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

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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 an 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 the effect of assisted hatching (AH) of embryos from assisted conception on live birth and multiple pregnancy rates.

Search methods

We searched the Cochrane Menstrual Disorders and Subfertility Group Specialised Register (August 2012), the Cochrane Central Register of Controlled Trials (CENTRAL) (August 2012), MEDLINE (1966 to August 2012) and EMBASE (1980 to August 2012).

Selection criteria

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

Data collection and analysis

Three authors independently performed quality assessments and data extraction.

Main results

Thirty-one trials reported clinical pregnancy data, including 1992 clinical pregnancies in 5728 women. There was no significant difference in the odds of live birth in the AH group compared with the control group (9 RCTs; odds ratio (OR) 1.03, 95% confidence interval (CI) 0.85 to 1.26, moderate quality evidence), with no evidence of significant heterogeneity (P = 0.38) or inconsistency (I^2 = 6%). Analysis of the clinical pregnancy rates from the nine studies which reported live birth showed a non-significant result (OR 1.03, 95% CI 0.85 to 1.25).

Analysis of all of the studies included in this update (31 RCTs) showed that the clinical pregnancy rate in women who underwent AH was slightly improved, but the level only just reached statistical significance (OR 1.13, 95% CI 1.01 to 1.27, moderate quality evidence). However,

it is important to note that the heterogeneity for this combined analysis for clinical pregnancy rate was statistically significant (P = 0.001) and the I^2 was 49%. Subgroup analysis of women who had had a previous failed attempt at IVF found improved clinical pregnancy rates in the women undergoing AH compared with the women in the control group (9 RCTs, n = 1365; OR 1.42, 95% CI 1.11 to 1.81) with I^2 = 20%.

Miscarriage rates per woman were similar in both groups (14 RCTs; OR 1.03, 95% CI 0.69 to 1.54, P = 0.90, moderate quality evidence). Multiple pregnancy rates per woman were significantly increased in women who were randomised to AH compared with women in the control groups (14 RCTs, 3447 women; OR 1.38, 95% CI 1.11 to 1.70, P = 0.004, low quality evidence).

Authors' conclusions

This update has demonstrated that whilst assisted hatching (AH) does appear to offer a significantly increased chance of achieving a clinical pregnancy, the extent to which it may do so only just reaches statistical significance. The 'take home' baby rate was still not proven to be increased by AH. The included trials provided insufficient data to investigate the impact of AH on several important outcomes. Most trials still failed to report on live birth rates.

PLAIN LANGUAGE SUMMARY

Assisted hatching of fertilised eggs to improve the chances of pregnancy in assisted conception (IVF and ICSI)

Assisted hatching is a technique sometimes used for IVF (in vitro fertilisation) and similar procedures. 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 womb so that pregnancy can begin. In this review of randomised controlled trials there was no evidence of a benefit in the live birth rate with assisted hatching although there was an increase in multiple pregnancy rates. There was some evidence that assisted hatching improves the chances of pregnancy in women for whom IVF has been repeatedly unsuccessful, but more research is needed.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Live birth

Live birth

Patient or population: Women undergoing assisted conception
Intervention: Assisted hatching compared with no assisted hatching

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Assisted hatching				
Live birth per woman randomised	**305 per 1000**	**311 per 1000** (271 to 356)	**OR 1.03** (0.85 to 1.26)	1921 (9 studies)	⊕⊕⊕⊖ **moderate**[1]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

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

[1] Many of the trials had some methodological limitations or missing information

Summary of findings 2. Multiple pregnancy

Multiple pregnancy

Patient or population: Women undergoing assisted reproduction
Intervention: Assisted hatching compared with no assisted hatching

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				

	Control	Assisted hatching			
Multiple pregnancy rate per woman randomised	**102 per 1000**	**136 per 1000** (112 to 162)	**OR 1.38** (1.11 to 1.7)	3447 (14 studies)	⊕⊕⊖⊖ **low**[1,2]

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

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

[1] There was methodological limitations or missing information in most trials
[2] I square statistic was 57%

Summary of findings 3. Clinical pregnancy

Clinical pregnancy

Patient or population: Women undergoing assisted reproduction
Intervention: Assisted hatching compared with no assisted hatching

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Assisted hatching				
Clinical pregnancy rate per woman randomised	**332 per 1000**	**360 per 1000** (334 to 387)	**OR 1.13** (1.01 to 1.27)	5728 (31 studies)	⊕⊕⊕⊖ **moderate**[1]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

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

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] There were methodological limitations or missing information in most of the trials

Summary of findings 4. Miscarriage

Miscarriage

Patient or population: Women undergoing assisted reproduction
Intervention: Assisted hatching compared with no assisted hatching

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Miscarriage				
Miscarriage per woman randomised	**45 per 1000**	**46 per 1000** (32 to 68)	**OR 1.03** (0.69 to 1.54)	2131 (14 studies)	⊕⊕⊕⊖ **moderate**[1]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

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

[1] There were methodological limitations or missing information in most of the trials

BACKGROUND

Description of the condition

The World Health Organization estimates that one in six couples experiences some delay in conception (WHO 1975), and an increasing number of couples require treatment by the assisted conception (AC) procedures of in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). In the UK in 2008, 12,211 successful births giving rise to 15,082 babies were achieved from 50,687 assisted conception cycles (24.1%), increasing from one live birth in seven cycles in 1992 to one in four (HFEA 2000; HFEA 2010).

The implantation rate of embryos resulting from IVF cycles is generally less than 20% (Gardner 2000; Lopata 1996), culminating in a generally low 'take home baby rate' (Sengoku 2000). This may be the result of poor embryo quality, poor endometrial receptivity, or both (Denker 1993). The human embryo is surrounded by an outer glycoprotein coat (zona pellucida) that, during fertilisation, prevents penetration by multiple sperm or sperm from other species (Bleil 1980). After fertilisation, the zona maintains the three-dimensional integrity of the uncompacted embryo, 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). The blastocyst-stage embryo eventually hatches out of this protective coat prior to implantation (Cole 1967).

Human embryos resulting from superovulation develop more slowly in vitro compared to embryos in vivo, manifest a relatively high degree of cytogenetic abnormalities and undergo cellular fragmentation; and only a small proportion achieve blastocyst-stage development (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 glycoproteins (ZP1, ZP2, ZP3) in an in vitro environment (Cohen 1991). Zona thickness appears to be influenced by a woman's age, hormone profile (high early proliferative phase follicle-stimulating hormone (FSH)), smoking and the cause of infertility, and correlates negatively with embryo implantation rates (Loret de Mola 1997). 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

Assisted hatching is a technique sometimes used for IVF and similar procedures. It involves thinning the coat surrounding a fertilised egg, or making a hole in it. This was thought to improve the chances of the embryo attaching to the womb so that pregnancy could begin. Artificially disrupting 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).

A variety of techniques have since been employed to assist embryo hatching, including partial mechanical zona dissection, zona drilling and zona thinning, making use of acid tyrodes, proteinases, piezon vibrator manipulators and lasers (Al-Nuaim 2002). In this update, one of the randomised controlled trials employs a new method of AH, namely that of mechanical expansion (Fang 2010).

Regardless of the AH technique employed, it is also important to distinguish whether the zona has remained unbreached such as in thinning (chemically or lasered), been fully breached (when a hole is made chemically, with a laser or mechanically), or has been completely removed (chemically). This distinction may have implications for whether an embryo is able to undergo normal zona expansion and escape following AH (Blake 2001), and also subsequent monozygotic twinning (da Costa 2001; Menezo 2003; Schieve 2000).

How the intervention might work

There are a variety of mechanisms by which AH could improve embryo implantation. The most obvious is that AH overcomes the zona pellucida hardening caused by IVF and cell culture or cryopreservation. Additionally, there is some evidence 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 in addition to messaging between the embryo and the endometrium.

Why it is important to do this review

For over a decade now, zona manipulation of some form has been offered to older women, those with high FSH levels, a high risk of zona hardening (as with in vitro oocyte maturation) and following repeated implantation failure (Al-Nuaim 2002). However, there remains considerable uncertainty over whether AH significantly improves IVF and ICSI success rates or whether it is associated with negative consequences. The previous update showed that AH results in a significant increase in clinical pregnancy rate when compared with no AH. AH failed to result in a statistically significant increase in live birth rate. However, few trials reported on live birth rate. We hoped that by updating this review and incorporating more studies more conclusive evidence of AH's effects on both clinical pregnancy and live birth rate, as well as other outcomes such as miscarriage and multiple pregnancy rates, could be achieved.

OBJECTIVES

To determine the effect of assisted hatching (AH) of embryos 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 (for example studies with evidence of inadequate sequence generation such as alternate days, patient numbers) as they are associated with a high risk of bias. Trials were only eligible for inclusion if data could be extracted per woman and not per cycle. We excluded trials which presented results as per cycle rather than per woman (unless it was clear in the text that per cycle and per woman were used interchangeably). Crossover trials were excluded as the design is not valid in this context.

Types of participants

The 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, use of frozen embryos, or where the primary study protocol referred to women with a poor prognosis.

Types of interventions

Trials were included that investigated any known method of AH after fertilisation. The techniques involved to disrupt the zona pellucida prior to embryo replacement were of the following forms:

- mechanical (including a new technique of hydrostatic pressure injection after thawing);
- chemical;
- laser.

Assisted hatching took place to the following extents:

- breaching the zona pellucida by a hole (by laser, chemical or mechanical means);
- thinning the zona pellucida (but no actual hole created);
- removing the whole of the zona pellucida.

In the trials, AH was performed on fresh embryos, cryopreserved embryos following thawing and prior to embryo transfer as well as vitrified-warmed embryos which were transferred at the cleavage stage. The effects of these interventions were compared to a control group in which AH was not performed.

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

Types of outcome measures

Primary outcomes

1. Live birth, defined as the birth of live offspring per woman
2. Multiple pregnancy rate per woman

Secondary outcomes

3. Clinical pregnancy, defined as the demonstration of fetal heart beats on ultrasound scan per woman

4. Miscarriage, loss of pregnancy up to 20 weeks gestation per woman

5. Monozygotic twinning

6. Ectopic pregnancy rate per woman

7. Congenital or chromosomal abnormalities

8. Failure to transfer any embryos per woman

9. Embryo damage

10. In vitro blastocyst development

Only trials which 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 as a reason for inclusion. It is not possible to pool implantation as the data are reported per cycle. We recorded live births as an event per woman and not by the number of infants delivered because of the high 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 and in consultation with the Menstrual Disorders and Subfertility Group Trials Search Co-ordinator.

Electronic searches

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

- Menstrual Disorders and Subfertility Group (MDSG) Specialised Register of Controlled Trials;
- Cochrane Central Register of Controlled Trials (CENTRAL) on *The Cochrane Library*;
- MEDLINE;
- EMBASE.

See: Appendix 1; Appendix 2

Searching other resources

We handsearched reference lists of 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 2011).

Selection of studies

Two authors scanned titles and abstracts from the first searches, and the same methods were adopted by another author for the second searches. Trials that appeared relevant were selected and formally assessed for inclusion independently by three authors using an inclusion and exclusion form. Trials excluded at this stage are detailed in the table 'Characteristics of excluded studies'.

Data extraction and management

Data were extracted from eligible studies using a data extraction proforma. Three authors independently performed all assessments of trial quality and data extraction using forms designed for the review (Appendix 3; Figure 1 and Figure 2). Discrepancies in quality assessment or data extraction were resolved by consensus during discussions with another author (MWS).

Figure 1. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

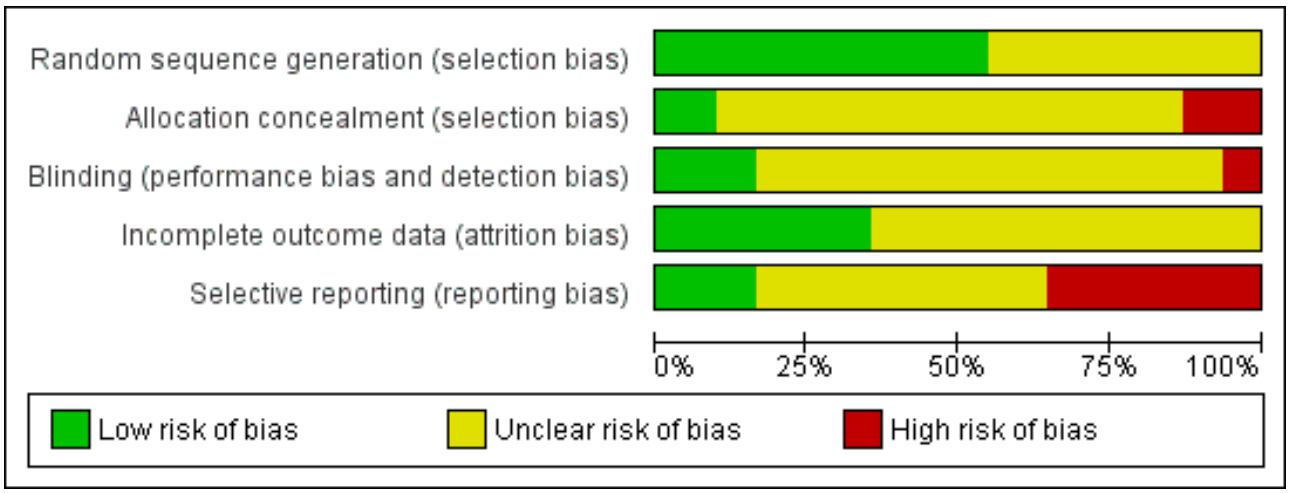


Figure 2. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.

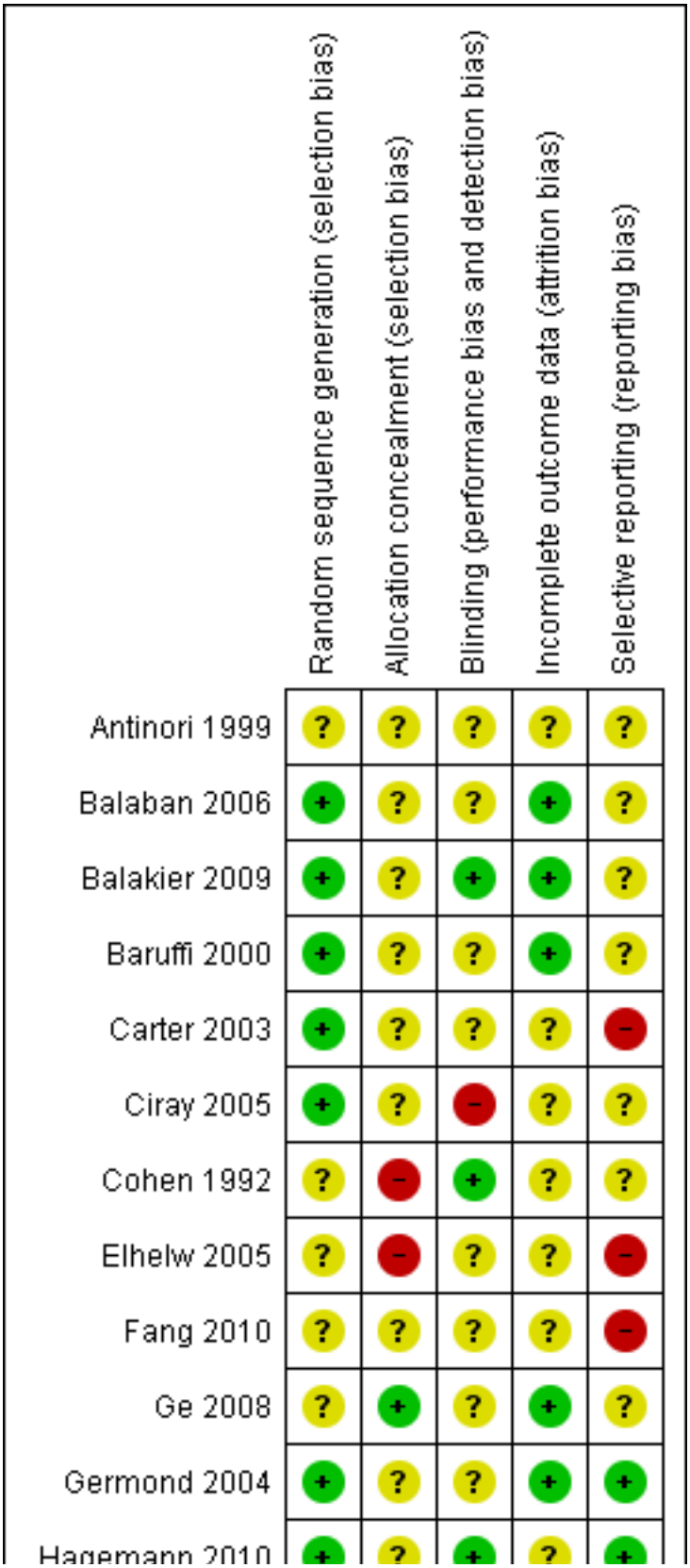
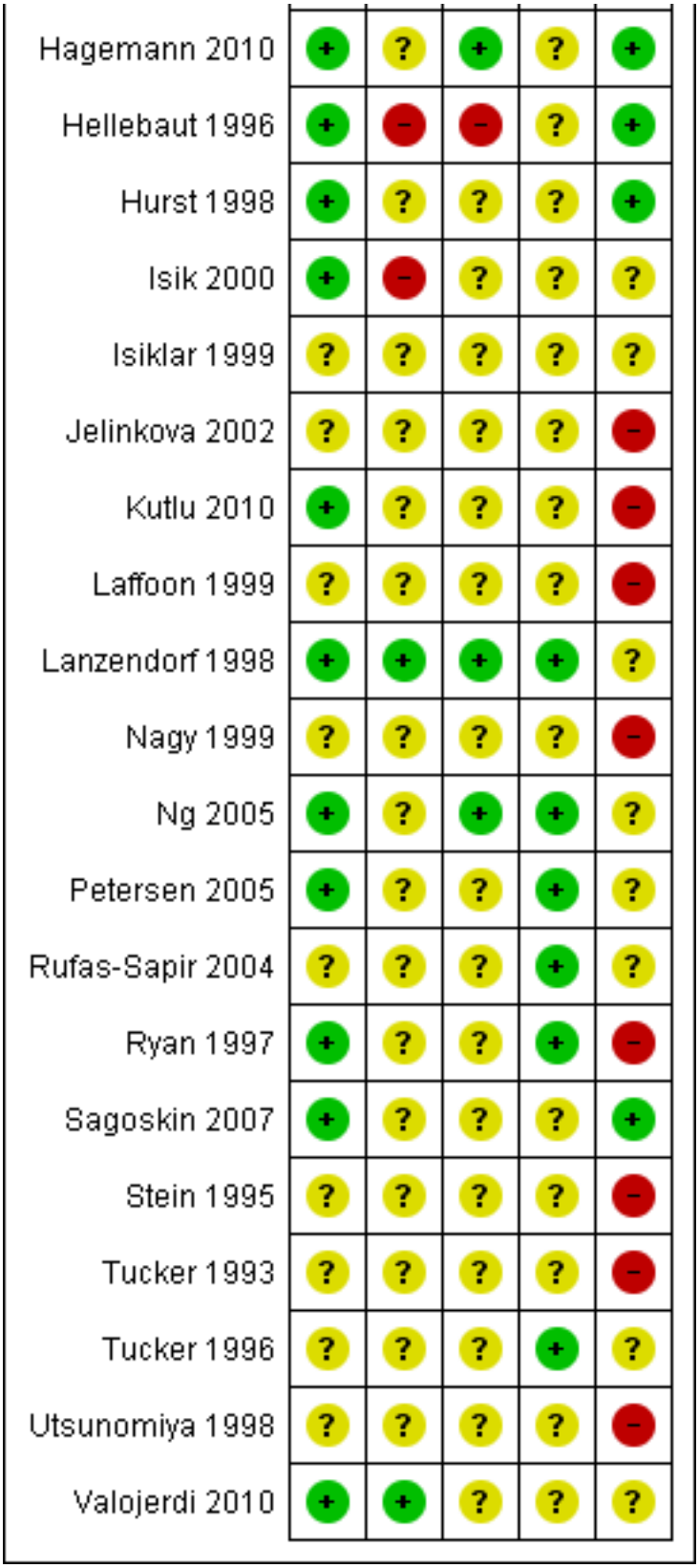


Figure 2. (Continued)



Assessment of risk of bias in included studies

Two review authors independently assessed the included studies for risk of bias using the Cochrane risk of bias assessment tool (www.cochrane-handbook.org) 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. Disagreements were resolved by discussion or by a third review author.

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, or it was unclear how allocation concealment was achieved, the trial was classed as being high risk or as having an unclear risk, respectively. For each trial we also determined whether an acceptable method of randomisation was described within the text, for example 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 it was unclear, or the trial did not appear to be randomised, the trial was classed as having an unclear risk or being at high risk of bias, respectively. We determined who was blinded in each trial. If participants and medical staff in the trial were blinded to the allocation, the trial was at low risk. If it was not stated or was clear that this was not the case, the trial was again classed as having an unclear risk or as being at 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 whereas each trial which did not was classed as high risk.

Measures of treatment effect

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

Unit of analysis issues

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

Dealing with missing data

Attempts were made to obtain additional information on trial methodology, actual original trial data, or both, by contacting the principal authors of the trials. Reminders were sent (where necessary) to authors if there was no reply four weeks after the initial request. Only data that were available 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 more variation than would be expected by chance. I^2 values were also examined and high values (> 40%) were taken to indicate substantial heterogeneity.

