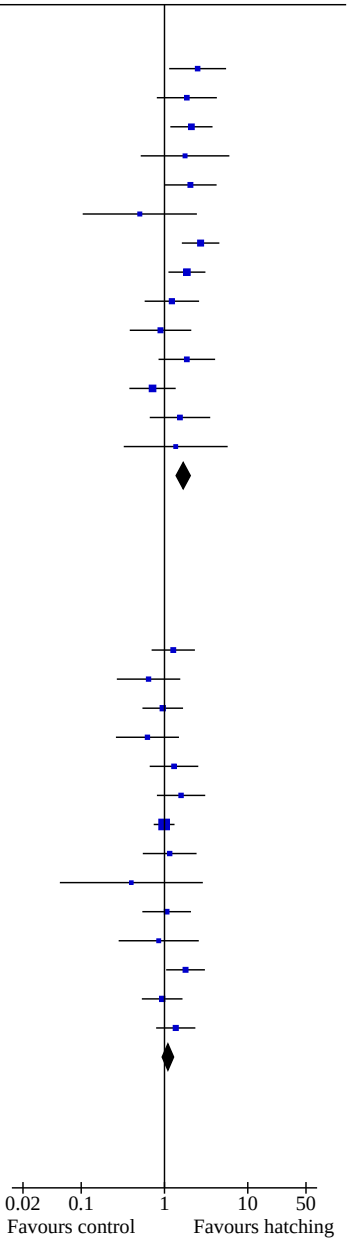
Analysis 3.4. (Continued)

Subtotal (95% CI)	**287**	**299**	**100.0%**	**1.30 [0.89 , 1.88]**	
Total events:	85	73			
Heterogeneity: Chi² = 8.15, df = 4 (P = 0.09); I² = 51%					
Test for overall effect: Z = 1.36 (P = 0.17)					
Test for subgroup differences: Chi² = 1.47, df = 2 (P = 0.48), I² = 0%					



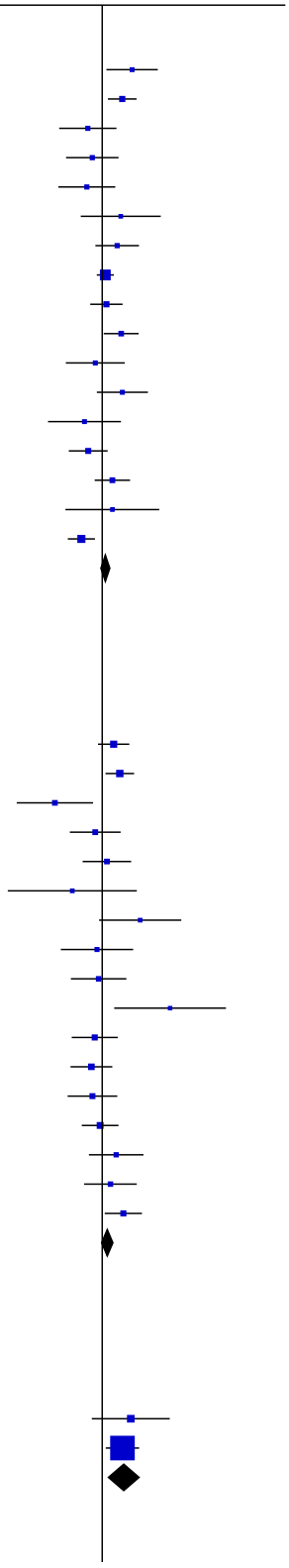
Analysis 3.5. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 5: Prognosis

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.5.1 Poor prognosis						
Abulsoud 2019	25	65	13	65	5.1%	2.50 [1.14 , 5.49]
Antinori 1999	19	73	11	69	5.3%	1.86 [0.81 , 4.25]
Cohen 1992	48	95	32	98	9.9%	2.11 [1.18 , 3.77]
Elhelw 2005	8	37	5	37	2.5%	1.77 [0.52 , 6.01]
Ge 2008	25	100	14	100	6.7%	2.05 [0.99 , 4.22]
Germond 2004	3	22	5	21	2.8%	0.51 [0.10 , 2.45]
González-Ortega 2015	61	154	29	149	11.3%	2.71 [1.62 , 4.56]
Jelinkova 2002	59	128	40	127	13.8%	1.86 [1.12 , 3.10]
Kutlu 2010	25	58	21	55	7.8%	1.23 [0.58 , 2.60]
Lanzendorf 1998	16	41	20	48	7.1%	0.90 [0.38 , 2.10]
Petersen 2005	21	75	13	75	5.9%	1.85 [0.85 , 4.05]
Rufas-Sapir 2004	22	104	28	103	14.1%	0.72 [0.38 , 1.36]
Stein 1995	15	72	12	82	5.6%	1.54 [0.67 , 3.54]
Utsunomiya 1998	5	27	4	28	2.0%	1.36 [0.32 , 5.73]
Subtotal (95% CI)		**1051**		**1057**	**100.0%**	**1.68 [1.38 , 2.04]**
Total events:	352		247			
Heterogeneity: Chi² = 17.29, df = 13 (P = 0.19); I² = 25%						
Test for overall effect: Z = 5.21 (P < 0.00001)						
3.5.2 Good prognosis						
Antinori 1999	33	96	30	103	6.4%	1.27 [0.70 , 2.32]
Balakier 2009	16	45	18	39	4.2%	0.64 [0.27 , 1.55]
Carter 2003	62	121	43	82	8.4%	0.95 [0.54 , 1.67]
Ciray 2005	17	76	12	38	4.2%	0.62 [0.26 , 1.49]
Cohen 1992	37	69	32	68	5.0%	1.30 [0.66 , 2.55]
Elnahas 2017	30	80	22	80	4.6%	1.58 [0.81 , 3.08]
Ge 2008	164	387	159	373	31.3%	0.99 [0.74 , 1.32]
Hellebaut 1996	23	60	21	60	4.3%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	1.0%	0.40 [0.06 , 2.89]
Kutlu 2010	42	73	37	66	5.5%	1.06 [0.54 , 2.08]
Laffoon 1999	9	28	10	28	2.3%	0.85 [0.28 , 2.58]
Nada 2018	46	158	28	150	6.8%	1.79 [1.05 , 3.06]
Sagoskin 2007	63	121	44	82	8.4%	0.94 [0.53 , 1.65]
Tucker 1993	49	110	40	108	7.5%	1.37 [0.79 , 2.35]
Subtotal (95% CI)		**1437**		**1284**	**100.0%**	**1.10 [0.94 , 1.29]**
Total events:	594		499			
Heterogeneity: Chi² = 10.77, df = 13 (P = 0.63); I² = 0%						
Test for overall effect: Z = 1.18 (P = 0.24)						
Test for subgroup differences: Chi² = 11.00, df = 1 (P = 0.0009), I² = 90.9%						



Analysis 3.6. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 6: Extent of assisted hatching