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, the authors aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies and by being alert for duplication of data. If there were 10 or more studies in an analysis, we planned to use a funnel plot to explore the possibility of small study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies).

Data synthesis

Studies were combined via meta-analysis using fixed-effect models for AH versus no AH using RevMan 5.1 software (RevMan 2011). 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 to the left of the centre-line.

Subgroup analysis and investigation of heterogeneity

The following subgroup analyses were undertaken for the 2012 update.

1. Results based on number of attempts: first or repeat attempt at assisted conception.
2. Results based on mode of assisted conception: IVF or ICSI.
3. Results based on method of assisted hatching: chemical, laser or mechanical.
4. Results based on prognosis of woman: good or poor.
5. Results based on extent of AH: thinning, breaching, complete removal of zona pellucida.
6. Results based on type of embryo: fresh or frozen.

Sensitivity analysis

We performed sensitivity analysis to examine the stability of results in relation to:

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

RESULTS

Description of studies

Results of the search

A total of 31 randomised controlled trials met the inclusion criteria. Several publications reported two or more different comparisons in different populations (Antinori 1999; Cohen 1992; Ge 2008; Germond 2004; Kutlu 2010; Petersen 2005). All included trials were in published reports (full papers or abstracts) and available in English. They recruited a total of 5728 women undergoing IVF or ICSI, 2933 women in the assisted hatching and 2795 women in the control groups.

Included studies

Study design and setting

We included a total of 31 studies, including seven new studies in this update (Balakier 2009; Fang 2010; Ge 2008; Germond 2004; Hagemann 2010; Kutlu 2010; Valojerdi 2010).

The trials were carried out in 16 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), Japan (Utsunomiya 1998), Israel (Rufas-Sapir 2004; Stein 1995), Iran (Valojerdi 2010), Canada (Balakier 2009) and Egypt (Elhelw 2005). One study was a European multicentre study involving women at IVF centres in Switzerland, France, Germany and Spain (Germond 2004).

Participants

The age of participants ranged from 27 to 40 years (where reported). Some trials had subgroup data within them (for example Ge 2008; Germond 2004; Kutlu 2010; Rufas-Sapir 2004; Stein 1995; and Tucker 1996 presented pregnancy for different age groups) whilst other studies only included women older than 35 years of age (for example Lanzendorf 1998) or less than 35 years old (Antinori 1999; Hurst 1998). Other studies included women of other specific age groups, for example 38 years old or younger (Balakier 2009; Hagemann 2010). Subgroup analysis based on the age of the women has not been achievable as studies did not categorise age groups in a universal way.

Twelve trials included women with a poor prognosis (Antinori 1999; Cohen 1992; Elhelw 2005; Ge 2008; Germond 2004; Hagemann 2010; Jelinkova 2002; Kutlu 2010; Petersen 2005; Rufas-Sapir 2004; Stein 1995; Utsunomiya 1998), 12 trials included women with a good prognosis (Antinori 1999; Balakier 2009; Carter 2003; Ciray 2005; Ge 2008; Germond 2004; Hellebaut 1996; Hurst 1998; Kutlu 2010; Laffoon 1999; Sagoskin 2007; Tucker 1993), and the remainder did not provide information.

Interventions

Nine trials were repeat cycles and five included women undergoing their first assisted reproductive technology (ART) cycle; 17 trials did not report whether the treatment cycle was a first or repeat cycle or were mixed cycles. Eight trials included women undergoing ICSI alone, 14 were IVF only, and the rest were unstated or mixed ICSI and IVF cycles. Twenty-three trials involved transfers of fresh embryos exclusively, six involved frozen or vitrified-warmed embryos only, and the remaining trials used a combination of fresh or frozen embryos.

Eleven trials employed chemical means for assisted hatching, five employed mechanical means and 15 employed laser.

Fifteen 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; Rufas-Sapir 2004; Ryan 1997; Sagoskin 2007; Stein 1995; Tucker 1996) while 12 utilised a non-breach thinning (Balaban 2006; Balakier 2009; Baruffi 2000; Ciray 2005; Elhelw 2005; Ge 2008; Kutlu 2010; Ng 2005; Petersen 2005; Tucker 1996; Utsunomiya 1998; Valojerdi 2010) and two performed a complete zona removal (Isik 2000; Jelinkova 2002). For one study this was unknown (Carter 2003), 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 a zona thickness of more than 12 µm as an inclusion criterion).

Twenty-four trials reported the interval between AH and embryo transfer (19 trials reported less than four hours; three trials, four to eight hours; and two were greater than eight hours).

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

Outcomes

The outcome measures utilised for this review were reported by a varying number of trials:

- nine trials reported live birth rate;
- 31 trials reported clinical pregnancy rate;
- 14 trials reported multiple pregnancy rate;
- 14 trials reported miscarriages;
- six trials reported monozygotic twinning;
- three trials reported ectopic pregnancy;
- two trials reported congenital, chromosomal abnormalities or both;
- four trials reported embryo damage;
- and no trials reported in vitro blastocyst development post AH.

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

Excluded studies

We excluded 58 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 and, in the remainder, the studies were not randomised. Three studies were found to be retrospective studies on close examination of the text.

Risk of bias in included studies

The overall methodological quality of the included trials was considered suboptimal, largely due to the lack of information on allocation and randomisation in many of the trials. Further details of the trials' risk of bias can be found in the table 'Characteristics of included studies'. Summaries of risk of bias for all the included studies are presented in Figure 1 and Figure 2.

Allocation

All 31 trials stated that randomised allocation had occurred. Ideally, studies should randomise women on the day of assessment of the embryos for suitability for embryo transfer. Regarding sequence generation, 17 studies were at low risk of selection bias, 14 studies had an unclear risk, and none of the studies was at high risk.

Four studies were at low risk of selection bias related to allocation concealment, 23 studies had an unclear risk, and four studies were

felt to be at high risk. Ge 2008, Lanzendorf 1998, Ng 2005 and Valojerdi 2010 gave details of adequate concealment of allocation.

Blinding

Although blinding was unlikely to influence findings for the primary review outcome (live birth), only five trials (Balakier 2009; Cohen 1992; Hagemann 2010; Lanzendorf 1998; Ng 2005) employed double blinding with both the woman and the outcome assessor being unaware of the allocation. In 24 studies it was unclear if blinding was used or who was blinded (participant or assessor), and in the remaining two studies there was no blinding.

Incomplete outcome data

No trial reported losses to follow up. One trial reported a loss of participants in the early stages of the trial but gave reasons and numbers for the new number of women in the control and AH groups.

A total of 17 studies were at low risk of bias related to incomplete outcome data, and 14 studies had an unclear risk.

Selective reporting

All pre-specified outcomes were reported within the outcomes of all of the studies. Studies which failed to report on live birth rate were rated as at high risk of reporting bias.

Other potential sources of bias

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

Twenty-four trials were reported in full published papers (Balaban 2006; Balakier 2009; Baruffi 2000; Ciray 2005; Cohen 1992; Fang 2010; Ge 2008; Germond 2004; Hagemann 2010; Hellebaut 1996; Hurst 1998; Isik 2000; Isiklar 1999; Jelinkova 2002; Kutlu 2010; Lanzendorf 1998; Nagy 1999; Ng 2005; Petersen 2005; Sagoskin 2007; Stein 1995; Tucker 1993; Tucker 1996; Valojerdi 2010). Seven trials were published in conference abstract form only (Antinori 1999; Carter 2003; Elhelw 2005; Laffoon 1999; Rufas-Sapir 2004; Ryan 1997; Utsunomiya 1998).

Effects of interventions

See: **Summary of findings for the main comparison** Live birth; **Summary of findings 2** Multiple pregnancy; **Summary of findings 3** Clinical pregnancy; **Summary of findings 4** Miscarriage

Primary outcomes

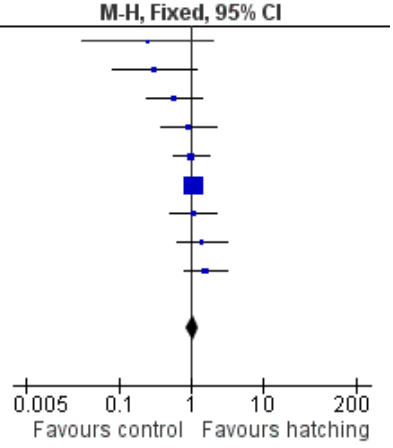
1. Live birth per woman

Few trials reported live birth data, with data available from only nine of the 31 trials. Overall, 595 live birth events were reported (that is not including individual births from multiple pregnancies), 313 in the AH group and 282 in the control group. Overall, there was no evidence of a significant difference between the odds of a live birth in women who underwent AH compared with those in the control group (9 RCTs, 1921 women; OR 1.03, 95% CI 0.85 to 1.26). There was no significant heterogeneity, with P = 0.38 and an I^2 of 6% (Analysis 1.1) (Figure 3).

Figure 3. Forest plot of comparison: 1 Live birth rate, outcome: 1.1 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	1.7%	0.24 [0.03, 2.03]
Germond 2004	3	84	8	74	4.2%	0.31 [0.08, 1.20]
Balakier 2009	13	45	16	39	6.3%	0.58 [0.24, 1.45]
Lanzendorf 1998	12	41	15	48	5.1%	0.91 [0.37, 2.26]
Sagoskin 2007	55	121	37	82	12.5%	1.01 [0.58, 1.78]
Ge 2008	156	487	144	473	51.4%	1.08 [0.82, 1.41]
Hellebaut 1996	21	60	20	60	6.7%	1.08 [0.51, 2.29]
Petersen 2005	17	75	13	75	5.2%	1.40 [0.62, 3.13]
Cohen 1992	34	69	26	68	6.9%	1.57 [0.80, 3.10]
Total (95% CI)		**995**		**926**	**100.0%**	**1.03 [0.85, 1.26]**
Total events	313		282			

Heterogeneity: Chi² = 8.54, df = 8 (P = 0.38); I² = 6%
Test for overall effect: Z = 0.32 (P = 0.75)



Subgroup analysis

1. First or repeat attempt at ART: for women undergoing their first attempt at ART, one trial showed no significant difference in live births between the AH and control groups (1 RCT, 20 women; OR 0.24, 95% CI 0.03 to 2.03, P = 0.19). Similarly for women with previous failed attempts at ART, no significant difference in live birth outcome between the AH and control groups was found (1 RCT, 150 women; OR 1.40, 95% CI 0.62 to 3.13, P = 0.42) (Analysis 1.2).
2. Assisted conception procedure: for women undergoing ICSI, there was no significant difference in live birth outcome between the AH and control groups (1 RCT, 150 women; OR 1.40, 95% CI 0.62 to 3.13, P = 0.42). The same applied to women who underwent IVF, there was no significant difference in live birth outcome between the two groups (3 RCTs, 241 women; OR 1.00, 95% CI 0.60 to 1.68, P = 0.09, I^2 of 58%) (Analysis 1.3).
3. Method of assisted hatching: for women undergoing a chemical method of assisted hatching, there was a no significant difference in live birth outcome between the AH and control

groups (4 RCTs, 366 women; OR 1.13, 95% CI 0.74 to 1.74, P = 0.37, I^2 of 5%). For women who underwent a laser method of AH, likewise there was no significant difference in live birth outcome between the groups (5 RCTs, 1555 women; OR 1.01, 95% CI 0.81 to 1.26, P = 0.27, I^2 of 23%). None of the trials which employed mechanical forms of AH reported on live births (Analysis 1.4).

4. Prognosis: for women in poor prognosis groups, there was no significant difference in live birth outcome between the AH and control groups (4 RCTs, 576 women; OR 1.46, 95% CI 0.99 to 2.15, P = 0.65, I^2 of 0%). The same was found for the women in good prognosis groups (5 RCTs, 1187 women; OR 0.94, 95% CI 0.74 to 1.19, P = 0.58, I^2 of 0%) (Analysis 1.5).

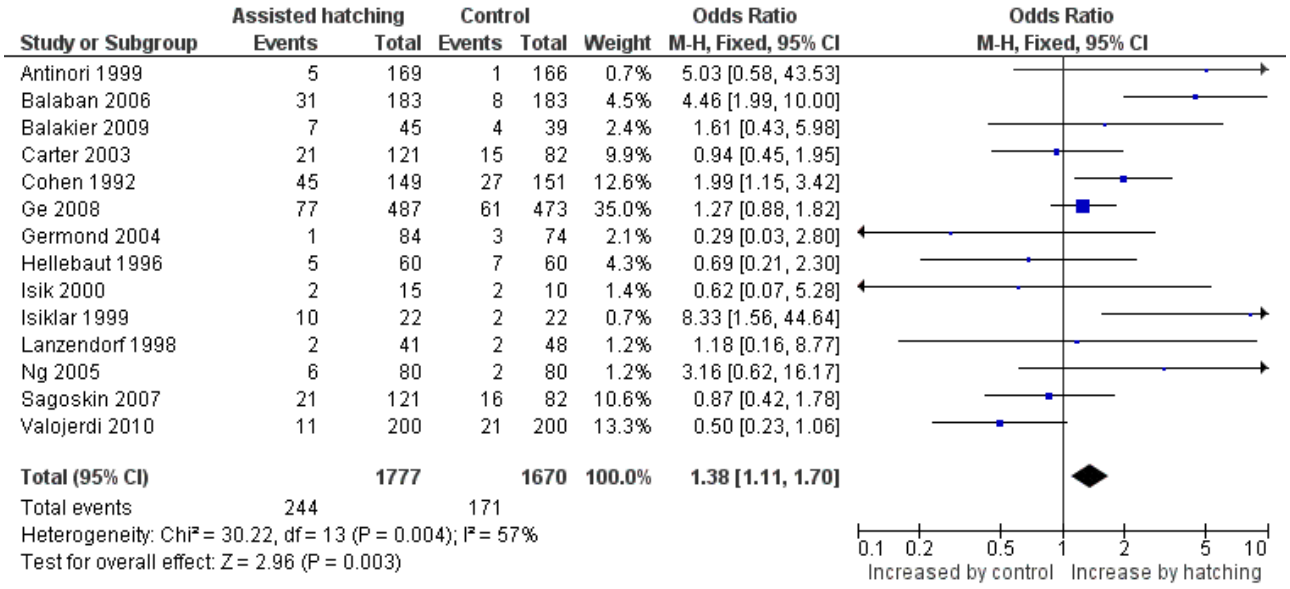
Sensitivity analysis

- Allocation concealment: limiting the analysis to those trials that reported allocation concealment left only two trials (Ge 2008; Lanzendorf 1998). There was no significant difference in live birth rate between the AH group and the control group (OR 1.06, 95% CI 0.81 to 1.38, P = 0.25).
- Method of randomisation: eight trials stated the method of randomisation (Balakier 2009; Ge 2008; Germond 2004; Hellebaut 1996; Hurst 1998; Lanzendorf 1998; Petersen 2005; Sagoskin 2007). Analysis of the data from these trials showed no statistically significant difference between the AH and control groups (OR 0.99, 95% CI 0.80 to 1.22, P = 0.19).

2. Multiple pregnancy per woman

Fourteen of the 31 trials reported on multiple pregnancy. Overall, 415 multiple pregnancies were reported in the 3447 women in the trials reporting on multiple pregnancies, with 244 multiple pregnancies occurring in the AH group and 171 in the control group. Overall, there was a significant increase in multiple pregnancy rates with AH compared to the controls (14 RCTs, 3447 women; OR 1.38, 95% CI 1.11 to 1.70, P = 0.004, I^2 of 57 %) (Figure 4) (Analysis 2.1).

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



Subgroup analysis

1. First attempt or repeat attempt at ART: for women undergoing their first attempt at ART, there were two trials which reported on multiple pregnancy rate, and these found no statistically significant difference between the AH and control groups (2 RCTs, 294 women; OR 0.62, 95% CI 0.12 to 3.19, P = 0.27). There were four trials where women had previous failed attempts at ART which reported multiple pregnancy rate. These also showed no statistically significance between AH and control groups with no significant heterogeneity (4 RCTs, 765 women; OR 1.12, 95% CI 0.70 to 1.80, P = 0.29, I^2 of 20%) (Analysis 2.2).
2. Assisted conception procedure: for women undergoing ICSI, there was evidence of a statistically significant difference in multiple pregnancy rate between the AH and control groups (2 RCTs, 391 women; OR 3.54, 95% CI 1.70 to 7.39, P = 0.09, I^2 of 65%). Similar results were found for women undergoing IVF (6 RCTs, 1126 women; OR 1.87, 95% CI 1.28 to 2.72, P = 0.17, I^2 of 36%) (Analysis 2.3).
3. Method of assisted hatching: there was an increase in multiple pregnancy rates, which bordered on statistical significance, for women in the trials undergoing the laser form of AH (9 RCTs, 2869 women; OR 1.27, 95% CI 1.00 to 1.61, P = 0.006, I^2 of 63%) and a significant increase in multiple pregnancies among women in the one trial undergoing a mechanical method of assisted hatching. For the laser trials, however, there was significant heterogeneity, and for the mechanical method only one trial reported on multiple pregnancy, so there was a wide CI (44 women; OR 8.33, 95% CI 1.56 to 44.64). No increase in multiple pregnancy rate was seen with the chemical method (4 RCTs, 534 women; OR 1.55, 95% CI 0.98 to 2.47, P = 0.35, I^2 = 10) (Analysis 2.4).
4. Prognosis: there was no evidence of significant differences between the AH and control groups in the rate of multiple

pregnancy amongst women with good prognosis (6 RCTs, 1569 women; OR 1.08, 95% CI 0.81 to 1.44, P = 0.69, I^2 of 0%). However, there was a significant difference in the AH group in women with a poor prognosis (5 RCTs, 883 women; OR 1.88, 95% CI 1.19 to 2.96, P = 0.48, I^2 of 0%), with no heterogeneity (Analysis 2.5).

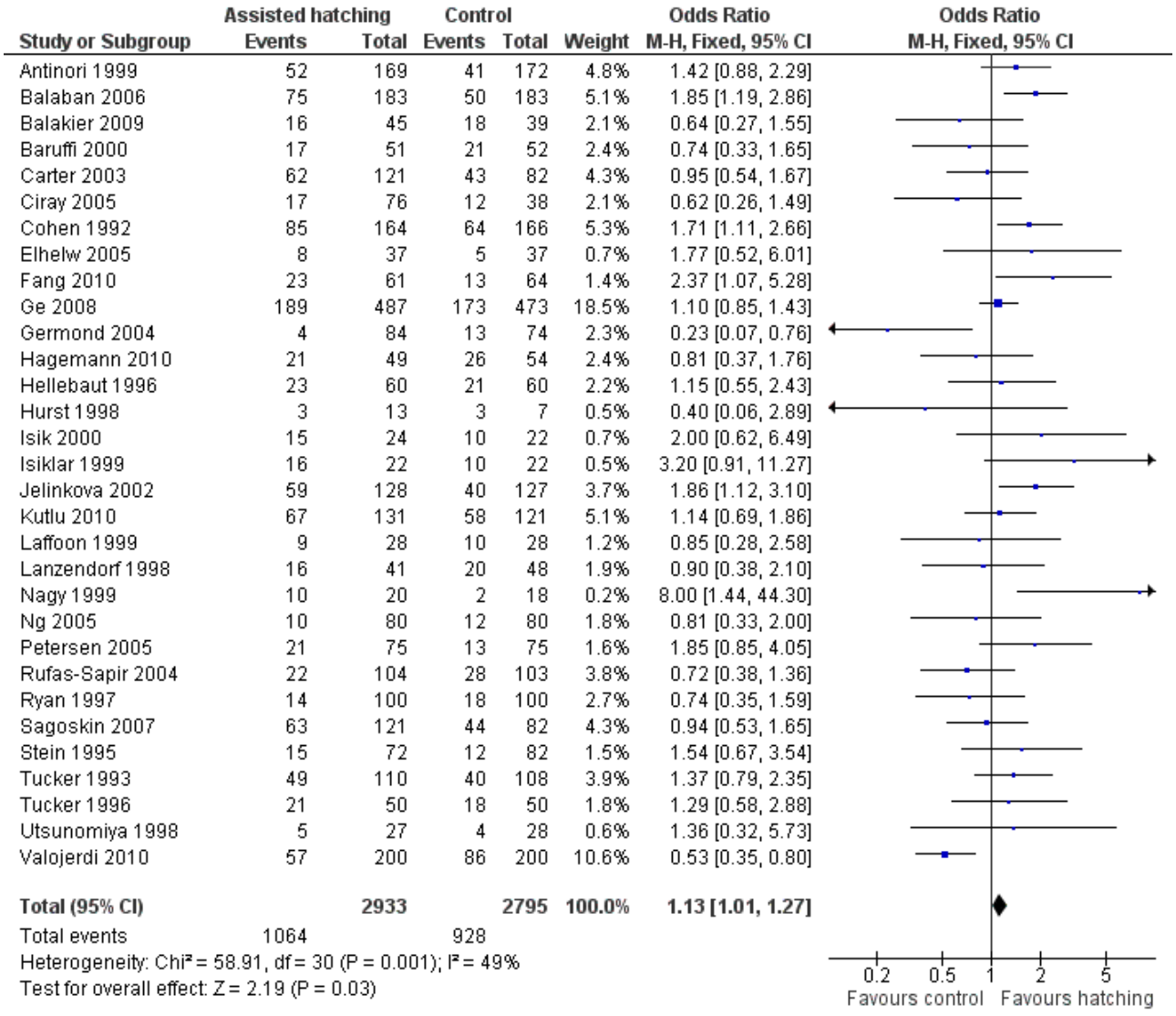
5. Degree of zona manipulation: for the one trial in which women underwent complete removal of the zona pellucida, there was no statistically significant increase in multiple pregnancy rate amongst women in the AH group compared to those in the control group (1 RCT, 25 women; OR 0.62, 95% CI 0.07 to 5.28, P = 0.66). The same applied to trials employing breaching (7 RCTs, 1249 women; OR 1.51, 95% CI 1.05 to 2.17, P = 0.06, I^2 of 51%). For trials employing thinning (5 RCTs, 1970 women; OR 1.39, 95% CI 1.05 to 1.84, P = 0.003, I^2 of 76%), there was a statistically significant increase in multiple pregnancy rates in the AH group compared to controls (Analysis 2.6).
6. Multiple pregnancy per pregnancy: overall the multiple pregnancy rate per clinical pregnancy achieved was statistically significant for women in the AH group compared to the control group (14 trials, OR 1.39, 95% CI 1.09 to 1.77, P = 0.07) (Analysis 2.7).

Secondary outcomes

3. Clinical pregnancy rate per woman

Thirty-one trials reported clinical pregnancy data, including 1992 clinical pregnancies in 5728 women. There were 1064 clinical pregnancies in the AH group and 928 in the control group. Overall, the OR for clinical pregnancy per woman randomised was 1.13 (95% CI 1.01 to 1.27) (Analysis 3.1), showing a borderline statistically significant difference overall favouring the AH group compared to controls. There was, however, evidence of heterogeneity in this analysis (P = 0.001, I^2 of 49%) indicating that, due to wide variation between trials, it may be inappropriate to perform a combined analysis (Figure 5).

Figure 5. Forest plot of comparison: 2 Clinical pregnancy, outcome: 2.1 Clinical pregnancy rate per woman randomised.



Among the nine trials that reported on live births there was no significant increase in clinical pregnancy for women in the AH group compared with the control group (OR 1.03, 95% CI 0.85 to 1.25, P = 0.18, I^2 of 29%) (Analysis 4.1).

Subgroup analysis

1. First or repeat attempt at ART: in the six trials with women experiencing their first cycle of IVF or ICSI there was no evidence of an improved clinical pregnancy rate between women in the AH group and women in the control group (6 RCTs, 650 women; OR 0.77, 95% CI 0.54 to 1.10, P = 0.19, I^2 of 32%). Amongst women, who had previously failed attempts at IVF or ICSI, there was evidence of an improved clinical pregnancy rate (9 trials, 1365 women; OR 1.42, 95% CI 1.11 to 1.81, P = 0.27, I^2 of 20%) (Analysis 3.2).
2. Assisted conception procedure: in the subgroup of women undergoing IVF, there was evidence of a statistically significantly improved clinical pregnancy rate in the AH group compared to the control group (14 RCTs, 2300 women; OR 1.29, 95% CI 1.08 to 1.54, P = 0.12, I^2 of 32%). The same applied to women undergoing ICSI cycles (8 RCTs, 1205 women; OR 1.34, 95% CI 1.05 to 1.71, P = 0.26, I^2 of 21%) (Analysis 3.3).
3. Method of assisted hatching: for women undergoing a chemical method of assisted hatching, there was evidence of an improved clinical pregnancy rate, which was statistically significant, amongst women in the AH group compared with those in the control group (11 RCTs, 1536 women; OR 1.33, 95% CI 1.08 to 1.64, P = 0.47, I^2 of 0%). In contrast, for women undergoing laser forms of assisted hatching, there was no evidence of a statistically significant improvement amongst women in the test group compared with those in the control group (15 RCTs, 3606 women; OR 1.04, 95% CI 0.90 to 1.19, P = 0.0008, I^2 of 62%). The same applied to women undergoing mechanical forms of AH (5 RCTs, 586 women; OR 1.30, 95% CI 0.89 to 1.88, P = 0.09, I^2 of 51) (Analysis 3.4).
4. Prognosis: for women in the poor prognosis group, a statistically significant better outcome in clinical pregnancy rate was found amongst those in the AH group compared to the control group (12 RCTs, 1675 women; OR 1.49, 95% 1.19 to 1.85, P = 0.37, I^2 of 8%), but there was no evidence of a statistically significant improvement amongst women with good prognosis (12 trials, 2253 women; OR 1.02, 95% CI 0.86 to 1.21, P = 0.89) (Analysis 3.5).
5. Degree of zona manipulation: for women undergoing complete removal of the zona pellucida, there was a statistically significant increase in clinical pregnancy rate amongst those in the AH group compared to the control group (2 RCTs, 301 women; OR 1.93, 95% CI 1.21 to 3.09, P = 0.70, I^2 of 0%). Although only two trials reported on this, the CI was not excessively wide and the OR may be of clinical relevance. Similarly, examining the effects of mechanical expansion of the zona pellucida, there was an improvement which was statistically significant in the AH group (1 RCT, 125 women; OR 2.37, 95% CI 1.07 to 5.28, P = 0.003), but only one trial examined this technique of AH. There was no significant difference between AH and control groups in trials which reported on zona pellucida thinning as a means of zona manipulation (12 RCTs, 2936 women; OR 1.05, 95% CI 0.90 to 1.23, P = 0.01, I^2 of 55%). Likewise, there was no significant difference between AH and control groups in trials which reported on zona pellucida piercing (breaching with a hole) as a means of zona manipulation (15 RCTs, 2163 women; OR 1.14, 95% CI 0.94 to 1.37, P = 0.03, I^2 of 45%). The heterogeneity of the latter two groups suggested too much variation amongst trials examining thinning and piercing, however (Analysis 3.6).
6. Fresh or frozen embryo transfer: in fresh embryo groups, there was a statistically significant increase amongst women in the AH group when compared with the control group (24 RCTs, 4050 women; OR 1.14, 95% CI 1.01 to 1.30, P = 0.33, I^2 of 10%). This was not the case for frozen embryo transfers (eight RCTs, 1478 women; OR 1.14, CI 0.90 to 1.44, P < 0.0001, I^2 of 81%) (Analysis 3.7).

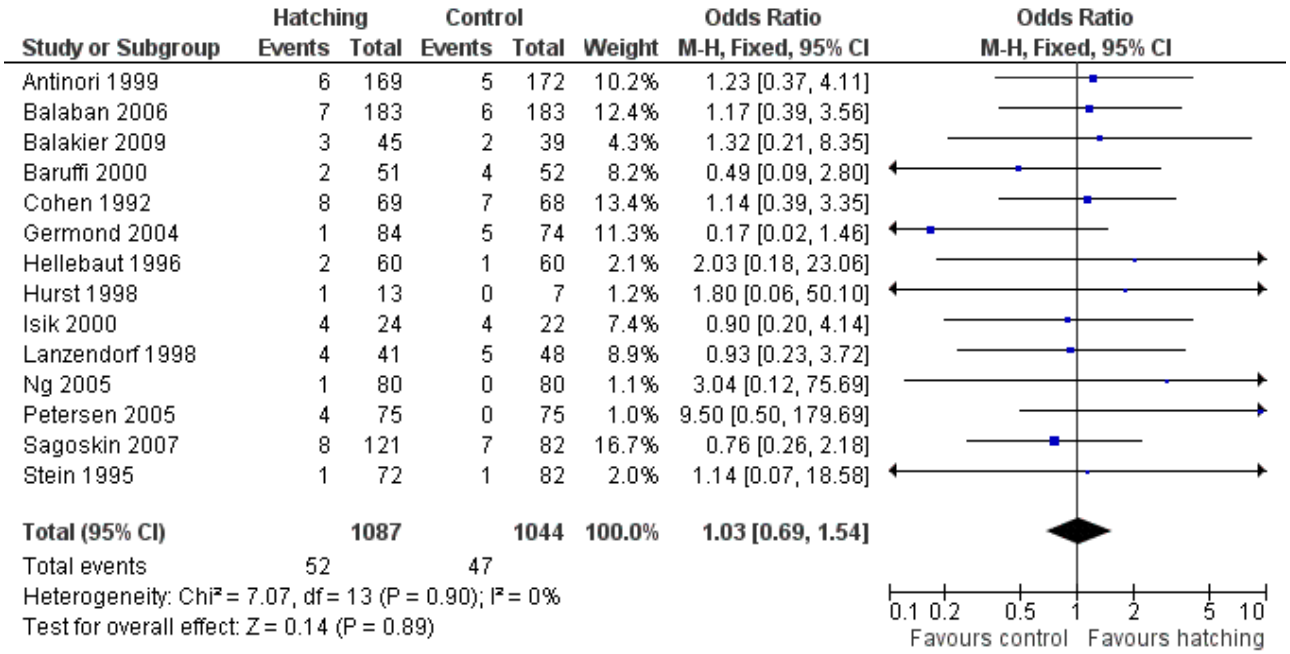
Sensitivity analysis

- Allocation concealment: limiting the analysis to trials which reported allocation concealment left only three trials (Ge 2008; Lanzendorf 1998; Ng 2005). There was no significant difference in clinical pregnancy rate in the AH group when compared to the control group (OR 1.05, 95% CI 0.83 to 1.35, P = 0.27).
- Method of randomisation: 16 trials stated an acceptable method of randomisation (Balaban 2006; Balakier 2009; Baruffi 2000; Carter 2003; Ciray 2005; Ge 2008; Germond 2004; Hagemann 2010; Hellebaut 1996; Hurst 1998; Isik 2000; Kutlu 2010; Petersen 2005; Ryan 1997; Sagoskin 2007; Valojerdi 2010). Analysis of the data from these trials showed no statistically significant difference between the AH and control groups (OR 0.96, 95% CI 0.83 to 1.11, P = 0.03).

4. Miscarriage per woman

Fourteen trials reported miscarriage rates, accounting for 2131 women. There were 99 miscarriages in total, 52 miscarriages occurring in the AH group and 47 in the control group. Overall, there was no significant difference in miscarriage rates between AH and control (14 RCTs, 2131 women; OR 1.03, 95% CI 0.69 to 1.54, P = 0.90, I^2 of 0%) (Figure 6) (Analysis 5.1).

Figure 6. Forest plot of comparison: 3 Miscarriage rate, outcome: 3.1 Miscarriage per woman randomised.



Subgroup analysis

1. First or repeat attempt at ART: for women undergoing their first attempt at ART, there was no evidence of a statistically significant difference in miscarriage rate between AH and control groups (3 RCTs, 264 women; OR 0.91, 95% CI 0.29 to 2.80, P = 0.64, I^2 of 0%). There was no evidence of heterogeneity in this group of trials. The same applied to women who had previously failed attempts at ART (4 RCTs, 663 women; OR 2.14, 95% CI 0.72 to 6.35, P = 0.59, I^2 of 0%) (Analysis 5.2).
2. Assisted conception procedure: for women undergoing ICSI, there was no evidence of a statistically significant difference in miscarriage rate between the AH and control groups (4 RCTs, 665 women; OR 1.20, 95% CI 0.58 to 2.43, P = 0.38, I^2 of 2%). There was no significant heterogeneity between studies. The same results were found for women undergoing IVF (6 RCTs; OR 1.28, 95% CI 0.65 to 2.52, P = 1.00, I^2 of 0%) (Analysis 5.3).
3. Method of assisted hatching: there was no statistically significant evidence of a difference in miscarriage rate between women who underwent a chemical means of AH and those in the control group, with no significant heterogeneity (5 RCTs, 412 women; OR 1.11, 95% CI 0.56 to 2.21, P = 0.98, I^2 of 0%). The same applied to women who underwent a laser means of AH when compared with the control group (8 RCTs, 1565 women; OR 0.98, 95% CI 0.59 to 1.63, P = 0.48, I^2 of 0%), and for women who underwent a mechanical means of AH when compared with the control group (one trial only) (Analysis 5.4).
4. Prognosis: for AH and control groups in the poor prognosis group there was no statistically significant difference in miscarriage rate, with no significant heterogeneity (6 RCTs, 830 women; OR 1.06, 95% CI 0.57 to 1.99, P = 0.40, I^2 of 2%). Likewise, for AH and control groups in the good prognosis group there was no statistically significant difference in miscarriage rate with no significant heterogeneity (5 RCTs, 626 women; OR 1.03, 95% CI 0.50 to 2.14, P = 0.94, I^2 of 0%).

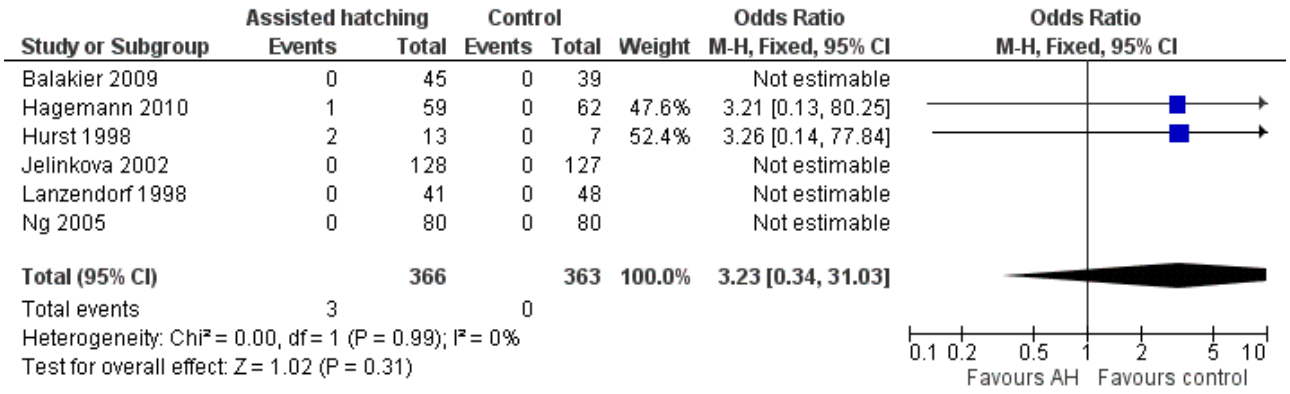
5. Ectopic pregnancy

Four 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 an absence of ectopic pregnancies.

6. Monozygotic twinning

Six trials reported data on monozygotic twinning (Figure 7): Hurst 1998 reported two monozygotic twins from the 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 an 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 7. Forest plot of comparison: 5 Monozygotic twinning rate, outcome: 5.1 Monozygotic twinning per woman randomised.



7. Congenital or chromosomal abnormalities

Two trials (Hurst 1998; Lanzendorf 1998) reported an absence of congenital or chromosomal abnormalities, and one trial (Hagemann 2010) reported fetal abnormalities in both the AH and the control groups.

8. Failure to transfer any embryos per woman

No trials reported data on this outcome.

9. Embryo damage

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

10. 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.

DISCUSSION

Summary of main results

Live birth

In this update, the primary outcome remained live birth rate. Yet only nine of the 31 studies reported this outcome, representing only 34% of all women randomised in the studies. Although the live birth rate may not be representative of all the studies in this review, these studies are representative of those with robust randomisation methods and were considered to be of good quality.

There is no evidence as yet that assisted hatching (AH) impacts on live birth rate, and subgroup analysis does not provide evidence of any effects. It was disappointing that the conclusions of the review were still limited by the paucity of available data in probably the most important and sought after statistic on the impact of AH on assisted conception, namely the 'take home baby rate'. This reflects the gap that currently exists between the practice of assisted conception and clinical obstetrics and the absence of a central database of patient records that would facilitate follow up of these women by authorised agencies, like the Human Fertilisation and Embryology Authority (HFEA) in the UK. That only nine of the included trials from nine authors reported live birth data suggests haste on the part of the other trialists to disseminate data limited to short-term outcomes, and to all intents and purposes these data are incomplete.

Multiple pregnancy

Overall, there was a statistically significant increase in multiple pregnancies per clinical pregnancy (38% increase in OR), indicating that AH does seem to increase the chances of multiple pregnancies. Given this significance in combination with the lack of concrete evidence of an increase in success at achieving live birth, it may bring us to consider the overall risks versus benefits of this technique.

The lack of reporting of live birth data in this group of studies is unfortunate as it limits the interpretation of the results, given this high multiple pregnancy rate, because as many as 5% of multiple pregnancies are lost between 20 and 40 weeks gestation. In addition, most studies were transferring two to four embryos although the numbers transferred were balanced between groups. The reason for 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 the planning of this procedure.

It is likely that reducing the number of embryos transferred to one will not completely eliminate monozygotic twinning. Implantation rate was not considered as an outcome in this update for two reasons. The pooling of embryo implantation data for meta-analysis is statistically problematic. Implantation is traditionally expressed 'per embryo transferred', without regard to 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 has the advantage of being more useful in aiding understanding of the 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 31 included trials reported on clinical pregnancy. There were 1992 pregnancies amongst the 5728 participants, 1064 in the AH

group and 928 in the control group. Similar to the previous update in 2007, this update has shown that, overall, AH does seem to increase the chance of achieving a clinical pregnancy, however the level to which it does so only just reaches statistical significance (OR 1.13, 95% CI 1.01 to 1.27).

Restricting analysis of clinical pregnancy rate to those trials that went on to report live birth, the clinical pregnancy result showed statistically insignificant differences between the AH and the control groups (OR 1.03, 95% CI 0.85 to 1.25, P = 0.18). Analysis of clinical pregnancy rate of the robust studies, which described allocation concealment and their method of randomisation as well as reporting on the live birth rate, gave a clinical pregnancy rate of 1.08 (95% CI 0.82 to 1.41, P = 0.60), again suggesting that AH may not give statistically significant increased chances of achieving clinical pregnancy.

Despite this, similar to 2007, further subgroup analysis of all 31 studies suggests that women undergoing IVF or ICSI cycles who have previously been unsuccessful may benefit from AH as well as those women with a poor prognosis. AH involving complete removal of the zona pellucida shows statistically significant differences in clinical pregnancy rates. The same applies for AH involving expansion of the zona pellucida; however, in this update, there was only one trial which employed this method. In contrast to the previous update, this update showed AH only had statistically significant effects among participants receiving fresh embryos for embryo transfer rather than AH using either fresh or frozen embryos for embryo transfer.

Miscarriage

This review did not find sufficient evidence to draw conclusions on the impact of AH on miscarriage rates overall or for any of the subgroups considered.

Other outcomes

The impact of AH on ectopic pregnancy, congenital and chromosomal abnormalities, blastocyst formation and embryo damage could unfortunately not be answered by this review because of the paucity of available data. This was disappointing as it leaves many unanswered questions about the 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, with a large sample size being investigated. The results of 5698 women in 31 trials are included in this review, leading to a generally acceptable level of evidence. However, failure of many trials to report on primary outcomes (live birth, multiple pregnancy) and variable levels of reporting on other outcomes will inevitably allow potential bias to be introduced into the analysis. This calls for standardised outcome reporting for future assisted conception trials.

Quality of the evidence

The overall quality of the evidence was low to moderate: please see Summary of findings for the main comparison; Summary of findings 2; Summary of findings 3; Summary of findings 4.

Potential biases in the review process

Three authors with varying levels of expertise undertook the search process several times in order to minimise the risk of authors introducing bias, and there was no conflict of interest.

Agreements and disagreements with other studies or reviews

Overall, the addition of the nine new trials in this update has not changed the findings regarding live birth that have been shown in previous reviews, namely that AH does not significantly increase the chances of a live birth. Clinical pregnancy rate again was shown to be slightly increased in women undergoing AH and this just reached a level of statistical significance.

AUTHORS' CONCLUSIONS

Implications for practice

Live birth is the primary outcome yet only nine trials reported on this. Therefore, there could be under reporting of live birth outcomes leading to this result (22 of the 31 trials did not report live birth rates). The addition of the new trials resulted in a further 2082 participants in this review update (36% of the 5728 participants). Subgroups including women who had previously had failed attempts at assisted reproduction and poor prognosis women did have increased clinical pregnancy rates in the assisted hatching (AH) groups, which reached significance.

There was a significant increase in multiple pregnancy rates. This significant increase in the rate of twinning raises concerns regarding the number of embryos transferred and AH. The statistically significant chance of a multiple pregnancy if a clinical pregnancy is achieved may bring the clinician to consider the overall safety of offering this procedure to women in the future, or offering the procedure only to specific subgroups for which AH may be favourable.

Implications for research

This review once again highlighted a wide range of currently unresolved issues that provide potential avenues for future research, including the need for high quality trials which report live births, clinical pregnancies and adverse events (including multiple pregnancies, miscarriages and long-term adverse outcomes), and which 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 multicentre trials with appropriate design, adequate power and appropriate duration of follow up) are undertaken to provide these urgently needed answers.

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Edmond Edi-Osagie contributed to designing the original review, publishing the protocol, data collection, developing a search strategy, undertaking searches, screening search results, organising retrieval of papers, screening retrieved papers against inclusion criteria, appraising quality of papers, abstracting data from papers, writing to authors of papers for additional information, data management for the review, interpretation of data, providing a methodological perspective, providing a clinical perspective, providing a policy perspective, writing the review, providing general advice on the review, and performing previous work that was the foundation of the review.