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.6.1 Thinning only						
Abulsoud 2019	25	65	13	65	2.0%	2.50 [1.14 , 5.49]
Balaban 2006	75	183	50	183	7.5%	1.85 [1.19 , 2.86]
Balakier 2009	16	45	18	39	3.2%	0.64 [0.27 , 1.55]
Baruffi 2000	17	51	21	52	3.5%	0.74 [0.33 , 1.65]
Ciray 2005	17	76	12	38	3.2%	0.62 [0.26 , 1.49]
Elhelw 2005	8	37	5	37	1.0%	1.77 [0.52 , 6.01]
Elnahas 2017	30	80	22	80	3.5%	1.58 [0.81 , 3.08]
Ge 2008	189	487	173	473	27.3%	1.10 [0.85 , 1.43]
Kutlu 2010	67	131	58	121	7.5%	1.14 [0.69 , 1.86]
Nada 2018	46	158	28	150	5.2%	1.79 [1.05 , 3.06]
Ng 2005	10	80	12	80	2.7%	0.81 [0.33 , 2.00]
Petersen 2005	21	75	13	75	2.4%	1.85 [0.85 , 4.05]
Safari 2017	7	30	11	32	2.1%	0.58 [0.19 , 1.78]
Shi 2016	40	82	57	96	6.8%	0.65 [0.36 , 1.18]
Tucker 1993	49	110	40	108	5.7%	1.37 [0.79 , 2.35]
Utsunomiya 1998	5	27	4	28	0.8%	1.36 [0.32 , 5.73]
Valojerdi 2010	57	200	86	200	15.6%	0.53 [0.35 , 0.80]
Subtotal (95% CI)		**1917**		**1857**	**100.0%**	**1.10 [0.96 , 1.26]**
Total events:	679		623			
Heterogeneity: Chi² = 37.51, df = 16 (P = 0.002); I² = 57%						
Test for overall effect: Z = 1.38 (P = 0.17)						
3.6.2 Breach by hole only						
Antinori 1999	52	169	41	172	11.7%	1.42 [0.88 , 2.29]
Cohen 1992	85	164	64	166	12.7%	1.71 [1.11 , 2.66]
Germond 2004	4	84	13	74	5.5%	0.23 [0.07 , 0.76]
Hagemann 2010	21	49	26	54	5.9%	0.81 [0.37 , 1.76]
Hellebaut 1996	23	60	21	60	5.4%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	1.2%	0.40 [0.06 , 2.89]
Isiklar 1999	16	22	10	22	1.1%	3.20 [0.91 , 11.27]
Laffoon 1999	9	28	10	28	2.8%	0.85 [0.28 , 2.58]
Lanzendorf 1998	16	41	20	48	4.7%	0.90 [0.38 , 2.10]
Nagy 1999	10	20	2	18	0.4%	8.00 [1.44 , 44.30]
Razi 2013	18	90	22	92	7.2%	0.80 [0.39 , 1.61]
Rufas-Sapir 2004	22	104	28	103	9.2%	0.72 [0.38 , 1.36]
Ryan 1997	14	100	18	100	6.4%	0.74 [0.35 , 1.59]
Sagoskin 2007	63	121	44	82	10.5%	0.94 [0.53 , 1.65]
Stein 1995	15	72	12	82	3.7%	1.54 [0.67 , 3.54]
Tucker 1996	21	50	18	50	4.3%	1.29 [0.58 , 2.88]
Wan 2014	49	96	36	102	7.1%	1.91 [1.08 , 3.38]
Subtotal (95% CI)		**1283**		**1260**	**100.0%**	**1.17 [0.98 , 1.39]**
Total events:	441		388			
Heterogeneity: Chi² = 29.47, df = 16 (P = 0.02); I² = 46%						
Test for overall effect: Z = 1.77 (P = 0.08)						
3.6.3 Complete removal of zona						
Isik 2000	16	24	10	22	13.8%	2.40 [0.73 , 7.92]
Jelinkova 2002	59	128	40	127	86.2%	1.86 [1.12 , 3.10]
Subtotal (95% CI)		**152**		**149**	**100.0%**	**1.93 [1.21 , 3.09]**
Total events:	75		50			
Heterogeneity: Chi² = 0.15, df = 1 (P = 0.70); I² = 0%						
Test for overall effect: Z = 2.76 (P = 0.006)						



Analysis 3.6. (Continued)

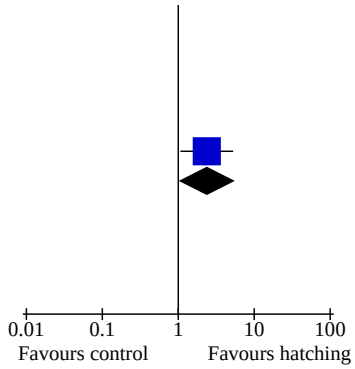
Heterogeneity: Chi² = 0.15, df = 1 (P = 0.70); I² = 0%
Test for overall effect: Z = 2.76 (P = 0.006)

3.6.4 Expansion of zona pellucida

Fang 2010	23	61	13	64	100.0%	2.37 [1.07 , 5.28]
Subtotal (95% CI)		**61**		**64**	**100.0%**	**2.37 [1.07 , 5.28]**
Total events:	23		13			

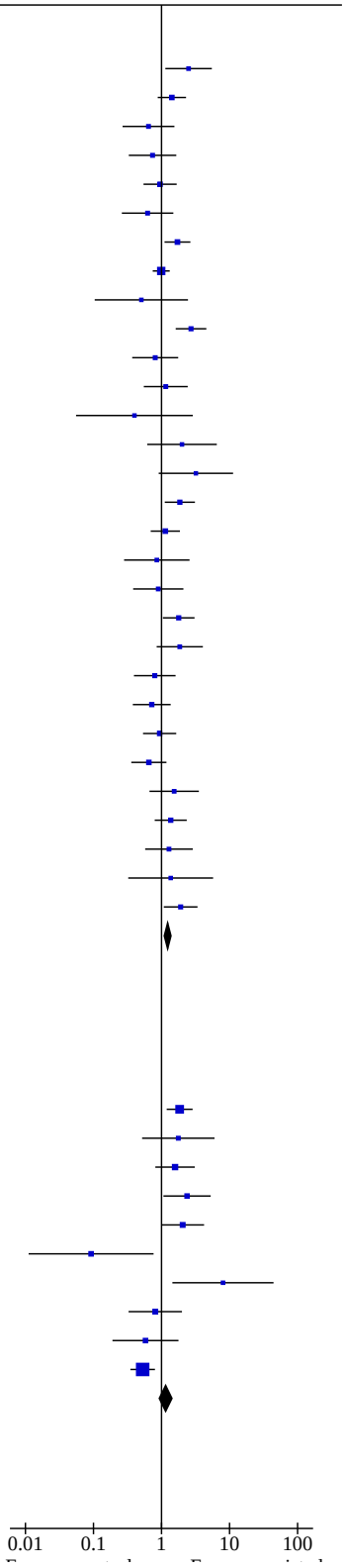
Heterogeneity: Not applicable
Test for overall effect: Z = 2.12 (P = 0.03)

Test for subgroup differences: Chi² = 8.18, df = 3 (P = 0.04), I² = 63.3%



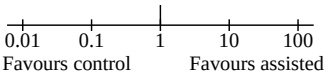
Analysis 3.7. Comparison 3: Clinical pregnancy: assisted hatching compared with no assisted hatching, Outcome 7: Fresh and frozen embryo transfer

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.7.1 Fresh embryo transfer						
Abulsoud 2019	25	65	13	65	1.5%	2.50 [1.14 , 5.49]
Antinori 1999	52	169	41	172	5.3%	1.42 [0.88 , 2.29]
Balakier 2009	16	45	18	39	2.3%	0.64 [0.27 , 1.55]
Baruffi 2000	17	51	21	52	2.6%	0.74 [0.33 , 1.65]
Carter 2003	62	121	43	82	4.7%	0.95 [0.54 , 1.67]
Ciray 2005	17	76	12	38	2.3%	0.62 [0.26 , 1.49]
Cohen 1992	85	164	64	166	5.7%	1.71 [1.11 , 2.66]
Ge 2008	164	387	159	373	17.4%	0.99 [0.74 , 1.32]
Germond 2004	3	22	5	21	0.8%	0.51 [0.10 , 2.45]
González-Ortega 2015	61	154	29	149	3.3%	2.71 [1.62 , 4.56]
Hagemann 2010	21	49	26	54	2.6%	0.81 [0.37 , 1.76]
Hellebaut 1996	23	60	21	60	2.4%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	0.6%	0.40 [0.06 , 2.89]
Isik 2000	15	24	10	22	0.7%	2.00 [0.62 , 6.49]
Isiklar 1999	16	22	10	22	0.5%	3.20 [0.91 , 11.27]
Jelinkova 2002	59	128	40	127	4.0%	1.86 [1.12 , 3.10]
Kutlu 2010	67	131	58	121	5.5%	1.14 [0.69 , 1.86]
Laffoon 1999	9	28	10	28	1.3%	0.85 [0.28 , 2.58]
Lanzendorf 1998	16	41	20	48	2.1%	0.90 [0.38 , 2.10]
Nada 2018	46	158	28	150	3.8%	1.79 [1.05 , 3.06]
Petersen 2005	21	75	13	75	1.7%	1.85 [0.85 , 4.05]
Razi 2013	18	90	22	92	3.3%	0.80 [0.39 , 1.61]
Rufas-Sapir 2004	22	104	28	103	4.1%	0.72 [0.38 , 1.36]
Sagoskin 2007	63	121	44	82	4.7%	0.94 [0.53 , 1.65]
Shi 2016	40	82	57	96	5.0%	0.65 [0.36 , 1.18]
Stein 1995	15	72	12	82	1.7%	1.54 [0.67 , 3.54]
Tucker 1993	49	110	40	108	4.2%	1.37 [0.79 , 2.35]
Tucker 1996	21	50	18	50	2.0%	1.29 [0.58 , 2.88]
Utsunomiya 1998	5	27	4	28	0.6%	1.36 [0.32 , 5.73]
Wan 2014	49	96	36	102	3.2%	1.91 [1.08 , 3.38]
Subtotal (95% CI)		**2735**		**2614**	**100.0%**	**1.23 [1.10 , 1.38]**
Total events:	1080		905			
Heterogeneity: Chi² = 48.79, df = 29 (P = 0.01); I² = 41%						
Test for overall effect: Z = 3.56 (P = 0.0004)						
3.7.2 Frozen embryo transfer only						
Balaban 2006	75	183	50	183	19.0%	1.85 [1.19 , 2.86]
Elhelw 2005	8	37	5	37	2.5%	1.77 [0.52 , 6.01]
Elnahas 2017	30	80	22	80	8.9%	1.58 [0.81 , 3.08]
Fang 2010	23	61	13	64	5.1%	2.37 [1.07 , 5.28]
Ge 2008	25	100	14	100	6.8%	2.05 [0.99 , 4.22]
Germond 2004	1	62	8	53	5.5%	0.09 [0.01 , 0.76]
Nagy 1999	10	20	2	18	0.7%	8.00 [1.44 , 44.30]
Ng 2005	10	80	12	80	6.8%	0.81 [0.33 , 2.00]
Safari 2017	7	30	11	32	5.3%	0.58 [0.19 , 1.78]
Valojerdi 2010 (1)	57	200	86	200	39.6%	0.53 [0.35 , 0.80]
Subtotal (95% CI)		**853**		**847**	**100.0%**	**1.15 [0.93 , 1.42]**
Total events:	246		223			
Heterogeneity: Chi² = 37.31, df = 9 (P < 0.0001); I² = 76%						
Test for overall effect: Z = 1.27 (P = 0.21)						
Test for subgroup differences: Chi² = 0.30, df = 1 (P = 0.58), I² = 0%						



Analysis 3.7. (Continued)

Test for subgroup differences: Chi² = 0.30, df = 1 (P = 0.58), I² = 0%



Footnotes

(1) vitrified-warmed embryo transfer

Comparison 4. Clinical pregnancies in trials that reported live births: assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Clinical pregnancies in trials reporting live births	14	2849	Odds Ratio (M-H, Fixed, 95% CI)	1.07 [0.92, 1.25]

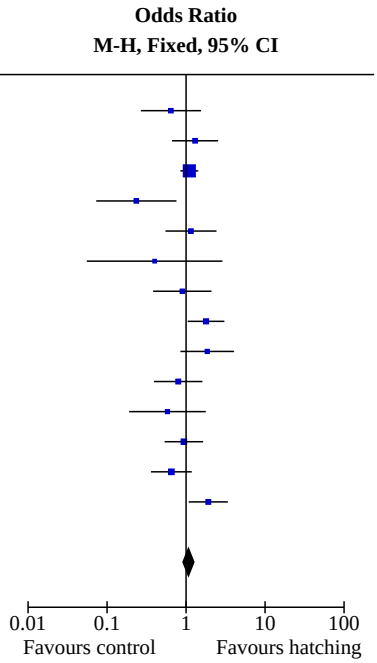
Analysis 4.1. Comparison 4: Clinical pregnancies in trials that reported live births: assisted hatching compared with no assisted hatching, Outcome 1: Clinical pregnancies in trials reporting live births

Study or Subgroup	Hatching Events	Hatching Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Balakier 2009	16	45	18	39	4.1%	0.64 [0.27 , 1.55]
Cohen 1992	37	69	32	68	5.0%	1.30 [0.66 , 2.55]
Ge 2008	189	487	173	473	35.9%	1.10 [0.85 , 1.43]
Germond 2004	4	84	13	74	4.4%	0.23 [0.07 , 0.76]
Hellebaut 1996	23	60	21	60	4.3%	1.15 [0.55 , 2.43]
Hurst 1998	3	13	3	7	1.0%	0.40 [0.06 , 2.89]
Lanzendorf 1998	16	41	20	48	3.8%	0.90 [0.38 , 2.10]
Nada 2018	46	158	28	150	6.8%	1.79 [1.05 , 3.06]
Petersen 2005	21	75	13	75	3.1%	1.85 [0.85 , 4.05]
Razi 2013	18	90	22	92	5.8%	0.80 [0.39 , 1.61]
Safari 2017	7	30	11	32	2.7%	0.58 [0.19 , 1.78]
Sagoskin 2007	63	121	44	82	8.4%	0.94 [0.53 , 1.65]
Shi 2016	40	82	57	96	9.0%	0.65 [0.36 , 1.18]
Wan 2014	49	96	36	102	5.7%	1.91 [1.08 , 3.38]
Total (95% CI)		**1451**		**1398**	**100.0%**	**1.07 [0.92 , 1.25]**
Total events:	532		491			

Heterogeneity: Chi² = 23.42, df = 13 (P = 0.04); I² = 45%

Test for overall effect: Z = 0.86 (P = 0.39)

Test for subgroup differences: Not applicable



Comparison 5. Miscarriage: assisted hatching compared with no assisted hatching

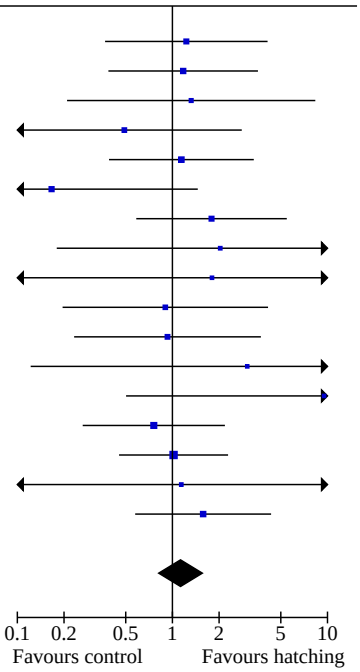
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.1 Miscarriage per woman randomised	17	2810	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [0.82, 1.56]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.2 First or repeat attempt	8		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.2.1 First attempt at IVF or ICSI	4	442	Odds Ratio (M-H, Fixed, 95% CI)	0.98 [0.51, 1.89]
5.2.2 Repeat attempt at IVF or ICSI	5	966	Odds Ratio (M-H, Fixed, 95% CI)	1.96 [0.90, 4.28]
5.3 Conception mode	10		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.3.1 ICSI only	4	665	Odds Ratio (M-H, Fixed, 95% CI)	1.20 [0.58, 2.47]
5.3.2 IVF only	6	896	Odds Ratio (M-H, Fixed, 95% CI)	1.28 [0.65, 2.52]
5.4 Hatching method	17		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.4.1 Chemical	5	412	Odds Ratio (M-H, Fixed, 95% CI)	1.11 [0.56, 2.21]
5.4.2 Laser	11	2244	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [0.78, 1.64]
5.4.3 Mechanical	1	154	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.07, 18.58]
5.5 Prognosis	11		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.5.1 Poor prognosis	7	1133	Odds Ratio (M-H, Fixed, 95% CI)	1.21 [0.70, 2.08]
5.5.2 Good prognosis	5	626	Odds Ratio (M-H, Fixed, 95% CI)	1.03 [0.50, 2.14]
5.6 Miscarriage per clinical pregnancy	15	777	Odds Ratio (M-H, Random, 95% CI)	0.94 [0.62, 1.43]

Analysis 5.1. Comparison 5: Miscarriage: assisted hatching compared with no assisted hatching, Outcome 1: Miscarriage per woman randomised