Lee Hooper developed the second search strategy, undertook the February 2002 searches, screened these search results, assessed inclusion of all potential studies, appraised the quality of and abstracted data from all included studies, analysed the data (meta-analysis in RevMan (RevMan 2008), subgrouping, sensitivity analyses, meta-regression in STATA), interpreted the data, provided a methodological perspective, provided a consumer perspective, wrote the methodology and results sections of the review, and edited the original review.

Phil McGinlay contributed to designing the original review, data collection for the review, developing a search strategy, undertaking searches, screening search results, organising retrieval of papers, screening retrieved papers against inclusion criteria, and appraising quality of papers. Mr McGinlay unfortunately passed away before completion of this review and although he was acknowledged as an author in the two initial versions of this review, in the 2007 version he was removed from the title list.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Antinori 1999

Methods	Randomisation stated, but method unclear or incorrect Allocation concealment unclear Unclear if single/multicentre Participants not blinded or unclear Assessor not blinded or unclear Unclear if 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) versus 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 or incorrect. Day not stated
Allocation concealment (selection bias)	Unclear risk	Unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear. Participants not blinded or unclear. Assessor not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear and no evidence of any losses
Selective reporting (reporting bias)	Unclear risk	This is a conference abstract. There is no evidence of a full paper, live birth was not reported

Balaban 2006

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

Balaban 2006 *(Continued)*

	versus No AH (laser thinning) n = 183 Unclear on how long before transfer, frozen-thawed embryos only
Outcomes	Primary: implantation rate, Secondary: clinical pregnancy, miscarriage and 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) All outcomes	Low risk	There were no losses to follow up and all women were analysed
Selective reporting (reporting bias)	Unclear risk	The original protocol was not viewed but all outcomes listed in the methods section were reported. Live birth was not reported

Balakier 2009

Methods	Single centre Unclear if power calculation performed ITT analysis unclear Published as full paper
Participants	84 women from Canada with no more than one unsuccessful previous IVF attempt, aged ≤ 37 years of age, and with a day 3 FSH ≤ 10mIU/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: the total length of laser cut was approximately 30-40µm, and about 60-80% of the outer layer of the zona pellucid was thinned without complete breaching, applying 2ms laser beams. versus control n = 39
Outcomes	Clinical pregnancy;Multiple pregnancies; Spontaneous miscarriages; Live births
Notes	

Balakier 2009 *(Continued)*

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	The study was double blinded to patients and medical personnel
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were no losses to follow up and all women were analysed
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth was reported

Baruffi 2000

Methods	Single centre Unclear if power calculation performed ITT analysis unclear Published as full paper
Participants	103 women from Brazil aged 37 years or less, 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 hours egg retrieval to AH; 0 hours AH to transfer), 51 women randomised, 141 embryos transferred versus 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, using a randomisation table
Allocation concealment (selection bias)	Unclear risk	No information in the text

Baruffi 2000 *(Continued)*

Blinding (performance bias and detection bias) All outcomes	Unclear risk	No information in the text
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were no losses to follow up and all women were analysed
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. Live birth not reported

Carter 2003

Methods	Single centre Unclear if power calculation performed Published as abstract and authors provided additional information
Participants	203 women from fertility clinic in US Age < 40 years FSH < 10, ovulatory menstrual cycles, day 3 ET with good embryo quality Women with more than one failed IVF cycle were excluded
Interventions	Laser hatching (n=121) versus No hatching (n=82)
Outcomes	Clinical pregnancy rate, multiple pregnancy rate
Notes	Additional information provided by authors Drop-outs were 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, method by computer generation on day three
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 drop-outs
Selective reporting (reporting bias)	High risk	This was a conference abstract only and was not published as a full paper although the authors did provide some additional information. Live birth was not reported

Ciray 2005

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 endometrosis 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) versus 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	Unclear risk	All women appear to have been analysed
Selective reporting (reporting bias)	Unclear risk	All outcomes reported but original protocol not viewed, live birth was not reported

Cohen 1992

Methods	Single centre Unclear if 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 tyrodes (chemical; complete zona breach hole; 68 to 72 hours 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 zonae pellucida, low developmental rate, excessive fragmentation), 15 women with FSH >15 (trial 3)

Cohen 1992 *(Continued)*

	No AH, 68 women with FSH <15 (trial 1), 83 women with poor prognosis (trial 2, thick zonae 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 form women in trials 1 and 2 only)
Notes	Attempted to contact author about this study. Reply received, but no additional information was offered No. of embryos AH 3.5; Control 3.4

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Pre-printed randomisation list day not stated
Allocation concealment (selection bias)	High risk	Allocation concealment inadequate
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants blinded Assessor blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed

Elhelw 2005

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) versus 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 article No data re no. of embryo transfer

Risk of bias

Bias	Authors' judgement	Support for judgement

Elhelw 2005 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Not stated, no details
Allocation concealment (selection bias)	High risk	Not used
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

Fang 2010

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 the zona pellucida via injected hydrostatic pressure AH: 61 women, 178 embryos Control: 64 women, 190 embryos
Outcomes	Clinical pregnancy and intrauterine implantation rates
Notes	Unclear if 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	Embryologists blinded to the group assignment, unclear if participants were too
Incomplete outcome data (attrition bias)	Unclear risk	Unclear

Fang 2010 *(Continued)*

All outcomes		
Selective reporting (reporting bias)	High risk	Original protocol not viewed. Live birth not reported

Ge 2008

Methods	Randomised controlled trial
Participants	760 women from China having IVF with fewer than five failed cycles of ART with normal baseline FSH concentration. Those participants with uterine abnormality or low fertilisation capacity (rate of fertilisation less than 20% and late ICSI following fertilisation failure of IVF) were excluded Mean age: Fresh, 31.08 in AH, 30.44 control; Frozen, 31.84 in AH, 30.66 control
Interventions	Laser thinning to about 50% of the 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 and live birth
Notes	Unclear if power calculation performed ITT not stated Published as full paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Women were randomised according to a randomisation list based on sequential numbers in sealed envelopes
Allocation concealment (selection bias)	Low risk	Both patients and the clinician were blinded to group allocation
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated in the text
Incomplete outcome data (attrition bias) All outcomes	Low risk	Fresh embryo transfer cycles: a 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: a total of 245 frozen-thawed cycles were also performed, of which 45 were excluded either because they didn't meet the criteria of the study or embryo transfer was abandoned
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed

Germond 2004

Methods	Multicentre RCT
Participants	153 women in four European IVF centres aged between 20 and 45 years old, having at least one functional ovary, having normal FSH and prolactin levels, having no clinically significant findings within six months before starting treatment, and having a normal uterine cavity
Interventions	Laser assisted hatching using diode laser AH: 56 women undergoing their first cycle of frozen-thawed embryos, 23 women who had a poor prognosis using fresh embryos Control: 53 women undergoing their first cycle of frozen-thawed embryos, 21 women who had a poor prognosis using fresh embryos
Outcomes	Clinical pregnancies, live births, miscarriages and 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

Hagemann 2010

Methods	Randomised, single centre, crossover trial
Participants	103 women in the United States under 38 years of age with any embryo with a zona pellucida thickness > 13µm and more than 2 previous failed IVF cycles Mean age: 32.1 years in the hatched group, 31.2 in the unhatched group
Interventions	AH performed by acidic Tyrode's solution AH: 49 women Control: 54 women

Hagemann 2010 *(Continued)*

Outcomes	Clinical intrauterine pregnancy rate, implantation rate, spontaneous pregnancy loss and live birth rate
Notes	Power calculation: study states it has inadequate power. The study as ultimately performed only had sufficient statistical power to identify 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)	Low risk	Randomisation was performed by the 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	The study arm to which participants belonged was blinded to care givers, with the exception of the IVF embryologyists, as well as to participants
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear
Selective reporting (reporting bias)	Low risk	Live birth reported (but results not included in this study as results were only given for both cycles combined and not for just the first cycle, which is the data we are using). No other evidence of reporting bias

Hellebaut 1996

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 hours egg retrieval to AH; 0.2 hours AH to transfer) versus 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
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 if power calculation performed ITT analysis unclear Published as full paper

Risk of bias

Bias	Authors' judgement	Support for judgement

Hellebaut 1996 *(Continued)*

Random sequence generation (selection bias)	Low risk	By computer on day of transfer
Allocation concealment (selection bias)	High risk	Allocation concealment inadequate
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. Authors responded to requests for details. No other evidence of bias, all outcomes stated were reported

Hurst 1998

Methods	Single centre randomised trial
Participants	20 women from North America undergoing IVF, either with no prior IVF (30 years or less, FSH < 10 IU/L, normal endometrium and sperm) or 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 tyrodes (chemical; complete zona breach hole; ? hours egg retrieval to AH; ? hours AH to transfer) versus 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 if 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	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

Hurst 1998 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	Low risk	Protocol not viewed but outcomes were reported and included live birth

Isik 2000

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 hours egg retrieval to AH; 0.5 to 1 hours AH to transfer) versus no AH AH: 24 women randomised, 71 embryos transferred Control: 22 women randomised, 63 embryos transferred
Outcomes	Implantation
Notes	Author response No. of embryos transferred, blastocyst transfer: AH 2.95 (0.9); Control 2.86 (0.8) Unclear if 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	Using a random number table on day three
Allocation concealment (selection bias)	High risk	Allocation not concealed
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 was not reported

Isiklar 1999

Methods	Single centre randomised trial
Participants	44 women from Turkey undergoing IVF Mean age not stated
Interventions	AH (mechanical; complete zona breach; ? hours egg retrieval to AH; ? hours AH to transfer) versus 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 if 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	Randomised on day three
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)	Unclear risk	This publication was in abstract form only, no full paper publication was identified. The authors do not report on live birth

Jelinkova 2002

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

Jelinkova 2002 *(Continued)*

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)	Unclear risk	Randomisation stated, but method and timing unclear
Allocation concealment (selection bias)	Unclear risk	Unclear, no details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Participants not blinded or unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not stated
Selective reporting (reporting bias)	High risk	Protocol not viewed, outcomes were reported on but do not include live birth

Kutlu 2010

Methods	Single centre, randomised trial
Participants	252 infertile couples having ART treatments at Medicana Camlica Hospital, Istanbul, Turkey. Subgrouped by prognosis: poor (n=113) or good (n=139)
Interventions	AH was performed by laser method AH: 73 women aged under 35 years, 58 women aged 35 or over Control: 66 women aged under 35, 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)	Unclear risk	Not stated within the text

Kutlu 2010 *(Continued)*

All outcomes		
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis unclear
Selective reporting (reporting bias)	High risk	Original protocol not viewed. Live birth not reported

Laffoon 1999

Methods	Single centre, randomised trial
Participants	56 women from North America aged less than 40 years undergoing IVF. Mean age not stated
Interventions	AH (mechanical; complete zona breach; ? hours egg retrieval to AH; ? hours AH to transfer) versus 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 Unclear if 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 was not reported

Lanzendorf 1998

Methods	Single centre, randomised trial

Lanzendorf 1998 *(Continued)*

Participants	94 women from North America aged at least 36 years (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 had been previously treated with IVF Mean age: control group 38.5 (0.26); AH group 38.3 (0.31)
Interventions	AH by acid tyrodes (chemical; complete zona breach; 55 hours egg retrieval to AH; ? hours AH to transfer) versus 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 if power calculation performed ITT analysis performed

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised, method stated
Allocation concealment (selection bias)	Low risk	Allocation concealment using 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. Authors did report on live birth

Nagy 1999

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; ? hours egg retrieval to AH; ? hours AH to transfer) with concomitant removal of damaged blastomeres versus 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

Nagy 1999 *(Continued)*

	No. of embryos: AH: 2.9, control: 3.2 Unclear if 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	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. The authors did not report on live birth

Ng 2005

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 and multiple pregnancy rates
Notes	No 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	Randomisation pm day of frozen embryo transfer, computergenerated randomisation in sealed envelopes on day of ET
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)	Low risk	double blinding until completion of the study

Ng 2005 *(Continued)*

All outcomes		
Incomplete outcome data (attrition bias) All outcomes	Low risk	No evidence of attrition bias
Selective reporting (reporting bias)	Unclear risk	Original protocol not viewed. The authors did not report on live birth

Petersen 2005

Methods	Randomised trial
Participants	150 Women from Brazil undergoing ART cycles All participants had one failed treatment cycle Mean age 34 years
Interventions	ICSI cycles only AH quarter-laser thinning versus control AH: 35 women with one previous implantation failure, 40 women with repeated implantation failures Control: 35 women with one previous implantation failure, 40 women with repeated implantation failures
Outcomes	Live birth, clinical pregnancy, miscarriage and multiple pregnancy
Notes	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 number table
Allocation concealment (selection bias)	Unclear risk	Unclear - a code ID to mask identity of the participant 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)	Unclear risk	Original protocol not viewed but authors did report on live birth

Rufas-Sapir 2004

Methods	Unknown randomisation method and allocation concealment. occurred on day of embryo transfer
Participants	207 women 3 consecutive failed IVF cycles All ages Undergoing IVF only
Interventions	Mechanical partial zonal dissection: complete breach technique versus control AH - 104 women Control - 103 women
Outcomes	Clinical pregnancy, miscarriage
Notes	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	
Selective reporting (reporting bias)	Unclear risk	Unclear

Ryan 1997

Methods	Single centre, randomised trial
Participants	200 women from Sydney Australia undergoing ART cycles
Interventions	AH: tyrodes complete breach - hole chemical means on both fresh and frozen-thawed embryos: 100 women Control: 100 women
Outcomes	Clinical pregnancy
Notes	Additional information was received from the 1st author regarding the definition of pregnancy. No further publication is planned

Ryan 1997 *(Continued)*

	Mean ET 2.17

Risk of bias

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

Sagoskin 2007

Methods	Randomised trial
Participants	199 women from USA undergoing IVF or ICSI Good prognosis group with only one 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 and multiple pregnancy rate
Notes	No author contact 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

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 in a 2:1 ratio of treatments to controls
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

Sagoskin 2007 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT unclear
Selective reporting (reporting bias)	Low risk	Live birth reported

Stein 1995

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; ? hours egg retrieval to AH; 1.5 hours AH to transfer) versus 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 if 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 authors did not report on live birth

Tucker 1993

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)

Tucker 1993 *(Continued)*

	Mean age: control group 34.2 (4.1); AH group 34.1 (4.8)
Interventions	AH with acid tyrodes thinning to 1/4; 72 hours egg retrieval to AH; 1 to 3 hours AH to transfer) versus 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 if 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 authors did not report on live birth

Tucker 1996

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 tyrodes (chemical; complete zona breach; 72 hours egg retrieval to AH; 4 hours AH to transfer) versus 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 if power calculation performed

Tucker 1996 *(Continued)*

	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 authors did not report on live birth

Utsunomiya 1998

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 Control: 28 women
Outcomes	Clinical pregnancy rate only (gestation sac on ultrasound)
Notes	No attempt to contact author No. of ET not stated Unclear if 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)	Unclear risk	Participants not blinded or unclear Assessor not blinded or unclear

Utsunomiya 1998 *(Continued)*

All outcomes		
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	ITT analysis is unclear
Selective reporting (reporting bias)	High risk	Published as a conference abstract only and did not report on live births

Valojerdi 2010

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); 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

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation list participants
Allocation concealment (selection bias)	Low risk	Sequential numbers in sealed envelopes (200 participants in each group)
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not stated
Selective reporting (reporting bias)	Unclear risk	Original protocol was not viewed but authors did not report on live birth

AH = assisted hatching
IVF = in vitro fertilisation
ICSI = intracytoplasmic sperm injection
ITT = intention-to-treat
Mean age given in years (standard deviation).
Note: only arms where all or no embryos transferred were treated with AH were accepted for data extraction.

ET= embryo transfer

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

Study	Reason for exclusion
Abdelmassih 2002	Pooled oocytes and then randomised; no per woman data provided
Antinori 1996a	Not a randomised controlled trial Mentions randomly selected not randomly allocated
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 two different culture media
Cieslak 1999	Comparison of two types of assisted hatching; no 'no assisted hatching' control group was used More than one cycle per woman
Cohen 1990	Not randomised
Debrock 2011	Primary outcome was implantation, results per embryo transfer and not per woman
Demirol 2003	No pregnancy data provided
Dirnfeld 2003	No hatching
Dokras 1994	No appropriate outcome measure
Domitrz 2000	Benefits of assisted hatching confounded by concurrent assessment of two different culture media
Ebner 2002	No per woman data
Edirisinghe 1999	Not randomised
Feng 2009	Not a prospective study. A retrospective study
Figueira 2012	eggs were from egg donors, not the womens' own eggs
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 The control group were from the period 1990 to 1993, while the assisted hatching group were from 1994 to 1996 (historical controls)
Hiraoka 2009	No control. Comparing a half thinning versus a quarter thinning.
Hur 2011	Not clear if 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
Komarovsky 2002	No per women data
Komarovsky 2003	No per women data
Lee 1999	Not randomised
Levron 2003	No per women data
Ma 2007	No per women data
Magli 1998	No per women data
Mahadevan 1998	Not randomised No concurrent controls
Mansour 2000	Randomisation by alternate days
Meldrum 1998	Not randomised No concurrent controls
Montag 1999	Not randomised No concurrent controls
Nagy 2003	No per woman data
Nakayama 1998	No appropriate outcome measure
Nakayama 1999	No per woman data
Ng 2008	No control. Compared 2 methods of laser
Obruca 1994	Not randomised No concurrent controls
Olivennes 1997	No per woman data
Peterson 2006	results per embryo transfer only no per woman data
Rienzi 2002	Assisted hatching was part of the ICSI method

Study	Reason for exclusion
Ringler 1999	It was not clear how many women were included in the study, or for how many cycles (only cycles were mentioned), and a mixture of participants and donated eggs were used for the study
Schoolcraft 1994	Not randomised Control and intervention groups recruited at different times
Shahin 2003	No per women data
Sifer 2005	Per cycle data only No per woman data
Szell 1998	Not randomised Benefits of assisted hatching confounded by concurrent assessment of two different culture media
Tao 1997	Not randomised Some of the women in the assisted hatching group were randomised, but most were allocated assisted hatching routinely, with no control option
Tucker 1991	Not randomised
Urman 2002	Alternate randomisation
Valojerdi 2008	Inadequate method of randomisation
Yano 2007	No per woman data, only per cycle data
Zech 1998	Numbers in tables do not add up correctly and the text and tables are contradictory on the age groups used in the prospective part of the study
Zhang 2009	Not a prospective study. A retrospective study.

HCG = human chorionic gonadotropin

DATA AND ANALYSES

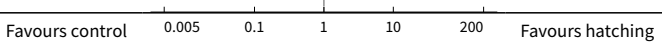
Comparison 1. Live birth: Assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth per woman randomised	9	1921	Odds Ratio (M-H, Fixed, 95% CI)	1.03 [0.85, 1.26]
2 First or repeat attempt	2		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 First attempt at IVF or ICSI	1	20	Odds Ratio (M-H, Fixed, 95% CI)	0.24 [0.03, 2.03]
2.2 Repeat attempt at IVF or ICSI	1	150	Odds Ratio (M-H, Fixed, 95% CI)	1.40 [0.62, 3.13]
3 Conception mode	4		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 ICSI only	1	150	Odds Ratio (M-H, Fixed, 95% CI)	1.40 [0.62, 3.13]
3.2 IVF only	3	241	Odds Ratio (M-H, Fixed, 95% CI)	1.00 [0.60, 1.68]
4 Hatching method	9		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Chemical	4	366	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [0.74, 1.74]
4.2 Laser	5	1555	Odds Ratio (M-H, Fixed, 95% CI)	1.01 [0.81, 1.26]
5 Prognosis	8		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.1 Poor prognosis	4	576	Odds Ratio (M-H, Fixed, 95% CI)	1.46 [0.99, 2.15]
5.2 Good prognosis	5	1187	Odds Ratio (M-H, Fixed, 95% CI)	0.94 [0.74, 1.19]

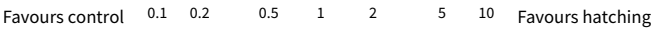
Analysis 1.1. Comparison 1 Live birth: Assisted hatching compared with no assisted hatching, Outcome 1 Live birth per woman randomised.