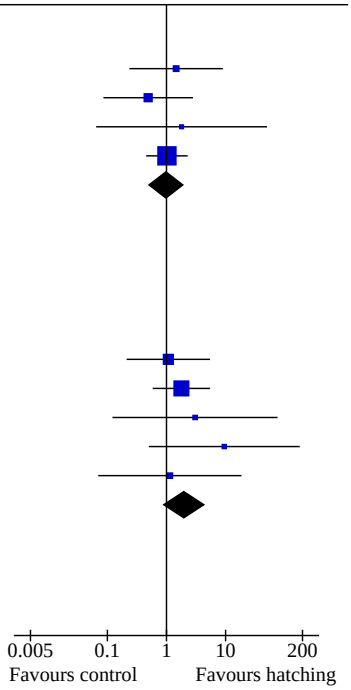
Study or Subgroup	Hatching Events	Hatching Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	6	169	5	172	6.9%	1.23 [0.37 , 4.11]
Balaban 2006	7	183	6	183	8.3%	1.17 [0.39 , 3.56]
Balakier 2009	3	45	2	39	2.9%	1.32 [0.21 , 8.35]
Baruffi 2000	2	51	4	52	5.5%	0.49 [0.09 , 2.80]
Cohen 1992	8	69	7	68	9.0%	1.14 [0.39 , 3.35]
Germond 2004	1	84	5	74	7.6%	0.17 [0.02 , 1.46]
González-Ortega 2015	9	154	5	149	6.9%	1.79 [0.58 , 5.46]
Hellebaut 1996	2	60	1	60	1.4%	2.03 [0.18 , 23.06]
Hurst 1998	1	13	0	7	0.8%	1.80 [0.06 , 50.10]
Isik 2000	4	24	4	22	5.0%	0.90 [0.20 , 4.14]
Lanzendorf 1998	4	41	5	48	6.0%	0.93 [0.23 , 3.72]
Ng 2005	1	80	0	80	0.7%	3.04 [0.12 , 75.69]
Petersen 2005	4	75	0	75	0.7%	9.50 [0.50 , 179.69]
Sagoskin 2007	8	121	7	82	11.3%	0.76 [0.26 , 2.18]
Shi 2016	13	82	15	96	16.8%	1.02 [0.45 , 2.29]
Stein 1995	1	72	1	82	1.3%	1.14 [0.07 , 18.58]
Wan 2014	10	96	7	102	8.8%	1.58 [0.58 , 4.33]
Total (95% CI)		**1419**		**1391**	**100.0%**	**1.13 [0.82 , 1.56]**
Total events:	84		74			

Heterogeneity: Chi² = 8.45, df = 16 (P = 0.93); I² = 0%
Test for overall effect: Z = 0.73 (P = 0.47)
Test for subgroup differences: Not applicable



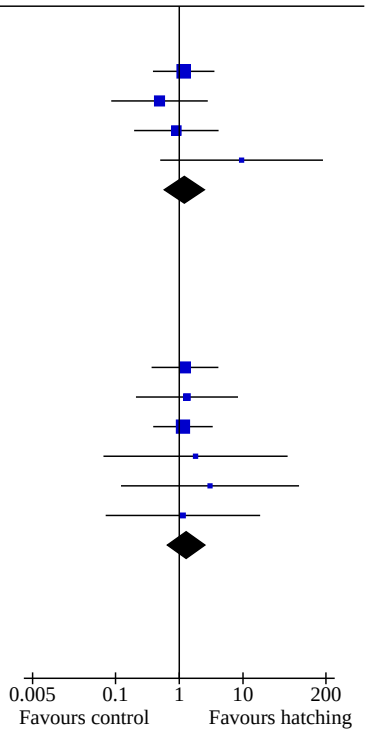
Analysis 5.2. Comparison 5: Miscarriage: assisted hatching compared with no assisted hatching, Outcome 2: First or repeat attempt

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
5.2.1 First attempt at IVF or ICSI						
Antinori 1999	3	72	2	69	10.9%	1.46 [0.24 , 8.99]
Baruffi 2000	2	51	4	52	21.2%	0.49 [0.09 , 2.80]
Hurst 1998	1	13	0	7	3.2%	1.80 [0.06 , 50.10]
Shi 2016	13	82	15	96	64.7%	1.02 [0.45 , 2.29]
Subtotal (95% CI)		**218**		**224**	**100.0%**	**0.98 [0.51 , 1.89]**
Total events:	19		21			
Heterogeneity: Chi² = 0.93, df = 3 (P = 0.82); I² = 0%						
Test for overall effect: Z = 0.07 (P = 0.95)						
5.2.2 Repeat attempt at IVF or ICSI						
Antinori 1999	3	96	3	103	29.6%	1.08 [0.21 , 5.46]
González-Ortega 2015	9	154	5	149	50.5%	1.79 [0.58 , 5.46]
Ng 2005	1	80	0	80	5.2%	3.04 [0.12 , 75.69]
Petersen 2005	4	75	0	75	5.0%	9.50 [0.50 , 179.69]
Stein 1995	1	72	1	82	9.7%	1.14 [0.07 , 18.58]
Subtotal (95% CI)		**477**		**489**	**100.0%**	**1.96 [0.90 , 4.28]**
Total events:	18		9			
Heterogeneity: Chi² = 1.88, df = 4 (P = 0.76); I² = 0%						
Test for overall effect: Z = 1.70 (P = 0.09)						
Test for subgroup differences: Chi² = 1.79, df = 1 (P = 0.18), I² = 44.1%						



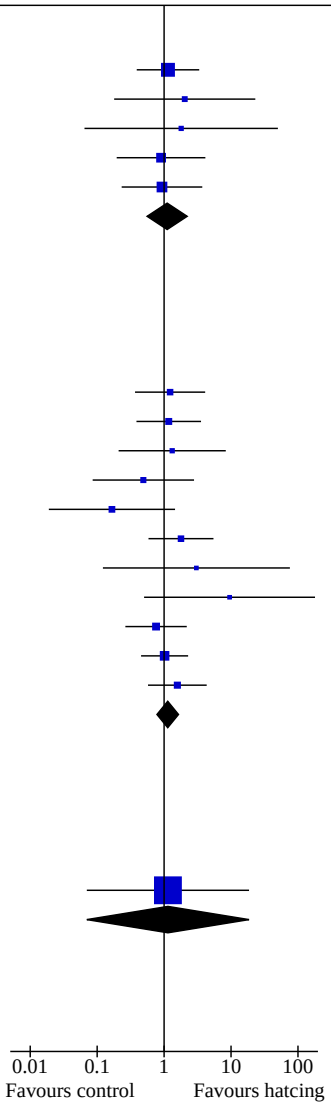
Analysis 5.3. Comparison 5: Miscarriage: assisted hatching compared with no assisted hatching, Outcome 3: Conception mode

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
5.3.1 ICSI only						
Balaban 2006	7	183	6	183	42.7%	1.17 [0.39 , 3.56]
Baruffi 2000	2	51	4	52	28.1%	0.49 [0.09 , 2.80]
Isik 2000	4	24	4	22	25.7%	0.90 [0.20 , 4.14]
Petersen 2005	4	75	0	75	3.5%	9.50 [0.50 , 179.69]
Subtotal (95% CI)		**333**		**332**	**100.0%**	**1.20 [0.58 , 2.47]**
Total events:	17		14			
Heterogeneity: Chi² = 3.06, df = 3 (P = 0.38); I² = 2%						
Test for overall effect: Z = 0.50 (P = 0.62)						
5.3.2 IVF only						
Antinori 1999	6	169	5	172	31.9%	1.23 [0.37 , 4.11]
Balakier 2009	3	45	2	39	13.3%	1.32 [0.21 , 8.35]
Cohen 1992	8	69	7	68	41.6%	1.14 [0.39 , 3.35]
Hurst 1998	1	13	0	7	3.8%	1.80 [0.06 , 50.10]
Ng 2005	1	80	0	80	3.3%	3.04 [0.12 , 75.69]
Stein 1995	1	72	1	82	6.1%	1.14 [0.07 , 18.58]
Subtotal (95% CI)		**448**		**448**	**100.0%**	**1.28 [0.65 , 2.52]**
Total events:	20		15			
Heterogeneity: Chi² = 0.37, df = 5 (P = 1.00); I² = 0%						
Test for overall effect: Z = 0.72 (P = 0.47)						
Test for subgroup differences: Chi² = 0.02, df = 1 (P = 0.90), I² = 0%						



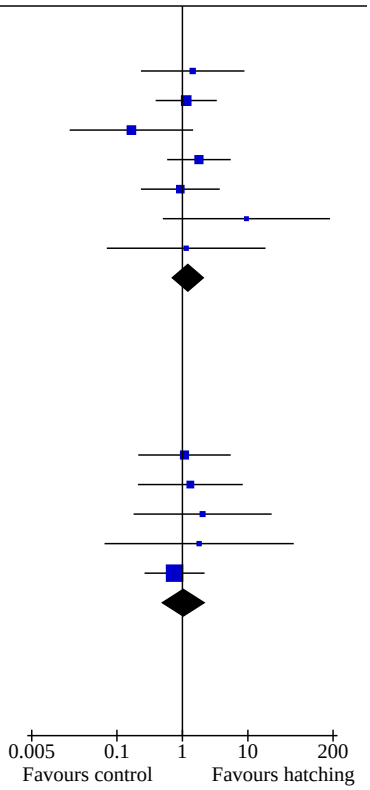
Analysis 5.4. Comparison 5: Miscarriage: assisted hatching compared with no assisted hatching, Outcome 4: Hatching method

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
5.4.1 Chemical						
Cohen 1992	8	69	7	68	40.5%	1.14 [0.39 , 3.35]
Hellebaut 1996	2	60	1	60	6.3%	2.03 [0.18 , 23.06]
Hurst 1998	1	13	0	7	3.7%	1.80 [0.06 , 50.10]
Isik 2000	4	24	4	22	22.6%	0.90 [0.20 , 4.14]
Lanzendorf 1998	4	41	5	48	27.0%	0.93 [0.23 , 3.72]
Subtotal (95% CI)		**207**		**205**	**100.0%**	**1.11 [0.56 , 2.21]**
Total events:	19		17			
Heterogeneity: Chi² = 0.46, df = 4 (P = 0.98); I² = 0%						
Test for overall effect: Z = 0.30 (P = 0.77)						
5.4.2 Laser						
Antinori 1999	6	169	5	172	9.0%	1.23 [0.37 , 4.11]
Balaban 2006	7	183	6	183	10.9%	1.17 [0.39 , 3.56]
Balakier 2009	3	45	2	39	3.8%	1.32 [0.21 , 8.35]
Baruffi 2000	2	51	4	52	7.2%	0.49 [0.09 , 2.80]
Germond 2004	1	84	5	74	9.9%	0.17 [0.02 , 1.46]
González-Ortega 2015	9	154	5	149	9.1%	1.79 [0.58 , 5.46]
Ng 2005	1	80	0	80	0.9%	3.04 [0.12 , 75.69]
Petersen 2005	4	75	0	75	0.9%	9.50 [0.50 , 179.69]
Sagoskin 2007	8	121	7	82	14.7%	0.76 [0.26 , 2.18]
Shi 2016	13	82	15	96	22.0%	1.02 [0.45 , 2.29]
Wan 2014	10	96	7	102	11.5%	1.58 [0.58 , 4.33]
Subtotal (95% CI)		**1140**		**1104**	**100.0%**	**1.13 [0.78 , 1.64]**
Total events:	64		56			
Heterogeneity: Chi² = 7.99, df = 10 (P = 0.63); I² = 0%						
Test for overall effect: Z = 0.66 (P = 0.51)						
5.4.3 Mechanical						
Stein 1995	1	72	1	82	100.0%	1.14 [0.07 , 18.58]
Subtotal (95% CI)		**72**		**82**	**100.0%**	**1.14 [0.07 , 18.58]**
Total events:	1		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.09 (P = 0.93)						
Test for subgroup differences: Chi² = 0.00, df = 2 (P = 1.00), I² = 0%						



Analysis 5.5. Comparison 5: Miscarriage: assisted hatching compared with no assisted hatching, Outcome 5: Prognosis

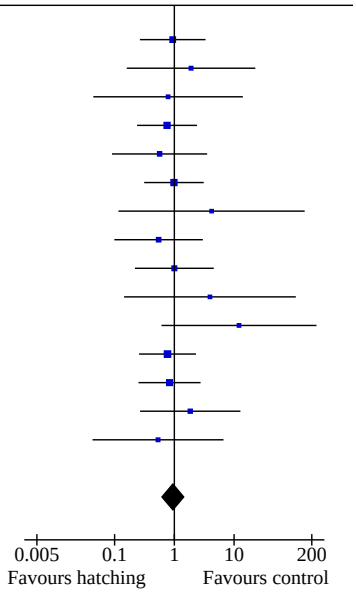
Study or Subgroup	Hatching Events	Hatching Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
5.5.1 Poor prognosis						
Antinori 1999	3	73	2	69	8.3%	1.44 [0.23 , 8.86]
Cohen 1992	8	69	7	68	26.2%	1.14 [0.39 , 3.35]
Germond 2004	1	84	5	74	22.1%	0.17 [0.02 , 1.46]
González-Ortega 2015	9	154	5	149	20.1%	1.79 [0.58 , 5.46]
Lanzendorf 1998	4	41	5	48	17.5%	0.93 [0.23 , 3.72]
Petersen 2005	4	75	0	75	2.0%	9.50 [0.50 , 179.69]
Stein 1995	1	72	1	82	3.9%	1.14 [0.07 , 18.58]
Subtotal (95% CI)		**568**		**565**	**100.0%**	**1.21 [0.70 , 2.08]**
Total events:	30		25			
Heterogeneity: Chi² = 5.75, df = 6 (P = 0.45); I² = 0%						
Test for overall effect: Z = 0.69 (P = 0.49)						
5.5.2 Good prognosis						
Antinori 1999	3	96	3	103	19.8%	1.08 [0.21 , 5.46]
Balakier 2009	3	45	2	39	14.2%	1.32 [0.21 , 8.35]
Hellebaut 1996	2	60	1	60	6.8%	2.03 [0.18 , 23.06]
Hurst 1998	1	13	0	7	4.0%	1.80 [0.06 , 50.10]
Sagoskin 2007	8	121	7	82	55.1%	0.76 [0.26 , 2.18]
Subtotal (95% CI)		**335**		**291**	**100.0%**	**1.03 [0.50 , 2.14]**
Total events:	17		13			
Heterogeneity: Chi² = 0.81, df = 4 (P = 0.94); I² = 0%						
Test for overall effect: Z = 0.08 (P = 0.94)						
Test for subgroup differences: Chi² = 0.12, df = 1 (P = 0.73), I² = 0%						



Analysis 5.6. Comparison 5: Miscarriage: assisted hatching compared with no assisted hatching, Outcome 6: Miscarriage per clinical pregnancy

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Antinori 1999	6	52	5	41	10.9%	0.94 [0.27 , 3.33]
Hellebaut 1996	2	23	1	21	2.8%	1.90 [0.16 , 22.68]
Stein 1995	1	15	1	12	2.1%	0.79 [0.04 , 14.03]
Balaban 2006	7	75	6	50	13.1%	0.75 [0.24 , 2.39]
Baruffi 2000	2	17	4	21	5.2%	0.57 [0.09 , 3.55]
Cohen 1992	8	37	7	32	13.3%	0.99 [0.31 , 3.10]
Hurst 1998	1	3	0	3	1.4%	4.20 [0.12 , 151.97]
Isik 2000	4	15	4	10	6.0%	0.55 [0.10 , 3.00]
Lanzendorf 1998	4	16	5	20	7.6%	1.00 [0.22 , 4.56]
Ng 2005	1	10	0	12	1.6%	3.95 [0.14 , 108.09]
Petersen 2005	4	21	0	23	2.0%	12.09 [0.61 , 239.51]
Sagoskin 2007	8	63	7	44	14.5%	0.77 [0.26 , 2.30]
González-Ortega 2015	9	61	5	29	12.2%	0.83 [0.25 , 2.75]
Balakier 2009	3	16	2	18	4.7%	1.85 [0.27 , 12.76]
Germond 2004	1	4	5	13	2.7%	0.53 [0.04 , 6.65]
Total (95% CI)		**428**		**349**	**100.0%**	**0.94 [0.62 , 1.43]**
Total events:	61		52			

Heterogeneity: Tau² = 0.00; Chi² = 6.26, df = 14 (P = 0.96); I² = 0%
Test for overall effect: Z = 0.27 (P = 0.79)
Test for subgroup differences: Not applicable



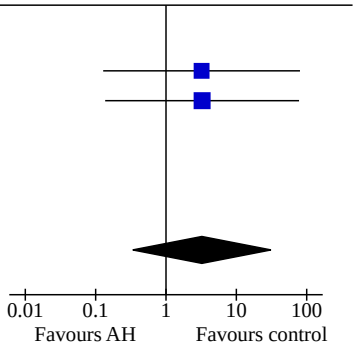
Comparison 6. Monozygotic twinning: assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6.1 Monozygotic twinning per woman randomised	6	729	Odds Ratio (M-H, Fixed, 95% CI)	3.23 [0.34, 31.03]

Analysis 6.1. Comparison 6: Monozygotic twinning: assisted hatching compared with no assisted hatching, Outcome 1: Monozygotic twinning per woman randomised