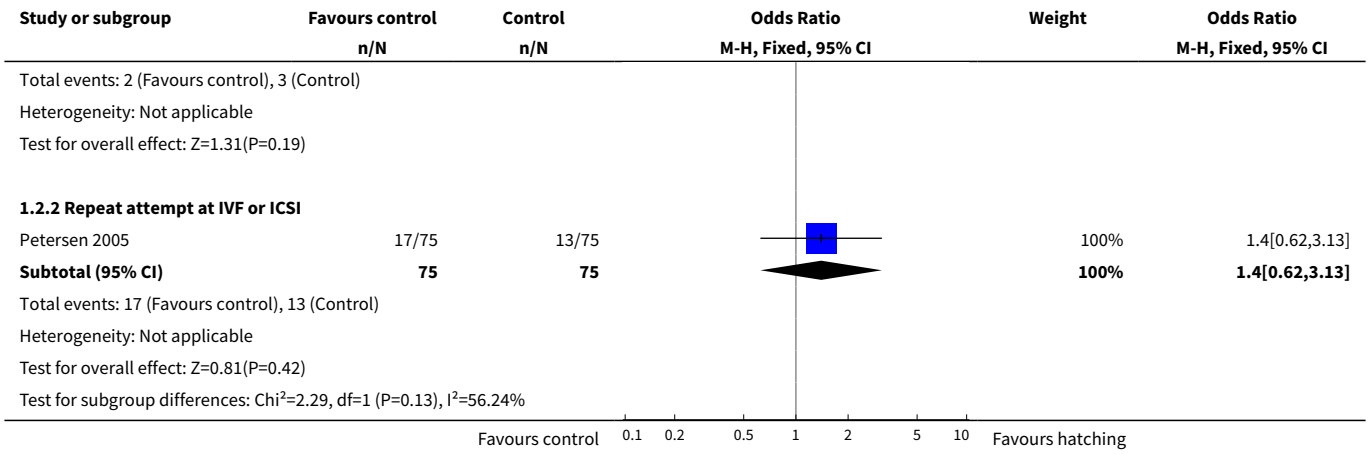
Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Hurst 1998	2/13	3/7		1.71%	0.24[0.03,2.03]
Germond 2004	3/84	8/74		4.25%	0.31[0.08,1.2]
Balakier 2009	13/45	16/39		6.31%	0.58[0.24,1.45]
Lanzendorf 1998	12/41	15/48		5.06%	0.91[0.37,2.26]
Sagoskin 2007	55/121	37/82		12.46%	1.01[0.58,1.78]
Ge 2008	156/487	144/473		51.41%	1.08[0.82,1.41]
Hellebaut 1996	21/60	20/60		6.73%	1.08[0.51,2.29]
Petersen 2005	17/75	13/75		5.2%	1.4[0.62,3.13]
Cohen 1992	34/69	26/68		6.88%	1.57[0.8,3.1]
Total (95% CI)	**995**	**926**		**100%**	**1.03[0.85,1.26]**
Total events: 313 (Assisted hatching), 282 (Control)					
Heterogeneity: Tau^2=0; Chi^2=8.54, df=8(P=0.38); I^2=6.28%					
Test for overall effect: Z=0.32(P=0.75)					



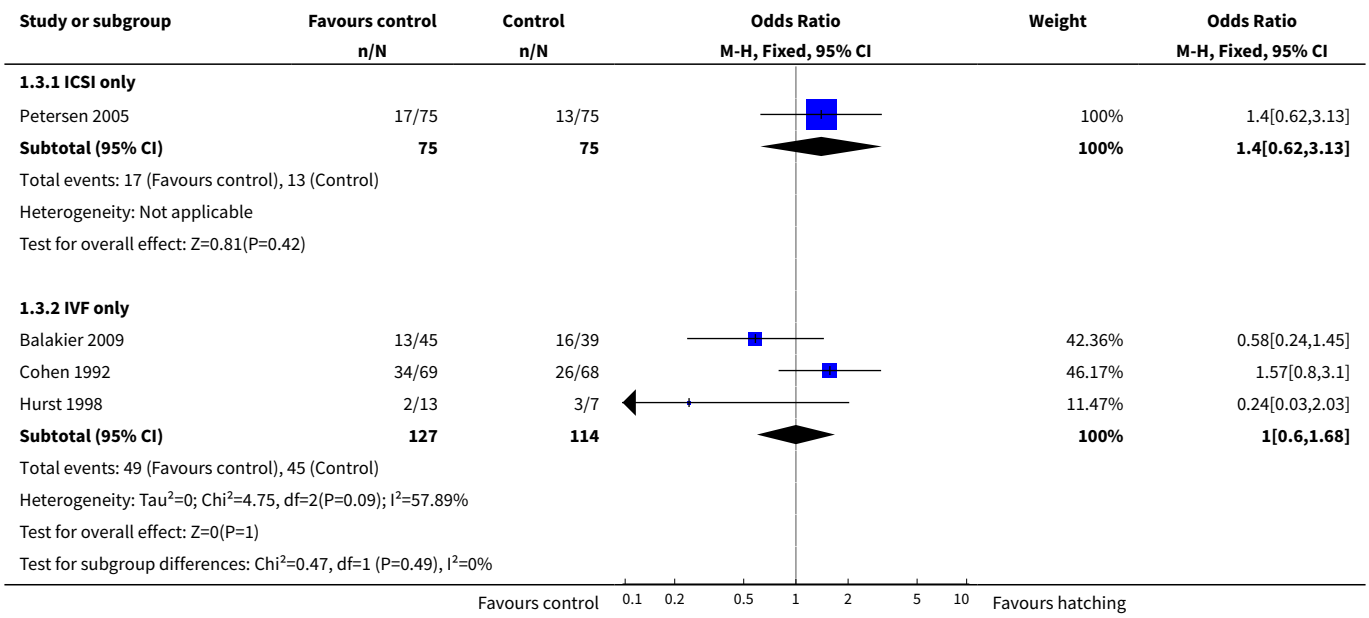
Analysis 1.2. Comparison 1 Live birth: Assisted hatching compared with no assisted hatching, Outcome 2 First or repeat attempt.

Study or subgroup	Favours control n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
1.2.1 First attempt at IVF or ICSI					
Hurst 1998	2/13	3/7		100%	0.24[0.03,2.03]
Subtotal (95% CI)	**13**	**7**		**100%**	**0.24[0.03,2.03]**

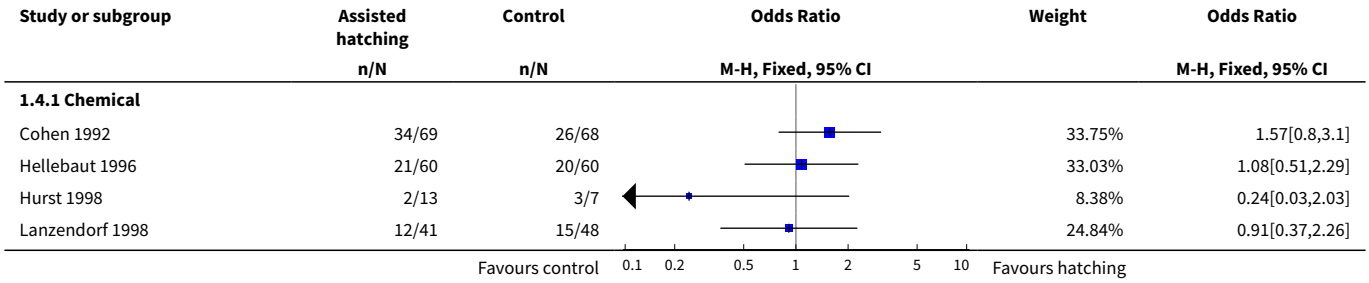


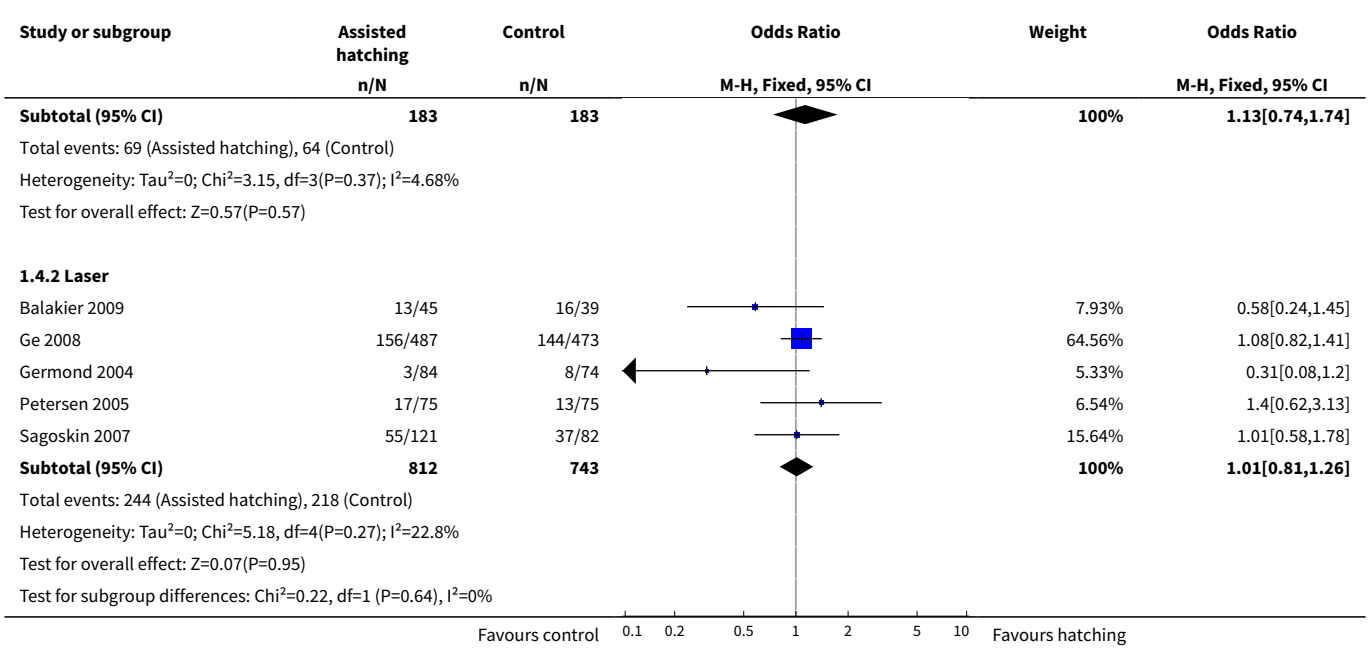


Analysis 1.3. Comparison 1 Live birth: Assisted hatching compared with no assisted hatching, Outcome 3 Conception mode.

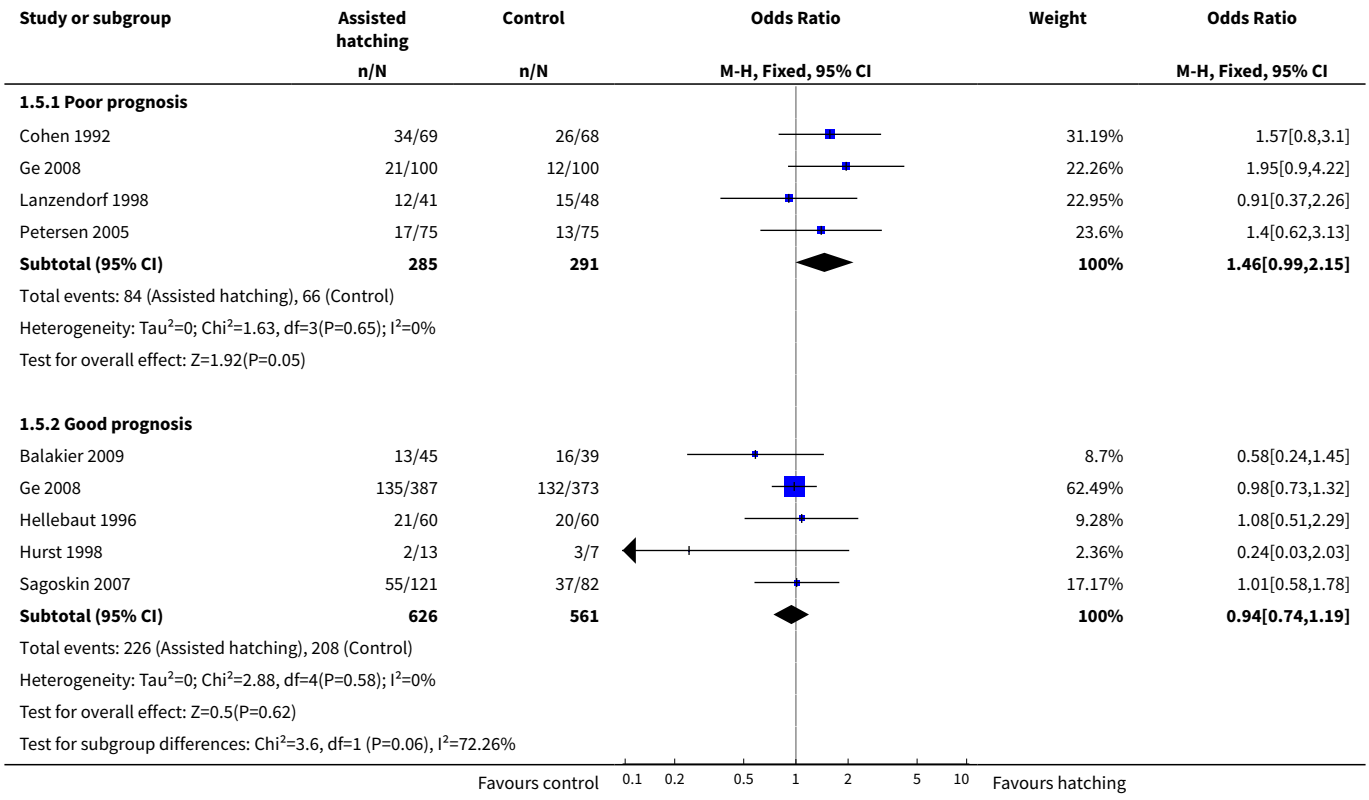


Analysis 1.4. Comparison 1 Live birth: Assisted hatching compared with no assisted hatching, Outcome 4 Hatching method.





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

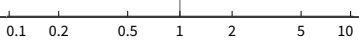


Comparison 2. Multiple pregnancy: Assisted hatching compared with no assisted hatching

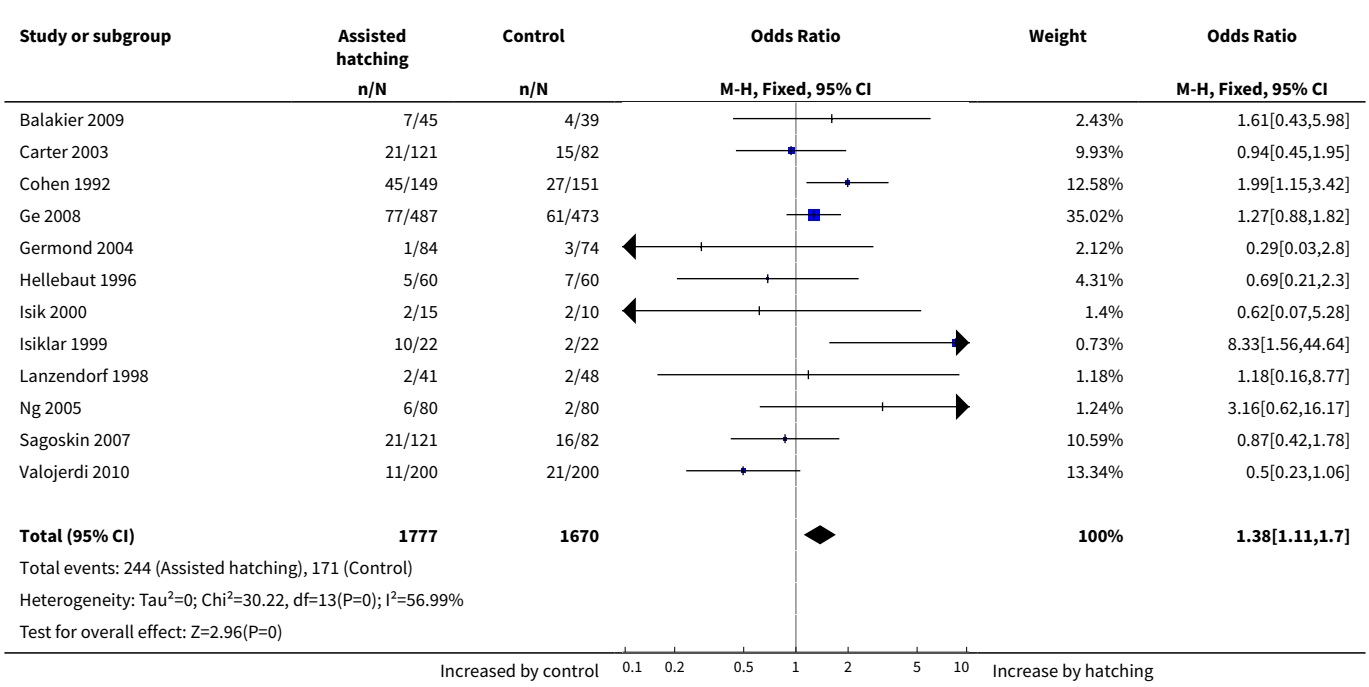
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Multiple pregnancy rate per woman randomised	14	3447	Odds Ratio (M-H, Fixed, 95% CI)	1.38 [1.11, 1.70]
2 First or repeat attempt	5		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 First attempt at IVF or ICSI	2	294	Odds Ratio (M-H, Fixed, 95% CI)	0.62 [0.12, 3.19]
2.2 Repeat attempt at IVF or ICSI	4	765	Odds Ratio (M-H, Fixed, 95% CI)	1.12 [0.70, 1.80]
3 Conception mode	8		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 ICSI only	2	391	Odds Ratio (M-H, Fixed, 95% CI)	3.54 [1.70, 7.39]
3.2 IVF only	6	1126	Odds Ratio (M-H, Fixed, 95% CI)	1.87 [1.28, 2.72]
4 Hatching method	14		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Chemical	4	534	Odds Ratio (M-H, Fixed, 95% CI)	1.55 [0.98, 2.47]
4.2 Laser	9	2869	Odds Ratio (M-H, Fixed, 95% CI)	1.27 [1.00, 1.61]
4.3 Mechanical	1	44	Odds Ratio (M-H, Fixed, 95% CI)	8.33 [1.56, 44.64]
5 Prognosis	9		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.1 Poor prognosis	5	883	Odds Ratio (M-H, Fixed, 95% CI)	1.88 [1.19, 2.96]
5.2 Good prognosis	6	1569	Odds Ratio (M-H, Fixed, 95% CI)	1.08 [0.81, 1.44]
6 Multiple pregnancy rate per woman grouped by extent of assisted hatching	13		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Thinning only	5	1970	Odds Ratio (M-H, Fixed, 95% CI)	1.39 [1.05, 1.84]
6.2 Breach by hole	7	1249	Odds Ratio (M-H, Fixed, 95% CI)	1.51 [1.05, 2.17]
6.3 Complete removal of zona	1	25	Odds Ratio (M-H, Fixed, 95% CI)	0.62 [0.07, 5.28]
7 Multiple pregnancy per pregnancy	14	1383	Odds Ratio (M-H, Fixed, 95% CI)	1.39 [1.09, 1.77]

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

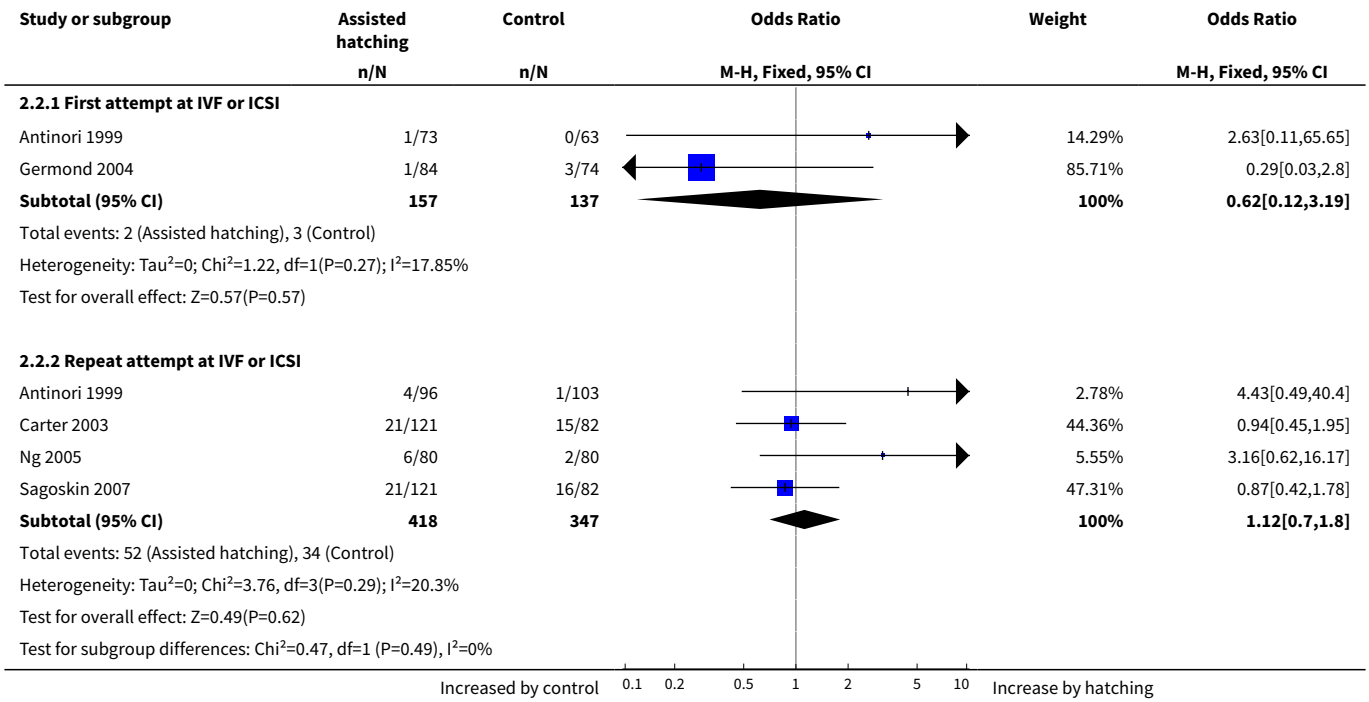
Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	5/169	1/166		0.66%	5.03[0.58,43.53]
Balaban 2006	31/183	8/183		4.47%	4.46[1.99,10]