Study or Subgroup	Assisted hatching Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Balakier 2009	0	45	0	39		Not estimable
Hagemann 2010	1	59	0	62	47.6%	3.21 [0.13 , 80.25]
Hurst 1998	2	13	0	7	52.4%	3.26 [0.14 , 77.84]
Jelinkova 2002	0	128	0	127		Not estimable
Lanzendorf 1998	0	41	0	48		Not estimable
Ng 2005	0	80	0	80		Not estimable
Total (95% CI)		**366**		**363**	**100.0%**	**3.23 [0.34 , 31.03]**
Total events:	3		0			

Heterogeneity: Chi² = 0.00, df = 1 (P = 0.99); I² = 0%
Test for overall effect: Z = 1.02 (P = 0.31)
Test for subgroup differences: Not applicable

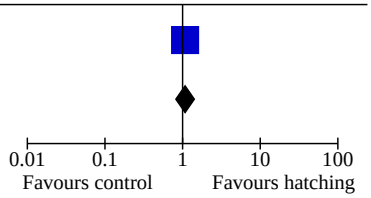


Comparison 7. Robust studies (randomisation method and allocation concealment stated and live birth reported): assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
7.1 Live births	1	960	Odds Ratio (M-H, Fixed, 95% CI)	1.08 [0.82, 1.41]
7.2 Clinical pregnancies	1	960	Odds Ratio (M-H, Fixed, 95% CI)	1.10 [0.85, 1.43]

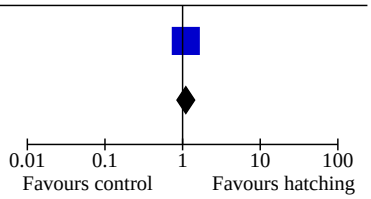
Analysis 7.1. Comparison 7: Robust studies (randomisation method and allocation concealment stated and live birth reported): assisted hatching compared with no assisted hatching, Outcome 1: Live births

	Assisted Hatching		Control			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Ge 2008	156	487	144	473	100.0%	1.08 [0.82 , 1.41]	
Total (95% CI)		**487**		**473**	**100.0%**	**1.08 [0.82 , 1.41]**	
Total events:	156		144				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.53 (P = 0.60)							
Test for subgroup differences: Not applicable							



Analysis 7.2. Comparison 7: Robust studies (randomisation method and allocation concealment stated and live birth reported): assisted hatching compared with no assisted hatching, Outcome 2: Clinical pregnancies

	Assisted Hatching		Control			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Ge 2008	189	487	173	473	100.0%	1.10 [0.85 , 1.43]	
Total (95% CI)		**487**		**473**	**100.0%**	**1.10 [0.85 , 1.43]**	
Total events:	189		173				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.71 (P = 0.48)							
Test for subgroup differences: Not applicable							



ADDITIONAL TABLES

Table 1. Mean age of participants in assisted hatching and control groups

Study	AH n, mean age (SD)	Control n, mean age (SD)	OR for clinical pregnancy
Abulsoud 2019	65, 39.2 (1.2)	65, 39.5 (1.2)	2.50 (1.14 to 5.49)
Antinori 1999: first IVF	73, 37.5	69, 36.0	1.27 (0.70 to 2.32)
Antinori 1999: repeat IVF	96, 27.5	103, 27	1.86 (0.81 to 4.25)
Balaban 2006	183, 32.4 (3.3)	183, 32.7 (3.1)	1.85 (1.19 to 2.86)

Table 1. Mean age of participants in assisted hatching and control groups *(Continued)*

Balakier 2009	45, 32.5 (3.8)	39, 33.8 (3.2)	0.64 (0.27 to 1.55)
Baruffi 2000	51, 31.8 (3.6)	52, 31.4 (3.6)	0.74 (0.33 to 1.65)
Carter 2003	121, 34 (3.3)	82, 34 (3.2)	0.95 (0.54 to 1.67)
Ciray 2005	60, 33.1 (4.2)	30, 34.0 (3.7)	0.62 (0.26 to 1.49)
Cohen 1992: FSH < 15	69, 36.50 (3.30)	68, 36.70 (3.70)	2.11 (1.18 to 3.77)
Cohen 1992: poor prognosis	80, 36.7 (4.3)	83, 35.3 (4.2)	1.30 (0.66 to 2.55)
Cohen 1992: FSH > 15	not stated	not stated	1.30 (0.66 to 2.55)
Elhelw 2005	not stated	not stated	1.77 (0.52 to 6.01)
Elnahas 2017	80, 31.0 (4.7)	80, 31.7 (4.9)	1.58 (0.81 to 3.08)
Fang 2010	61, 32.3 (3.4)	64, 32.1 (3.6)	2.37 (1.07 to 5.28)
Ge 2008: fresh embryo	387, 31.08 (4.68)	373, 30.44 (4.15)	0.99 (0.74 to 1.32)
Ge 2008: frozen embryo	100, 31.84 (3.85)	100, 30.66 (4.42)	2.05 (0.99 to 4.22)
Germond 2004: first cycle of frozen-thawed embryos	62, 32.8 (4.2)	53, 32.6 (3.8)	0.09 (0.01 to 0.76)
Germond 2004: poor prognosis, first cycle of fresh embryos	22, 39.3 (2.9)	21, 38.3 (3.4)	0.51 (0.10 to 2.45)
González-Ortega 2015	154, 38.5 (2.8)	149, 37.3 (4.2)	2.71 (1.62 to 4.56)
Hagemann 2010	59, 32.1 (3.0)	62, 31.2 (3.5)	0.81 (0.37 to 1.76)
Hellebaut 1996	60, 30.9 (4.3)	60, 30.8 (3.9)	1.15 (0.55 to 2.43)
Hurst 1998	13, 30.0 (0.9)	7, 30.0 (0.8)	0.40 (0.06 to 2.89)
Isik 2000	24, 30.5 (5.2)	22, 29.1 (3.6)	2.0 (0.62 to 6.49)
Isiklar 1999	not stated	not stated	3.20 (0.91 to 11.27)
Jelinkova 2002	128, 32.3 (4.24)	129, 32.1 (3.16)	1.86 (1.12 to 3.10)
Kutlu 2010: good prognosis	73, 29.9 (2.9)	66, 28.9 (3.4)	1.06 (0.54 to 2.08)
Kutlu 2010: poor prognosis	58, 38.0 (2.3)	55, 37.4 (2.4)	1.23 (0.58 to 2.60)
Laffoon 1999	not stated	not stated	0.85 (0.28 to 2.58)
Lanzendorf 1998	41, 38.30 (0.31)	48, 38.50 (0.26)	0.90 (0.38 to 2.10)
Nada 2018	158, 31.3 (4.1)	150, 32.6 (2.4)	1.79 (1.05 to 3.07)
Nagy 1999	20, 32.0 (4.0)	20, 31.4 (3.7)	8.0 (1.44 to 44.3)

Table 1. Mean age of participants in assisted hatching and control groups *(Continued)*

Ng 2005	80, 34.0 (range 25 to 40)	80, 34.0 (range 26 to 40)	0.81 (0.33 to 2.00)
Petersen 2005: 1 previous implantation failure	35, 34.6 (4.6)	35, 34.1 (5.3)	1.15 (0.41 to 3.19)
Petersen 2005: several previous implantation failures	40, 35.7 (3.8)	40, 35.3 (5.1)	4.11 (1.04 to 16.29)
Razi 2013	90, 32.9 (0.5)	92, 31.6 (0.4)	0.7 (0.3 to 1.6)
Rufas-Sapir 2004	not stated	not stated	0.72 (0.38 to 1.36)
Ryan 1997	not stated	not stated	0.74 (0.35 to 1.59)
Safari 2017	30, 30.6 (5.6)	32, 29.2 (5.3)	0.58 (0.19 to 1.78)
Sagoskin 2007	118, 34.0 (3.3)	81, 34.0 (3.2)	0.94 (0.53 to1.65)
Shi 2016	82, 37.2 (2.22)	96, 36.97 (1.96)	0.65 (0.36 to 1.18)
Stein 1995	not stated	not stated	1.54 (0.67 to 3.54)
Tucker 1993	110, 34.1 (4.8)	108, 34.2 (4.1)	1.37 (0.79 to 2.35)
Tucker 1996	50, 35.3 (4.2)	50, 33.5 (4.3)	0.74 (0.35 to 1.59)
Utsunomiya 1998	not stated	not stated	1.36 (0.32 to 5.73)
Valojerdi 2010	200, 30.86 (5.82)	200, 29.85 (5.14)	0.53 (0.35 to 0.80)
Wan 2014	96, 33.1 (3.7)	102, 32.6 (3.4)	1.91 (1.08 to 3.38)