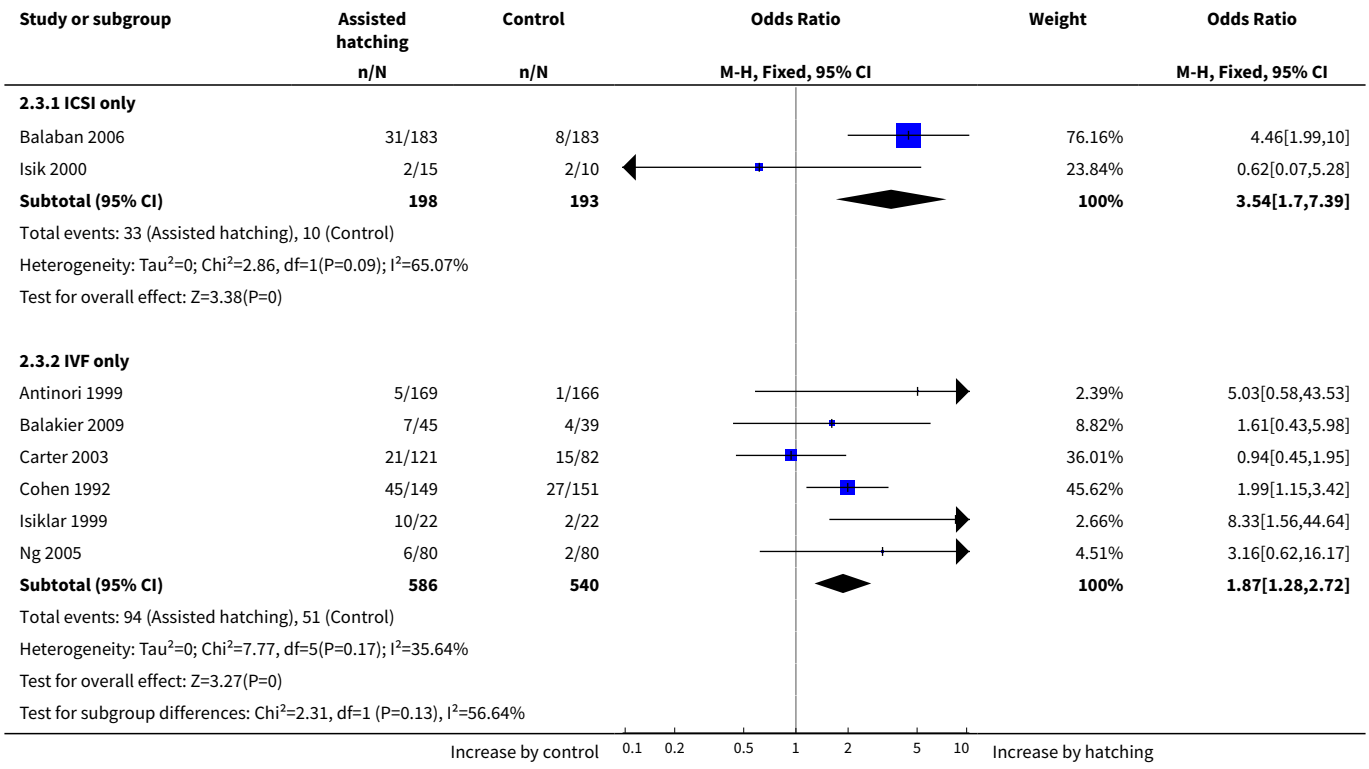
Increased by control — Increase by hatching



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

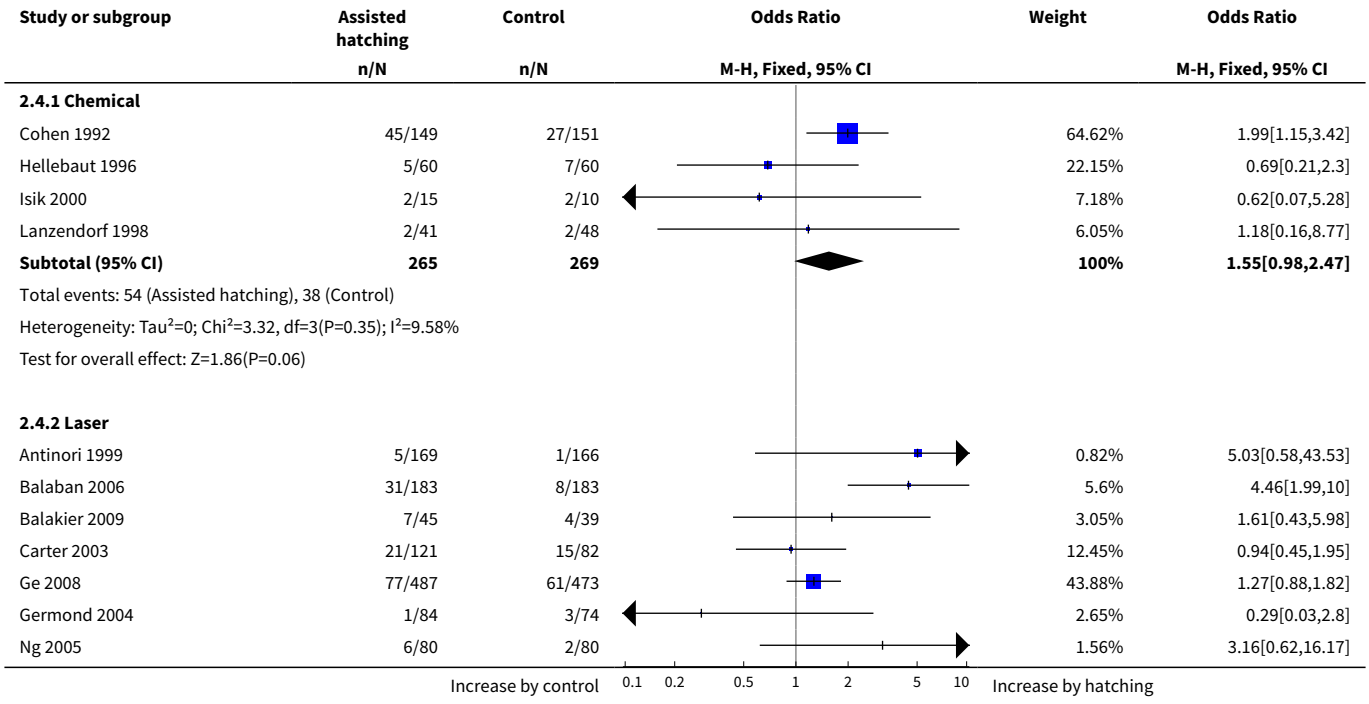


Analysis 2.3. Comparison 2 Multiple pregnancy: Assisted hatching compared with no assisted hatching, Outcome 3 Conception mode.

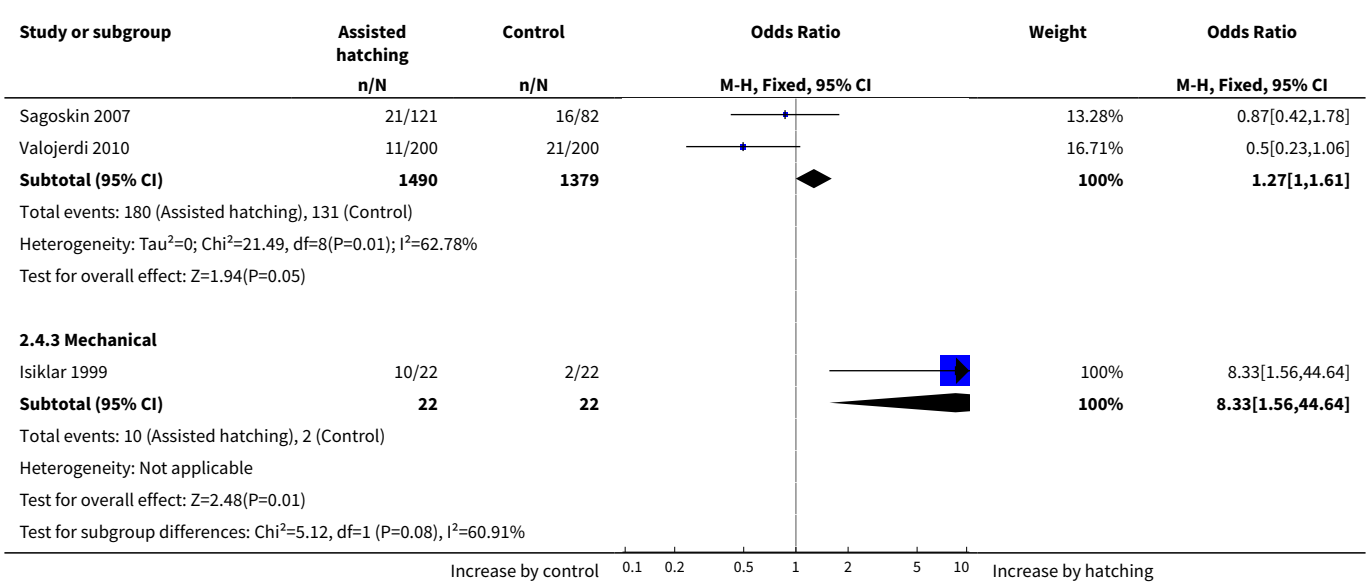


Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
2.3.1 ICSI only					
Balaban 2006	31/183	8/183		76.16%	4.46[1.99,10]
Isik 2000	2/15	2/10		23.84%	0.62[0.07,5.28]
Subtotal (95% CI)	**198**	**193**		**100%**	**3.54[1.7,7.39]**
Total events: 33 (Assisted hatching), 10 (Control)					
Heterogeneity: Tau^2=0; Chi^2=2.86, df=1(P=0.09); I^2=65.07%					
Test for overall effect: Z=3.38(P=0)					
2.3.2 IVF only					
Antinori 1999	5/169	1/166		2.39%	5.03[0.58,43.53]
Balakier 2009	7/45	4/39		8.82%	1.61[0.43,5.98]
Carter 2003	21/121	15/82		36.01%	0.94[0.45,1.95]
Cohen 1992	45/149	27/151		45.62%	1.99[1.15,3.42]
Isiklar 1999	10/22	2/22		2.66%	8.33[1.56,44.64]
Ng 2005	6/80	2/80		4.51%	3.16[0.62,16.17]
Subtotal (95% CI)	**586**	**540**		**100%**	**1.87[1.28,2.72]**
Total events: 94 (Assisted hatching), 51 (Control)					
Heterogeneity: Tau^2=0; Chi^2=7.77, df=5(P=0.17); I^2=35.64%					
Test for overall effect: Z=3.27(P=0)					
Test for subgroup differences: Chi^2=2.31, df=1 (P=0.13), I^2=56.64%					

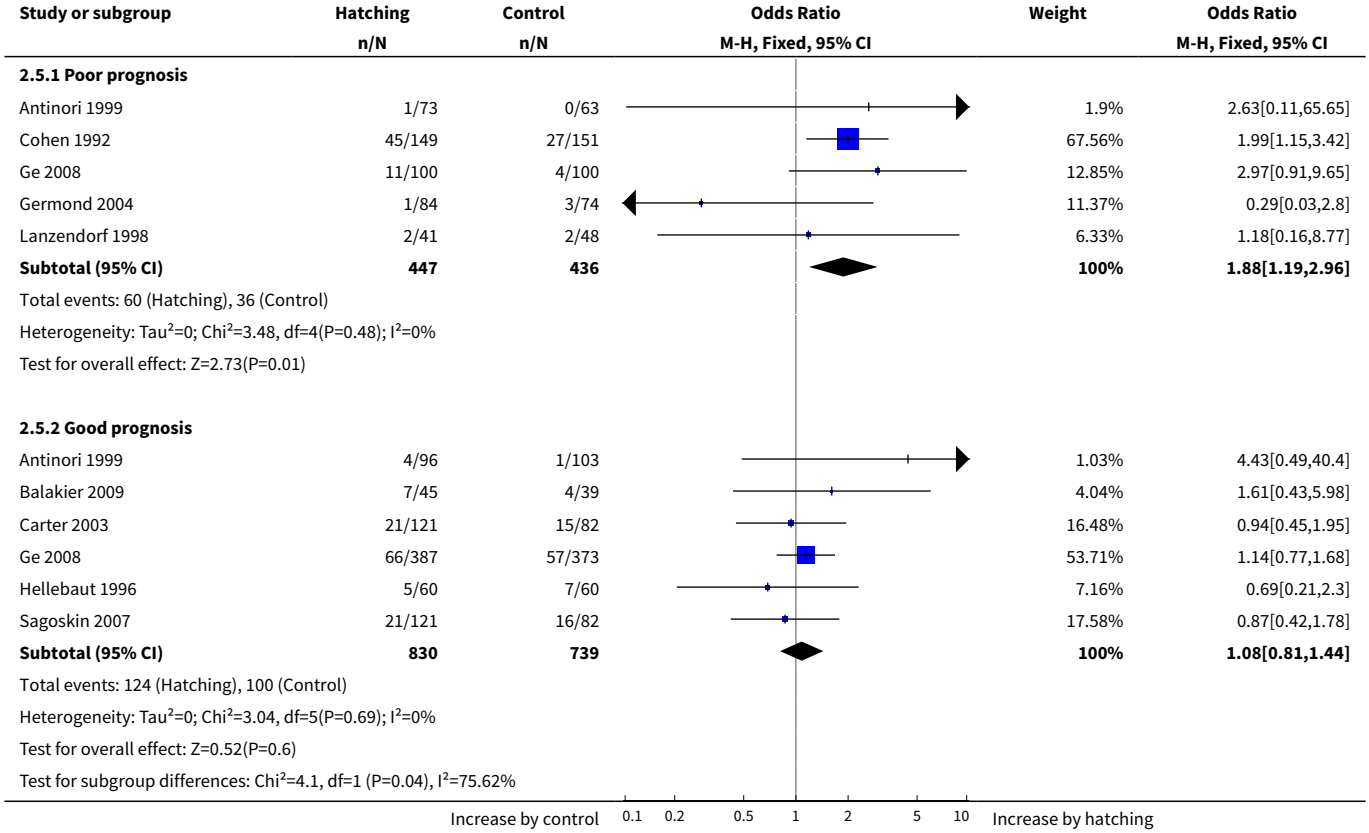
Analysis 2.4. Comparison 2 Multiple pregnancy: Assisted hatching compared with no assisted hatching, Outcome 4 Hatching method.



Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
2.4.1 Chemical					
Cohen 1992	45/149	27/151		64.62%	1.99[1.15,3.42]
Hellebaut 1996	5/60	7/60		22.15%	0.69[0.21,2.3]
Isik 2000	2/15	2/10		7.18%	0.62[0.07,5.28]
Lanzendorf 1998	2/41	2/48		6.05%	1.18[0.16,8.77]
Subtotal (95% CI)	**265**	**269**		**100%**	**1.55[0.98,2.47]**
Total events: 54 (Assisted hatching), 38 (Control)					
Heterogeneity: Tau^2=0; Chi^2=3.32, df=3(P=0.35); I^2=9.58%					
Test for overall effect: Z=1.86(P=0.06)					
2.4.2 Laser					
Antinori 1999	5/169	1/166		0.82%	5.03[0.58,43.53]
Balaban 2006	31/183	8/183		5.6%	4.46[1.99,10]
Balakier 2009	7/45	4/39		3.05%	1.61[0.43,5.98]
Carter 2003	21/121	15/82		12.45%	0.94[0.45,1.95]
Ge 2008	77/487	61/473		43.88%	1.27[0.88,1.82]
Germond 2004	1/84	3/74		2.65%	0.29[0.03,2.8]
Ng 2005	6/80	2/80		1.56%	3.16[0.62,16.17]

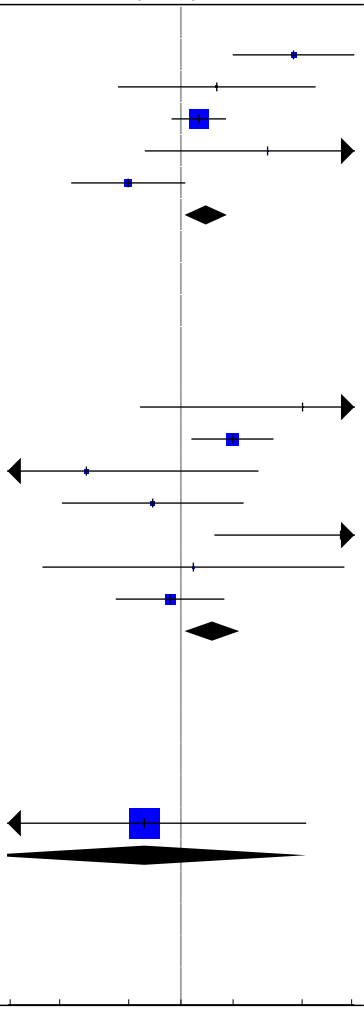


Analysis 2.5. Comparison 2 Multiple pregnancy: Assisted hatching compared with no assisted hatching, Outcome 5 Prognosis.



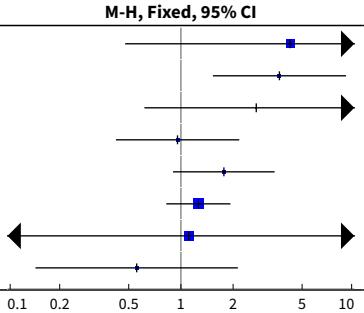
Analysis 2.6. Comparison 2 Multiple pregnancy: Assisted hatching compared with no assisted hatching, Outcome 6 Multiple pregnancy rate per woman grouped by extent of assisted hatching.

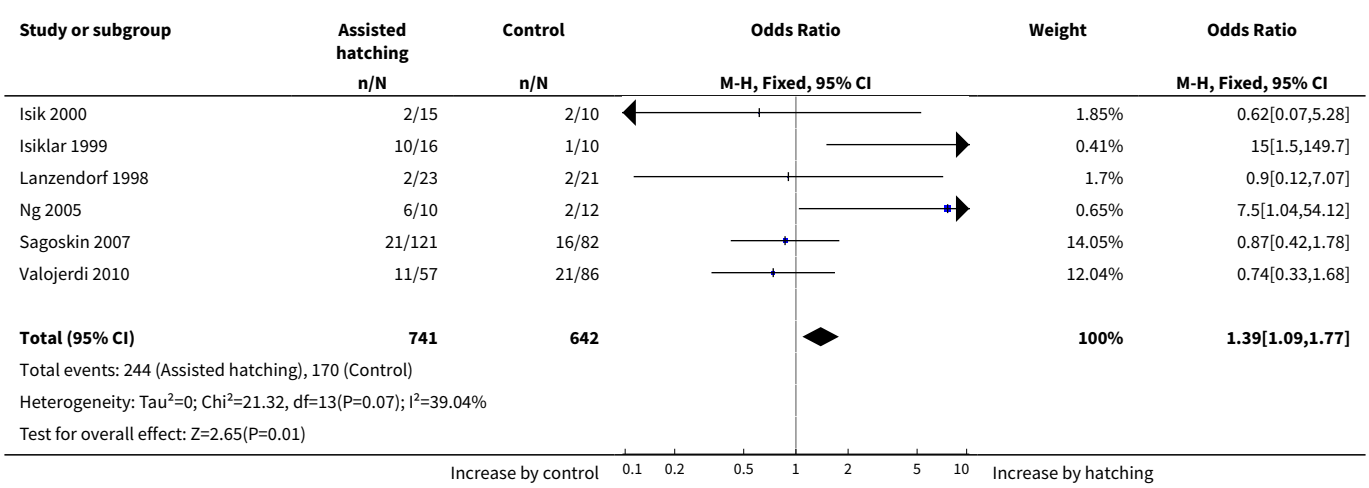
Study or subgroup	Hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
2.6.1 Thinning only					
Balaban 2006	31/183	8/183		7.9%	4.46[1.99,10]
Balakier 2009	7/45	4/39		4.31%	1.61[0.43,5.98]
Ge 2008	77/487	61/473		61.98%	1.27[0.88,1.82]
Ng 2005	6/80	2/80		2.2%	3.16[0.62,16.17]
Valojerdi 2010	11/200	21/200		23.61%	0.5[0.23,1.06]
Subtotal (95% CI)	**995**	**975**		**100%**	**1.39[1.05,1.84]**
Total events: 132 (Hatching), 96 (Control)					
Heterogeneity: Tau²=0; Chi²=16.4, df=4(P=0); I²=75.61%					
Test for overall effect: Z=2.33(P=0.02)					
2.6.2 Breach by hole					
Antinori 1999	5/169	1/166		2.05%	5.03[0.58,43.53]
Cohen 1992	45/149	27/151		39.1%	1.99[1.15,3.42]
Germond 2004	1/84	3/74		6.58%	0.29[0.03,2.8]
Hellebaut 1996	5/60	7/60		13.4%	0.69[0.21,2.3]
Isiklar 1999	10/22	2/22		2.28%	8.33[1.56,44.64]
Lanzendorf 1998	2/41	2/48		3.66%	1.18[0.16,8.77]
Sagoskin 2007	21/121	16/82		32.93%	0.87[0.42,1.78]
Subtotal (95% CI)	**646**	**603**		**100%**	**1.51[1.05,2.17]**
Total events: 89 (Hatching), 58 (Control)					
Heterogeneity: Tau²=0; Chi²=12.16, df=6(P=0.06); I²=50.67%					
Test for overall effect: Z=2.22(P=0.03)					
2.6.3 Complete removal of zona					
Isik 2000	2/15	2/10		100%	0.62[0.07,5.28]
Subtotal (95% CI)	**15**	**10**		**100%**	**0.62[0.07,5.28]**
Total events: 2 (Hatching), 2 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.44(P=0.66)					
Test for subgroup differences: Chi²=0.7, df=1 (P=0.7), I²=0%					



Analysis 2.7. Comparison 2 Multiple pregnancy: Assisted hatching compared with no assisted hatching, Outcome 7 Multiple pregnancy per pregnancy.

Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	5/52	1/41		0.9%	4.26[0.48,37.95]
Balaban 2006	31/75	8/50		5.02%	3.7[1.53,8.96]
Balakier 2009	7/16	4/18		1.89%	2.72[0.62,12.04]
Carter 2003	21/62	15/43		10.44%	0.96[0.42,2.17]
Cohen 1992	45/78	27/62		11.35%	1.77[0.9,3.47]
Ge 2008	77/189	61/173		33.65%	1.26[0.82,1.93]
Germond 2004	1/4	3/13		0.94%	1.11[0.08,15.04]
Hellebaut 1996	5/23	7/21		5.11%	0.56[0.14,2.13]





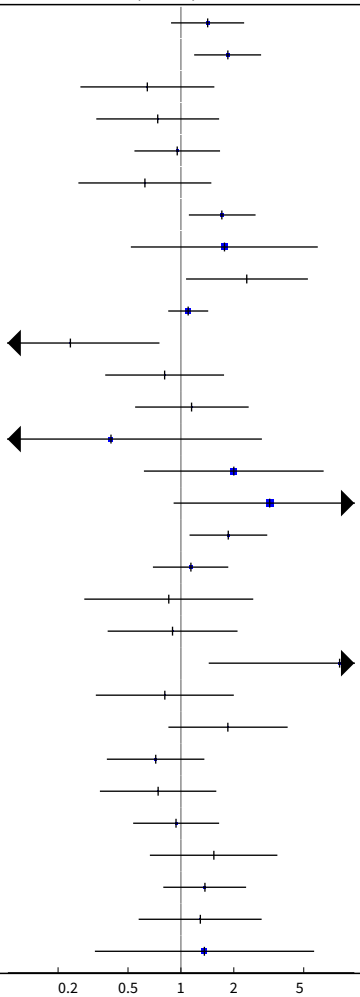
Comparison 3. Clinical pregnancy: Assisted hatching compared with no assisted hatching

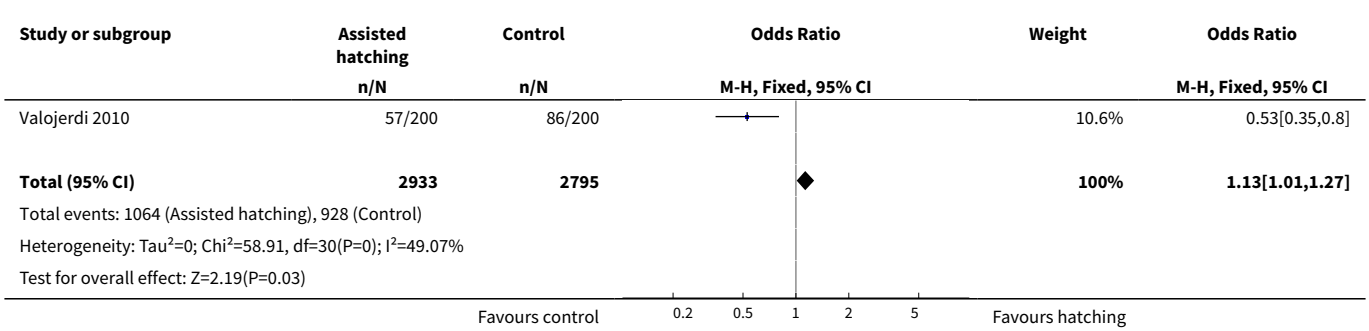
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy rate per woman randomised	31	5728	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [1.01, 1.27]
2 First or repeat attempt	14		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 First attempt at IVF or ICSI	6	650	Odds Ratio (M-H, Fixed, 95% CI)	0.77 [0.54, 1.10]
2.2 Repeat attempt at IVF or ICSI	9	1365	Odds Ratio (M-H, Fixed, 95% CI)	1.42 [1.11, 1.81]
3 Conception mode	22		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 ICSI only	8	1205	Odds Ratio (M-H, Fixed, 95% CI)	1.34 [1.05, 1.71]
3.2 IVF only	14	2300	Odds Ratio (M-H, Fixed, 95% CI)	1.29 [1.08, 1.54]
4 Hatching method	31		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Chemical	11	1536	Odds Ratio (M-H, Fixed, 95% CI)	1.33 [1.08, 1.64]
4.2 Laser	15	3606	Odds Ratio (M-H, Fixed, 95% CI)	1.04 [0.90, 1.19]
4.3 Mechanical	5	586	Odds Ratio (M-H, Fixed, 95% CI)	1.30 [0.89, 1.88]
5 Prognosis	20		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.1 Poor prognosis	12	1675	Odds Ratio (M-H, Fixed, 95% CI)	1.49 [1.19, 1.85]
5.2 Good prognosis	12	2253	Odds Ratio (M-H, Fixed, 95% CI)	1.02 [0.86, 1.21]
6 Extent of assisted hatching	30		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Thinning only	12	2936	Odds Ratio (M-H, Fixed, 95% CI)	1.05 [0.90, 1.23]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6.2 Breach by hole only	15	2163	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.94, 1.37]
6.3 Complete removal of zona	2	301	Odds Ratio (M-H, Fixed, 95% CI)	1.93 [1.21, 3.09]
6.4 Expansion of zona pellucida	1	125	Odds Ratio (M-H, Fixed, 95% CI)	2.37 [1.07, 5.28]
7 Fresh and frozen embryo transfer	30		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
7.1 Fresh embryo transfer	24	4050	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [1.01, 1.30]
7.2 Frozen embryo transfer only	8	1478	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.90, 1.44]

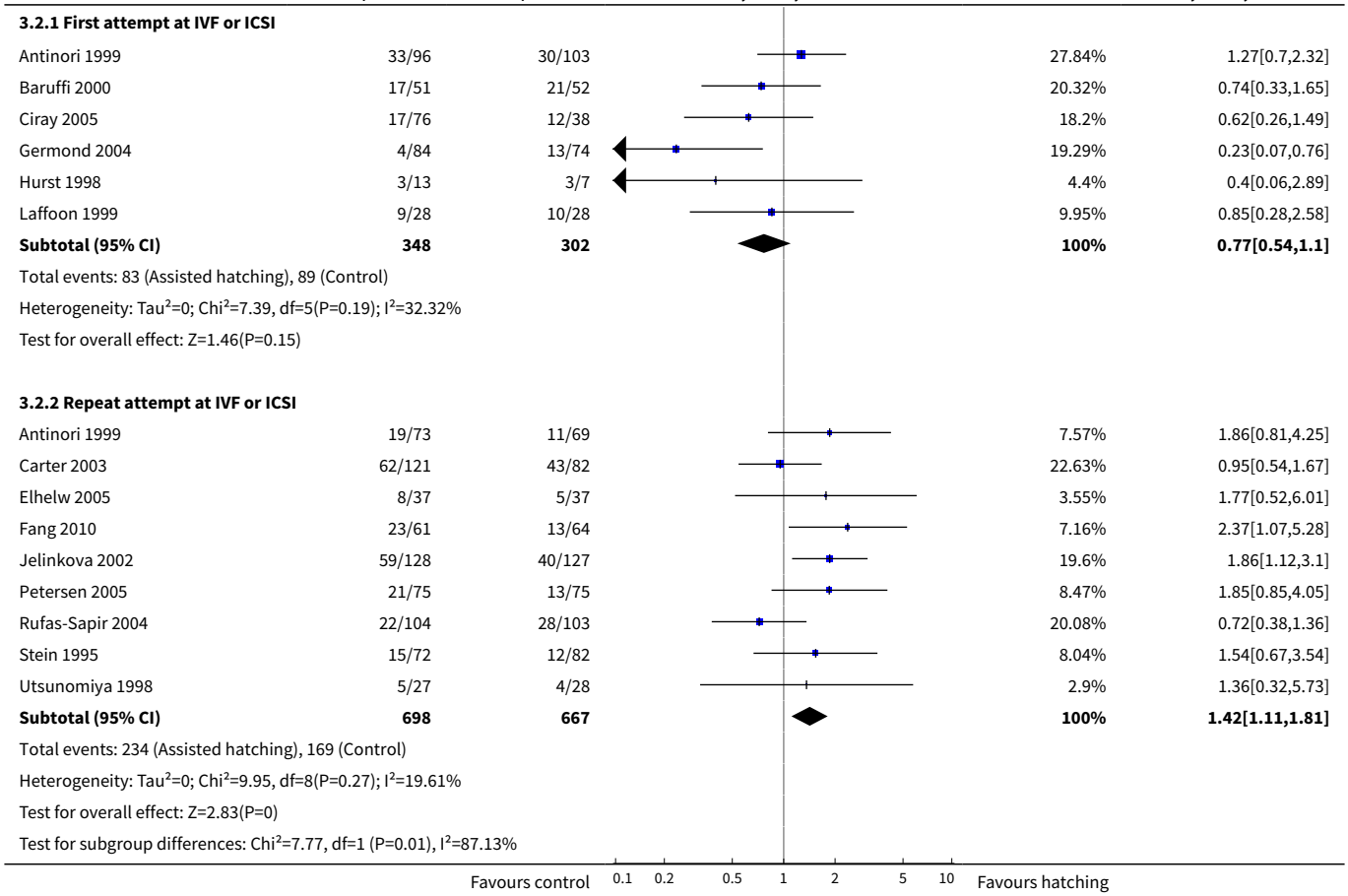
Analysis 3.1. Comparison 3 Clinical pregnancy: Assisted hatching compared with no assisted hatching, Outcome 1 Clinical pregnancy rate per woman randomised.

Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	52/169	41/172		4.85%	1.42[0.88,2.29]
Balaban 2006	75/183	50/183		5.08%	1.85[1.19,2.86]
Balakier 2009	16/45	18/39		2.14%	0.64[0.27,1.55]
Baruffi 2000	17/51	21/52		2.39%	0.74[0.33,1.65]
Carter 2003	62/121	43/82		4.31%	0.95[0.54,1.67]
Ciray 2005	17/76	12/38		2.14%	0.62[0.26,1.49]
Cohen 1992	85/164	64/166		5.28%	1.71[1.11,2.66]
Elhelw 2005	8/37	5/37		0.68%	1.77[0.52,6.01]
Fang 2010	23/61	13/64		1.36%	2.37[1.07,5.28]
Ge 2008	189/487	173/473		18.51%	1.1[0.85,1.43]
Germond 2004	4/84	13/74		2.27%	0.23[0.07,0.76]
Hagemann 2010	21/49	26/54		2.44%	0.81[0.37,1.76]
Hellebaut 1996	23/60	21/60		2.23%	1.15[0.55,2.43]
Hurst 1998	3/13	3/7		0.52%	0.4[0.06,2.89]
Isik 2000	15/24	10/22		0.67%	2[0.62,6.49]
Isiklar 1999	16/22	10/22		0.47%	3.2[0.91,11.27]
Jelinkova 2002	59/128	40/127		3.73%	1.86[1.12,3.1]
Kutlu 2010	67/131	58/121		5.08%	1.14[0.69,1.86]
Laffoon 1999	9/28	10/28		1.17%	0.85[0.28,2.58]
Lanzendorf 1998	16/41	20/48		1.94%	0.9[0.38,2.1]
Nagy 1999	10/20	2/18		0.18%	8[1.44,44.3]
Ng 2005	10/80	12/80		1.81%	0.81[0.33,2]
Petersen 2005	21/75	13/75		1.61%	1.85[0.85,4.05]
Rufas-Sapir 2004	22/104	28/103		3.82%	0.72[0.38,1.36]
Ryan 1997	14/100	18/100		2.67%	0.74[0.35,1.59]
Sagoskin 2007	63/121	44/82		4.33%	0.94[0.53,1.65]
Stein 1995	15/72	12/82		1.53%	1.54[0.67,3.54]
Tucker 1993	49/110	40/108		3.86%	1.37[0.79,2.35]
Tucker 1996	21/50	18/50		1.8%	1.29[0.58,2.88]
Utsunomiya 1998	5/27	4/28		0.55%	1.36[0.32,5.73]



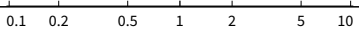


Analysis 3.2. Comparison 3 Clinical pregnancy: Assisted hatching compared with no assisted hatching, Outcome 2 First or repeat attempt.



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

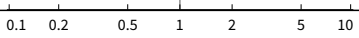
Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.3.1 ICSI only					
Balaban 2006	75/183	50/183		26.14%	1.85[1.19,2.86]
Baruffi 2000	17/51	21/52		12.28%	0.74[0.33,1.65]
Ciray 2005	17/76	12/38		11%	0.62[0.26,1.49]
Elhelw 2005	8/37	5/37		3.47%	1.77[0.52,6.01]
Isik 2000	15/24	10/22		3.47%	2[0.62,6.49]
Kutlu 2010	67/131	58/121		26.1%	1.14[0.69,1.86]
Petersen 2005	21/75	13/75		8.29%	1.85[0.85,4.05]
Tucker 1996	21/50	18/50		9.25%	1.29[0.58,2.88]
Subtotal (95% CI)	**627**	**578**		**100%**	**1.34[1.05,1.71]**
Total events: 241 (Assisted hatching), 187 (Control)					
Heterogeneity: Tau^2=0; Chi^2=8.86, df=7(P=0.26); I^2=21%					
Test for overall effect: Z=2.38(P=0.02)					
3.3.2 IVF only					
Antinori 1999	52/169	41/172		13%	1.42[0.88,2.29]
Balakier 2009	16/45	18/39		5.74%	0.64[0.27,1.55]
Carter 2003	62/121	43/82		11.55%	0.95[0.54,1.67]
Cohen 1992	85/164	64/166		14.16%	1.71[1.11,2.66]
Fang 2010	23/61	13/64		3.65%	2.37[1.07,5.28]
Hagemann 2010	21/49	26/54		6.53%	0.81[0.37,1.76]
Hurst 1998	3/13	3/7		1.39%	0.4[0.06,2.89]
Isiklar 1999	16/22	10/22		1.26%	3.2[0.91,11.27]
Jelinkova 2002	59/128	40/127		10.01%	1.86[1.12,3.1]
Laffoon 1999	9/28	10/28		3.14%	0.85[0.28,2.58]
Ng 2005	10/80	12/80		4.85%	0.81[0.33,2]
Rufas-Sapir 2004	22/104	28/103		10.25%	0.72[0.38,1.36]
Stein 1995	15/72	12/82		4.11%	1.54[0.67,3.54]
Tucker 1993	49/110	40/108		10.35%	1.37[0.79,2.35]
Subtotal (95% CI)	**1166**	**1134**		**100%**	**1.29[1.08,1.54]**
Total events: 442 (Assisted hatching), 360 (Control)					
Heterogeneity: Tau^2=0; Chi^2=19.24, df=13(P=0.12); I^2=32.42%					
Test for overall effect: Z=2.82(P=0)					
Test for subgroup differences: Chi^2=0.07, df=1 (P=0.8), I^2=0%					



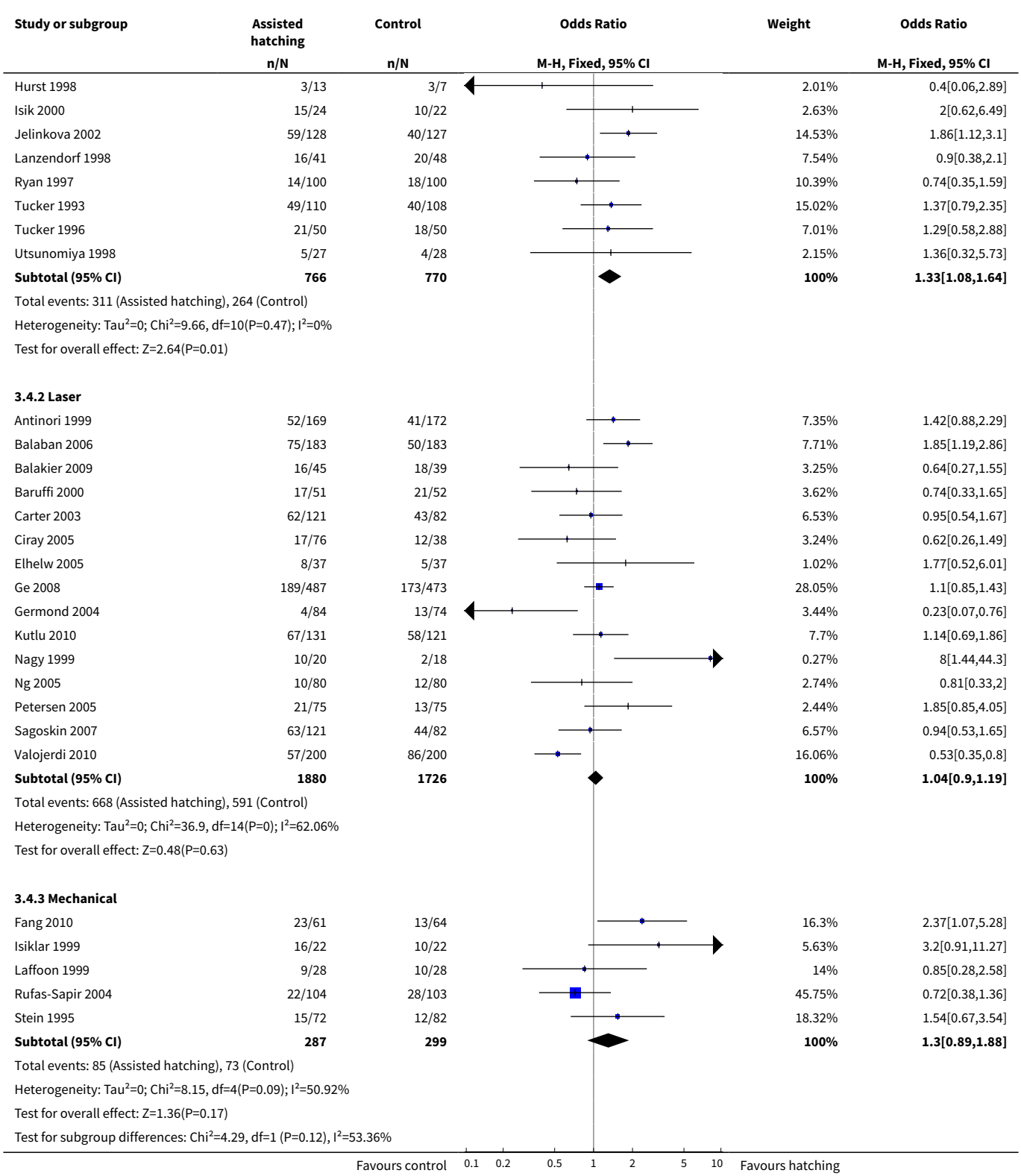
Favours control — Favours treatment

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

Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.4.1 Chemical					
Cohen 1992	85/164	64/166		20.56%	1.71[1.11,2.66]
Hagemann 2010	21/49	26/54		9.49%	0.81[0.37,1.76]
Hellebaut 1996	23/60	21/60		8.69%	1.15[0.55,2.43]

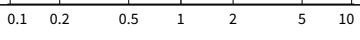


Favours control — Favours hatching



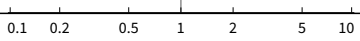
Analysis 3.5. Comparison 3 Clinical pregnancy: Assisted hatching compared with no assisted hatching, Outcome 5 Prognosis.