Table 2. Prognostic factors in included trials

Study ID	Balanced age between groups	Balances no. of embryos transferred	Prognosis: poor/ good	FSH levels	Blastocyst transfer	Complete/partial AH	Frozen cycles
Abulsoud 2019	Yes	Yes	Poor	No data	No	Thinning	Fresh
Antinori 1999	AH mean 1.5 years older	Yes	Good and poor subgroups	No data	No	Complete hole	Not stated
Balaban 2006	Yes	Yes	Unselected	< 10	No	Thinning	Frozen
Balakier 2009	AH mean 1.3 years older	Yes	Good	< 10	No	Thinning	Fresh
Baruffi 2000	Yes	Yes	Good	No data	No	Thinning	Fresh
Carter 2003	Yes	Yes	Good	< 10	No	Not stated	Fresh
Ciray 2005	Yes	Yes	Good	< 15	No	Thinning	Fresh
Cohen 1992	Yes	Yes	Unstated	≤ 15 and > 15 subgroups	No	Complete hole	Fresh
Elhelw 2005	Yes	No data	Poor	No data	No	Thinning	Frozen
Elnahas 2017	Yes	No data	Good	No data	No	Thinning	Frozen
Fang 2010	Yes	Yes	Not stated	No data	No	Mechanical expansion	Frozen thawed
Ge 2008	Yes	Yes	Mixed	No data	No	Thinning	Fresh and frozen subgroups
Germond 2004	Yes	Yes	Mixed, in subgroups	Between 3 and 12	No	Complete hole	Fresh and frozen subgroups
González-Ortega 2015	Yes	Yes	Poor	> 12	No	Partial	Fresh
Hagemann 2010	Mean age data given only for combined cycles 1 and 2	Yes	Under 38 years, > 2 previously failed cycles, ZP thickness > 13 micrometers	No data	No	20 micrometer diameter opening	Fresh

Table 2. Prognostic factors in included trials *(Continued)*

Hellebaut 1996	Yes	Yes	Good	No data	No	Complete hole	Fresh
Hurst 1998	Yes	Yes	Good	< 10	No	Complete hole	Fresh
Isik 2000	AH mean 1.4 years older	Yes	Unstated	< 10	Yes	Removal complete	Fresh
Isiklar 1999	No data	Yes	Unstated	No data	Yes	Complete hole	Fresh
Jelinkova 2002	Yes	Yes	Poor	No data	Yes	Removal complete	Fresh
Kutlu 2010	Yes	Yes	Good and poor subgroups	No data	No	Complete hole	Fresh
Laffoon 1999	No data	No data	Good	No data	No	Complete hole	Fresh
Lanzendorf 1998	No	Yes	Poor	No data	No	Complete hole	Fresh
Nada 2018	Yes	Yes	Good	AH 5.4 ± 1.3 No AH 6.0 ± 1.1	No	Thinning	Fresh
Nagy 1999	Yes	Yes	Unstated	No data	No	Thinning	Frozen-thaw cycle
Ng 2005	Yes	Higher proportion of controls received 3 embryos	Unstated	< 11	No	Thinning	Frozen-thaw cycle
Petersen 2005	Yes	Yes	Poor	No data	No	Thinning	Fresh
Razi 2013	Yes	Yes	Unstated	No data	No	Partial	Fresh
Rufas-Sapir 2004	No data	Yes	Poor	No data	No	Complete hole	Fresh
Ryan 1997	No data	No data	Unstated	No data	No	Complete hole	Both
Safari 2017	Yes	Yes	Unstated	AH 6.4 ± 2.3 No AH 5.6 ± 2.1	No	Thinning	Frozen
Sagoskin 2007	Yes	Yes	Good	< 10	No	Hole	Fresh

Table 2. Prognostic factors in included trials *(Continued)*

Shi 2016	Yes	Yes	Advanced maternal age	< 10	No	Zona Thinning	Fresh
Stein 1995	No data	No data	Poor	No data	No	Complete hole	Fresh
Tucker 1993	Yes	Yes	Good	< 15	No	Thinning	Fresh
Tucker 1996	AH mean 1.8 years older	Yes	Not stated	No data	No	Complete hole	Fresh
Utsunomiya 1998	No data	No data	Poor	No data	No	Thinning	Fresh
Valojerdi 2010	Yes	Yes	Not stated	No data	No	Partially thinned	Vitrified-warmed embryo
Wan 2014	Yes	Yes	Previously unsuccessful 1 fresh cycle	No data	Yes	Partial	Vitrified-warmed embryo

AH: assisted hatching.
ET: embryo transfer.
FSH: follicle-stimulating hormone.

APPENDICES

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

PROCITE platform

Searched 27 May 2020

Keywords CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "Embryo" or "in-vitro fertilization" or "Embryo Transfer" or "ET" or "Blastocyst" or "implantation" or "poor implantation" or "poor prognostic patients" or "recurrent implantation failure" or "repeated implantation failure" or Title CONTAINS"IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "Embryo" or "in-vitro fertilization" or "Embryo Transfer" or "ET" or "Blastocyst" or "implantation" or "poor implantation" or "poor prognostic patients" or "recurrent implantation failure" or "repeated implantation failure"

AND

Keywords CONTAINS "assisted hatching" or "assisted hatching techniques" or "assisted zona hatching" or "zona drilling" or "zona free" or "zona laser" or "zona pellucida dissection" or "zona pellucida removal techniques" or "zona thinning" or "mechanical assisted hatching" or "Chemical hatching" or "Chemically activated" or "laser-assisted hatching" or "laser assisted" or "laser drilling" or "Laser hatching" or "Tyrodes" or "thinning" or Title CONTAINS "assisted hatching" or "assisted hatching techniques" or "assisted zona hatching" or "zona drilling" or "zona free" or "zona laser" or "zona pellucida dissection" or "zona pellucida removal techniques" or "zona thinning" or "mechanical assisted hatching" or "Chemical hatching" or "Chemically activated" or "laser-assisted hatching" or "laser assisted" or "laser drilling" or "Laser hatching" or "Tyrodes" or "thinning"

(184 records)

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

Web platform

Searched 27 May 2020

#1 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES 1076

#2 MESH DESCRIPTOR Fertilization in Vitro EXPLODE ALL TREES 2028

#3 MESH DESCRIPTOR Sperm Injections, Intracytoplasmic EXPLODE ALL TREES 530

#4 (vitro fertili?ation):TI,AB,KY 3336

#5 ivf:TI,AB,KY 5516

#6 icsi:TI,AB,KY 2661

#7 (intracytoplasmic sperm injection*):TI,AB,KY 1859

#8 (embryo* or blastocyst*):TI,AB,KY 7494

#9 implantation*:TI,AB,KY 17443

#10 (assisted reproducti*):TI,AB,KY 1375

#11 (poor prognos*):TI,AB,KY 3520

#12 (recur* adj3 implant*):TI,AB,KY 190

#13 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 29082

#14 (zona* adj5 (dissect* or tyrode* or proteinase* or piezon* or krypton* or yag*)):TI,AB,KY 30

#15 (zona* adj5 (pellucid* or manipulate* or disrupt* or thin* or drill*)):TI,AB,KY 226

#16 (mechanical adj5 zona*):TI,AB,KY 3

#17 (chemical adj5 zona*):TI,AB,KY 4

#18 (laser adj5 zona*):TI,AB,KY 47

#19 hatch*:TI,AB,KY 391

#20 pzd:TI,AB,KY 6

#21 microfertili?ation:TI,AB,KY 5

#22 (micro fertili?ation):TI,AB,KY 1

#23 #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 523

#24 #13 AND #23 347

Appendix 3. MEDLINE search strategy

Ovid platform

Searched from 1946 to 27 May 2020

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (41278)
2 in vitro fertili?ation.tw. (23107)
3 ivf-et.tw. (2311)
4 icsi.tw. (8369)
5 intracytoplasmic sperm injection$.tw. (7167)
6 (embryo$ or blastocyst$).tw. (355005)
7 implantation$.tw. (166687)
8 ART.tw. (101884)
9 assisted reproducti$.tw. (14867)
10 (ivf or et).tw. (286418)
11 or/1-10 (887220)
12 (assist$ adj5 hatch$).tw. (397)
13 (zona$ adj5 (dissect$ or tyrode$ or proteinase$ or piezon$ or krypton$ or yag$)).ti,ab,sh. (183)
14 (zona$ adj5 (pellucid$ or manipulat$ or disrupt$ or thin$ or drill$)).ti,ab,sh. (5425)
15 (mechanical adj5 zona$).tw. (60)
16 (chemical$ adj5 zona$).tw. (53)
17 (laser adj5 zona$).tw. (137)
18 pzd.tw. (72)
19 or/12-18 (5815)
20 11 and 19 (2897)
21 randomized controlled trial.pt. (506126)
22 controlled clinical trial.pt. (93684)
23 randomized.ab. (480049)
24 placebo.tw. (213550)
25 clinical trials as topic.sh. (191286)
26 randomly.ab. (333535)
27 trial.ti. (218599)
28 (crossover or cross-over or cross over).tw. (84663)
29 or/21-28 (1319801)
30 exp animals/ not humans.sh. (4700877)
31 29 not 30 (1213169)
32 20 and 31 (159)

Appendix 4. Embase search strategy

Ovid platform

Searched from 1980 to 27 May 2020

1 (assist$ adj5 hatch$).tw. (696)
2 (zona$ adj5 (dissect$ or tyrode$ or proteinase$ or piezon$ or krypton$ or yag$)).ti,ab,sh. (356)
3 (zona$ adj5 (pellucid$ or manipulat$ or disrupt$ or thin$ or drill$)).ti,ab,sh. (6079)
4 (mechanical adj5 zona$).tw. (74)
5 (chemical$ adj5 zona$).tw. (52)
6 (laser adj5 zona$).tw. (226)

7 pzd.tw. (94)
8 or/1-7 (6757)
9 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (68949)
10 in vitro fertili?ation.tw. (30147)
11 ivf-et.tw. (3196)
12 icsi.tw. (15993)
13 intracytoplasmic sperm injection$.tw. (9566)
14 (ivf or et).tw. (690080)
15 (embryo$ or blastocyst$).tw. (392034)
16 implantation$.tw. (238479)
17 ART.tw. (127295)
18 assisted reproducti$.tw. (22584)
19 or/9-18 (1398644)
20 8 and 19 (3633)
21 Clinical Trial/ (963034)
22 Randomized Controlled Trial/ (598954)
23 exp randomization/ (86801)
24 Single Blind Procedure/ (38819)
25 Double Blind Procedure/ (169202)
26 Crossover Procedure/ (62950)
27 Placebo/ (335995)
28 Randomi?ed controlled trial$.tw. (227639)
29 Rct.tw. (36951)
30 random allocation.tw. (1997)
31 randomly allocated.tw. (34904)
32 allocated randomly.tw. (2533)
33 (allocated adj2 random).tw. (812)
34 Single blind$.tw. (24525)
35 Double blind$.tw. (201612)
36 ((treble or triple) adj blind$).tw. (1133)
37 placebo$.tw. (301171)
38 prospective study/ (598878)
39 or/21-38 (2176633)
40 case study/ (68827)
41 case report.tw. (400435)
42 abstract report/ or letter/ (1092786)
43 or/40-42 (1551618)
44 39 not 43 (2123502)
45 20 and 44 (312)

Appendix 5. PsycINFO search strategy

Ovid platform

Searched from 1806 to 27 May 2020

1 exp Embryo/ or exp Reproductive Technology/ or exp Infertility/ (5151)
2 in vitro fertili?ation.tw. (744)
3 ivf-et.tw. (19)
4 icsi.tw. (72)
5 intracytoplasmic sperm injection$.tw. (56)
6 (embryo$ or blastocyst$).tw. (11093)
7 implantation$.tw. (4383)
8 ART.tw. (44913)
9 assisted reproducti$.tw. (968)
10 (ivf or et).tw. (140592)
11 or/1-10 (202023)
12 (assist$ adj5 hatch$).tw. (5)
13 (zona$ adj5 (pellucid$ or manipulat$ or disrupt$ or thin$ or drill$)).ti,ab,sh. (23)
14 pzd.tw. (5)
15 12 or 13 or 14 (33)
16 11 and 15 (8)

WHAT'S NEW

Date	Event	Description
7 April 2021	Amended	Searching sections corrected in methods and appendices

HISTORY

Protocol first published: Issue 1, 2000
Review first published: Issue 4, 2003

Date	Event	Description
5 October 2020	New search has been performed	Review authors have updated the Cochrane Review
5 October 2020	New citation required but conclusions have not changed	The addition of 8 trials has not led to a change in Review conclusions (Abulsoud 2019; Elnahas 2017; González-Ortega 2015; Nada 2018; Razi 2013; Safari 2017; Shi 2016; Wan 2014)
30 May 2013	Amended	Minor correction to review title (format only)
8 August 2012	New citation required but conclusions have not changed	Seven new studies added; no change to conclusions
8 August 2012	New search has been performed	Review updated August 2012. Seven new studies in this update (Balakier 2009; Fang 2010; Ge 2008; Germond 2004; Hagemann 2010; Kutlu 2010; Valojerdi 2010)
17 June 2008	New search has been performed	New search identified 4 new randomised controlled trials, which have been added. Conclusions have not changed
15 May 2008	Amended	Converted to new review format
18 September 2007	New citation required and conclusions have changed	Substantive amendments made

CONTRIBUTIONS OF AUTHORS

Mourad Seif contributed to conceiving the review, designing the review, publishing the protocol, co-ordinating the review, collecting data for the review, developing a search strategy, undertaking searches, screening search results, organising retrieval of papers, screening retrieved papers against inclusion criteria, arbitrating on quality and data extraction, interpreting data, providing a methodological perspective, providing a clinical perspective, providing a policy perspective, editing the review, providing general advice on the review, and performing previous work that was the foundation of the review.

Muhammad A Akhtar updated this review by extracting data, conducting analysis, and editing the review in detail.

Lauren Lacey updated this review by completing new searches, retrieving papers, screening retrieved papers against inclusion criteria, extracting data, conducting analysis, and editing the review in detail.

Sibte Hassan updated this review by completing new searches, retrieving papers, and screening retrieved papers against inclusion criteria.

Sebastian Franik updated this review by providing support in analysis and editing the review.

DECLARATIONS OF INTEREST

MAA, SF, SH and LL have no interests to declare. MS has received travel and accommodation support for conferences unrelated to the topic of this review.

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Internal sources

- Central Manchester and Manchester Children's University Trust, UK
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External sources

- Ministry of Health, New Zealand
- Dr. Demián Glujovsky, Editor, Cochrane, Other

  Translated González-Ortega et al., 2015 (published in Spanish) and extracted data from the paper for the purposes of this review.
- Dr. Jasmine Lee, Other

  Translated Lu et al., 2016 (published in Chinese) and extracted data for the purposes of this review.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

For the 2005 update, we investigated the following subgroups.

- Age (when reported in the studies) ≥ 35 years.
- First cycle versus previous failed cycles of IVF, ICSI, or both.
- ICSI only cycles.
- Chemical versus laser versus mechanical.
- Thinning versus breach with hole versus complete removal.

For the 2007 update, the subgroup of poor prognosis women (age ≥ 35, poor ovulation induction, previous failed cycles, or referred to as poor prognosis women in the protocol) and new subgroups of fresh and frozen embryo transfer cycles were added.

No new subgroups were added to the 2020 update.

For the 2020 update, the review was reformatted in line with current recommended Cochrane guidance for reporting outcomes.

For the 2020 update, we added exclusion criterion: biopsied embryos were excluded (for purposes of PGS/PGD) during assisted reproduction because essentially they have largely been affected by assisted hatched with a hole made in them routinely at Day 3.

For the 2020 update, we specified which specific outcomes would be subject to sensitivity analysis. Sensitivity analysis was conducted for our primary outcomes and for clinical pregnancy, as those are the most important clinical outcomes.

INDEX TERMS

Medical Subject Headings (MeSH)

Abortion, Spontaneous [epidemiology]; Bias; Embryo Implantation [*physiology]; *Fertilization in Vitro; Live Birth [epidemiology]; Pregnancy Outcome; *Pregnancy Rate; Pregnancy, Multiple [statistics & numerical data]; Publication Bias; Randomized Controlled Trials as Topic; Sperm Injections, Intracytoplasmic; Zona Pellucida [*physiology]

MeSH check words

Female; Humans; Pregnancy