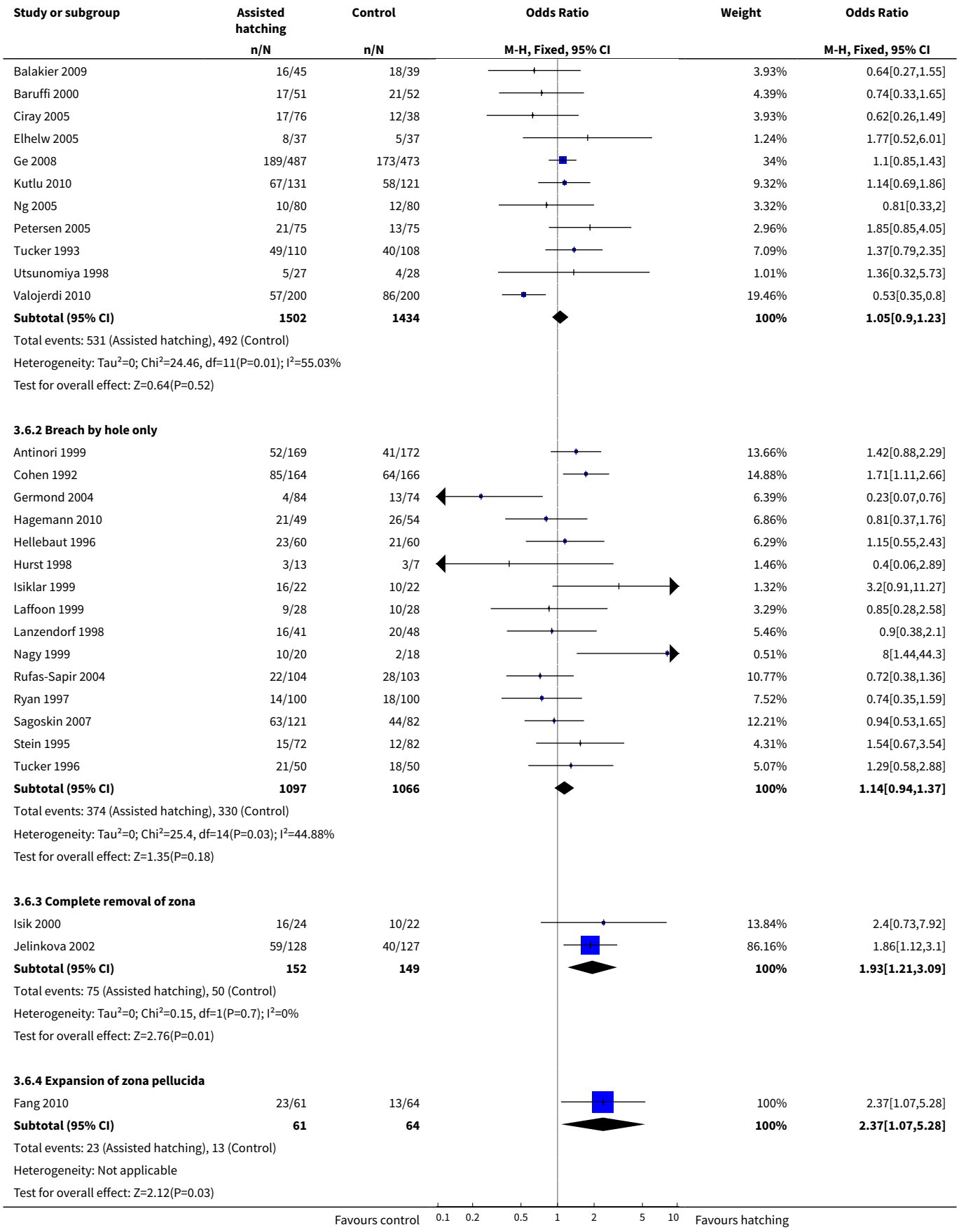
Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.5.1 Poor prognosis					
Antinori 1999	19/73	11/69		6.36%	1.86[0.81,4.25]
Cohen 1992	48/95	32/98		11.85%	2.11[1.18,3.77]
Elhelw 2005	8/37	5/37		2.98%	1.77[0.52,6.01]
Ge 2008	25/100	14/100		7.98%	2.05[0.99,4.22]
Germond 2004	3/22	5/21		3.36%	0.51[0.1,2.45]
Jelinkova 2002	59/128	40/127		16.45%	1.86[1.12,3.1]
Kutlu 2010	25/58	21/55		9.32%	1.23[0.58,2.6]
Lanzendorf 1998	16/41	20/48		8.54%	0.9[0.38,2.1]
Petersen 2005	21/75	13/75		7.11%	1.85[0.85,4.05]
Rufas-Sapir 2004	22/104	28/103		16.86%	0.72[0.38,1.36]
Stein 1995	15/72	12/82		6.75%	1.54[0.67,3.54]
Utsunomiya 1998	5/27	4/28		2.43%	1.36[0.32,5.73]
Subtotal (95% CI)	**832**	**843**		**100%**	**1.49[1.19,1.85]**
Total events: 266 (Assisted hatching), 205 (Control)					
Heterogeneity: Tau^2=0; Chi^2=11.9, df=11(P=0.37); I^2=7.56%					
Test for overall effect: Z=3.55(P=0)					
3.5.2 Good prognosis					
Antinori 1999	33/96	30/103		7.2%	1.27[0.7,2.32]
Balakier 2009	16/45	18/39		4.71%	0.64[0.27,1.55]
Carter 2003	62/121	43/82		9.47%	0.95[0.54,1.67]
Ciray 2005	17/76	12/38		4.71%	0.62[0.26,1.49]
Cohen 1992	37/69	32/68		5.67%	1.3[0.66,2.55]
Ge 2008	164/387	159/373		35.36%	0.99[0.74,1.32]
Hellebaut 1996	23/60	21/60		4.91%	1.15[0.55,2.43]
Hurst 1998	3/13	3/7		1.14%	0.4[0.06,2.89]
Kutlu 2010	42/73	37/66		6.25%	1.06[0.54,2.08]
Laffoon 1999	9/28	10/28		2.57%	0.85[0.28,2.58]
Sagoskin 2007	63/121	44/82		9.53%	0.94[0.53,1.65]
Tucker 1993	49/110	40/108		8.48%	1.37[0.79,2.35]
Subtotal (95% CI)	**1199**	**1054**		**100%**	**1.02[0.86,1.21]**
Total events: 518 (Assisted hatching), 449 (Control)					
Heterogeneity: Tau^2=0; Chi^2=5.69, df=11(P=0.89); I^2=0%					
Test for overall effect: Z=0.23(P=0.82)					
Test for subgroup differences: Chi^2=7.1, df=1 (P=0.01), I^2=85.91%					



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 n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.6.1 Thinning only					
Balaban 2006	75/183	50/183		9.34%	1.85[1.19,2.86]





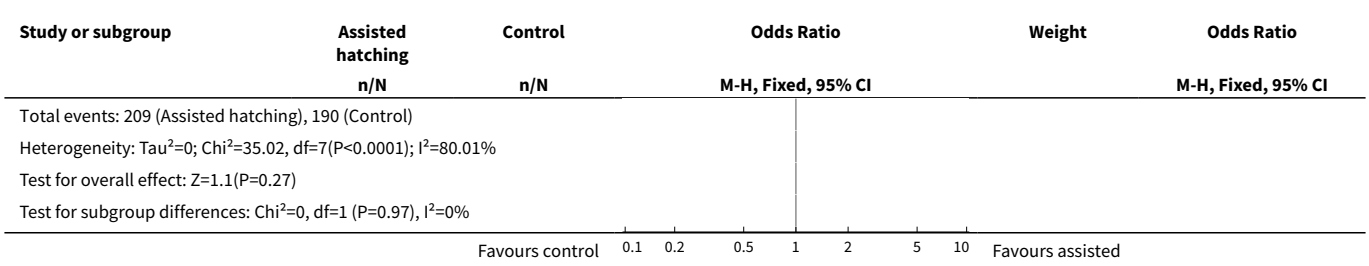
Study or subgroup	Assisted hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Test for subgroup differences: Chi²=9.16, df=1 (P=0.03), I²=67.25%					

Favours control 0.1 0.2 0.5 1 2 5 10 Favours hatching

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 n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.7.1 Fresh embryo transfer					
Antinori 1999	52/169	41/172		6.58%	1.42[0.88,2.29]
Balakier 2009	16/45	18/39		2.91%	0.64[0.27,1.55]
Baruffi 2000	17/51	21/52		3.24%	0.74[0.33,1.65]
Carter 2003	62/121	43/82		5.84%	0.95[0.54,1.67]
Ciray 2005	17/76	12/38		2.9%	0.62[0.26,1.49]
Cohen 1992	85/164	64/166		7.17%	1.71[1.11,2.66]
Ge 2008	164/387	159/373		21.82%	0.99[0.74,1.32]
Germond 2004	3/22	5/21		1.03%	0.51[0.1,2.45]
Hagemann 2010	21/49	26/54		3.31%	0.81[0.37,1.76]
Hellebaut 1996	23/60	21/60		3.03%	1.15[0.55,2.43]
Hurst 1998	3/13	3/7		0.7%	0.4[0.06,2.89]
Isik 2000	15/24	10/22		0.91%	2[0.62,6.49]
Isiklar 1999	16/22	10/22		0.64%	3.2[0.91,11.27]
Jelinkova 2002	59/128	40/127		5.06%	1.86[1.12,3.1]
Kutlu 2010	67/131	58/121		6.89%	1.14[0.69,1.86]
Laffoon 1999	9/28	10/28		1.59%	0.85[0.28,2.58]
Lanzendorf 1998	16/41	20/48		2.63%	0.9[0.38,2.1]
Petersen 2005	21/75	13/75		2.19%	1.85[0.85,4.05]
Rufas-Sapir 2004	22/104	28/103		5.19%	0.72[0.38,1.36]
Sagoskin 2007	63/121	44/82		5.88%	0.94[0.53,1.65]
Stein 1995	15/72	12/82		2.08%	1.54[0.67,3.54]
Tucker 1993	49/110	40/108		5.23%	1.37[0.79,2.35]
Tucker 1996	21/50	18/50		2.44%	1.29[0.58,2.88]
Utsunomiya 1998	5/27	4/28		0.75%	1.36[0.32,5.73]
Subtotal (95% CI)	**2090**	**1960**		**100%**	**1.14[1.01,1.3]**
Total events: 841 (Assisted hatching), 720 (Control)					
Heterogeneity: Tau²=0; Chi²=25.47, df=23(P=0.33); I²=9.71%					
Test for overall effect: Z=2.04(P=0.04)					
3.7.2 Frozen embryo transfer only					
Balaban 2006	75/183	50/183		22.13%	1.85[1.19,2.86]
Elhelw 2005	8/37	5/37		2.94%	1.77[0.52,6.01]
Fang 2010	23/61	13/64		5.93%	2.37[1.07,5.28]
Ge 2008	25/100	14/100		7.87%	2.05[0.99,4.22]
Germond 2004	1/62	8/53		6.36%	0.09[0.01,0.76]
Nagy 1999	10/20	2/18		0.79%	8[1.44,44.3]
Ng 2005	10/80	12/80		7.87%	0.81[0.33,2]
Valojerdi 2010	57/200	86/200		46.11%	0.53[0.35,0.8]
Subtotal (95% CI)	**743**	**735**		**100%**	**1.14[0.9,1.44]**

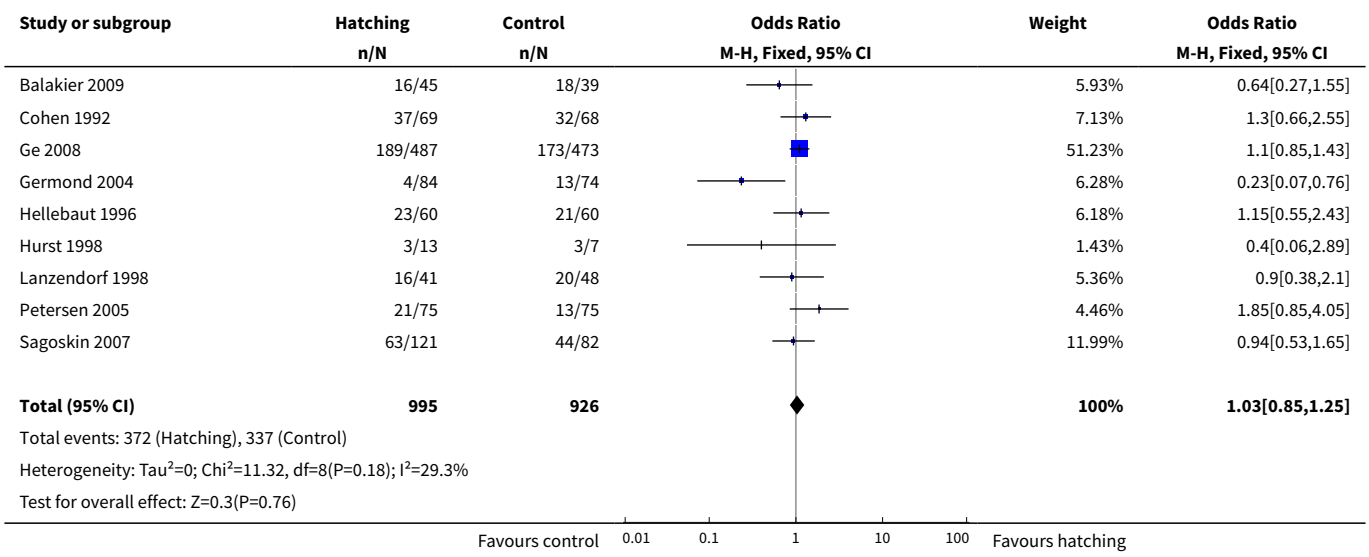
Favours control 0.1 0.2 0.5 1 2 5 10 Favours assisted



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

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical Pregnancies in trials reporting live births	9	1921	Odds Ratio (M-H, Fixed, 95% CI)	1.03 [0.85, 1.25]

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



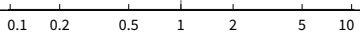
Comparison 5. Miscarriage: Assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Miscarriage per woman randomised	14	2131	Odds Ratio (M-H, Fixed, 95% CI)	1.03 [0.69, 1.54]

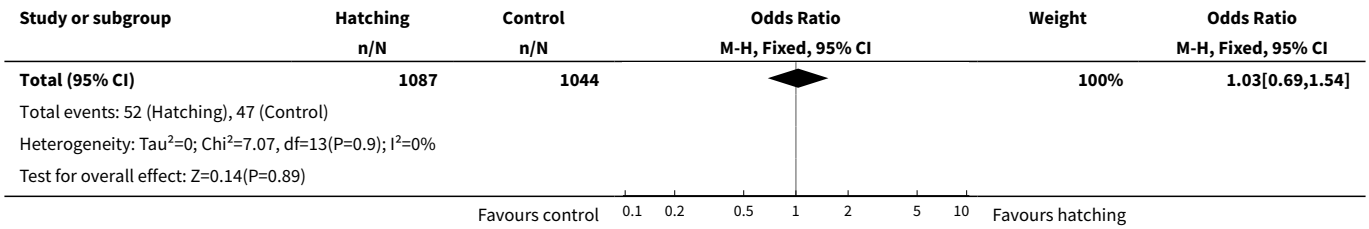
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2 First or repeat attempt	6		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 First attempt at IVF or ICSI	3	264	Odds Ratio (M-H, Fixed, 95% CI)	0.91 [0.29, 2.80]
2.2 Repeat attempt at IVF or ICSI cycle	4	663	Odds Ratio (M-H, Fixed, 95% CI)	2.14 [0.72, 6.35]
3 Conception mode	10		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 ICSI only	4	665	Odds Ratio (M-H, Fixed, 95% CI)	1.20 [0.58, 2.47]
3.2 IVF only	6	896	Odds Ratio (M-H, Fixed, 95% CI)	1.28 [0.65, 2.52]
4 Hatching method	14		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Chemical	5	412	Odds Ratio (M-H, Fixed, 95% CI)	1.11 [0.56, 2.21]
4.2 Laser	8	1565	Odds Ratio (M-H, Fixed, 95% CI)	0.98 [0.59, 1.63]
4.3 Mechanical	1	154	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.07, 18.58]
5 Prognosis	10		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.1 Poor prognosis	6	830	Odds Ratio (M-H, Fixed, 95% CI)	1.06 [0.57, 1.99]
5.2 Good prognosis	5	626	Odds Ratio (M-H, Fixed, 95% CI)	1.03 [0.50, 2.14]
6 Miscarriage per clinical pregnancy	14	687	Odds Ratio (M-H, Random, 95% CI)	0.96 [0.62, 1.50]

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

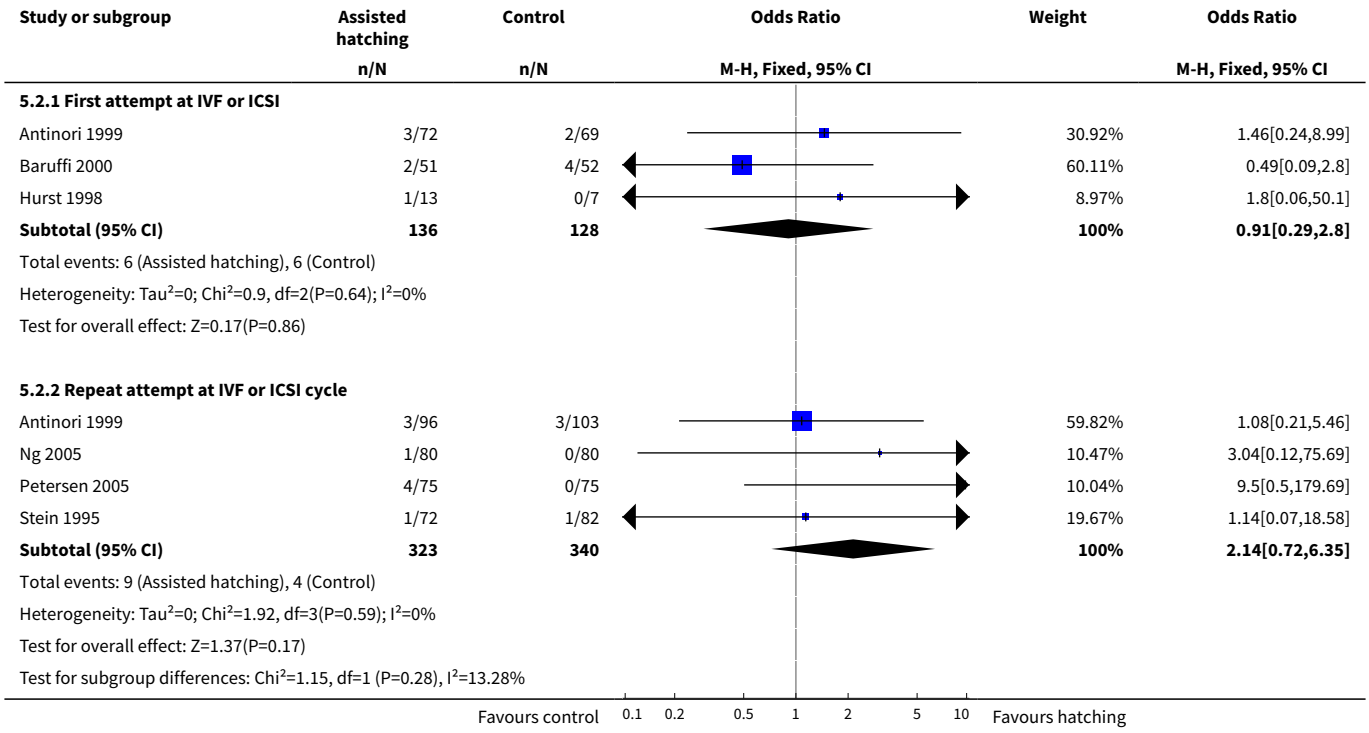
Study or subgroup	Hatching n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Antinori 1999	6/169	5/172		10.24%	1.23[0.37,4.11]
Balaban 2006	7/183	6/183		12.36%	1.17[0.39,3.56]
Balakier 2009	3/45	2/39		4.28%	1.32[0.21,8.35]
Baruffi 2000	2/51	4/52		8.15%	0.49[0.09,2.8]
Cohen 1992	8/69	7/68		13.35%	1.14[0.39,3.35]
Germond 2004	1/84	5/74		11.25%	0.17[0.02,1.46]
Hellebaut 1996	2/60	1/60		2.07%	2.03[0.18,23.06]
Hurst 1998	1/13	0/7		1.22%	1.8[0.06,50.1]
Isik 2000	4/24	4/22		7.45%	0.9[0.2,4.14]
Lanzendorf 1998	4/41	5/48		8.9%	0.93[0.23,3.72]
Ng 2005	1/80	0/80		1.05%	3.04[0.12,75.69]
Petersen 2005	4/75	0/75		1.01%	9.5[0.5,179.69]
Sagoskin 2007	8/121	7/82		16.69%	0.76[0.26,2.18]
Stein 1995	1/72	1/82		1.97%	1.14[0.07,18.58]



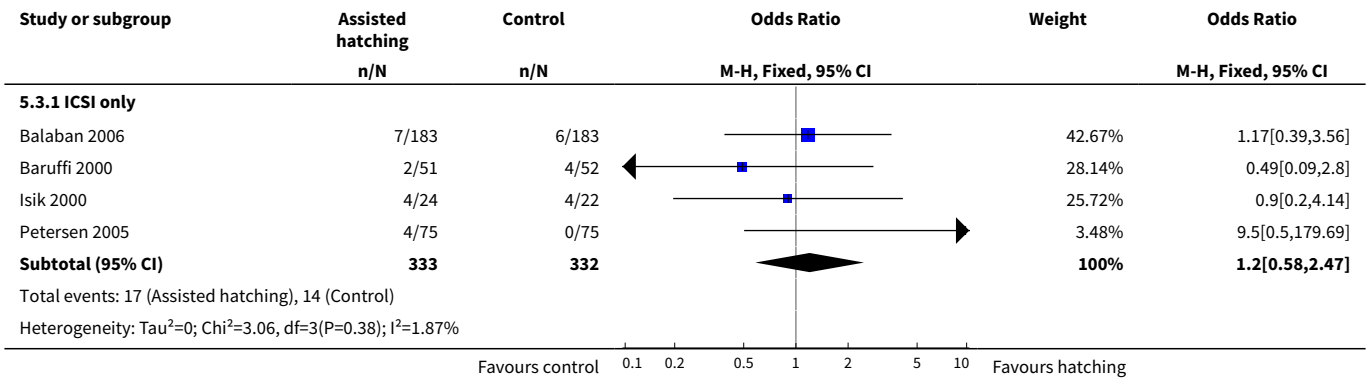
Favours control — Favours hatching

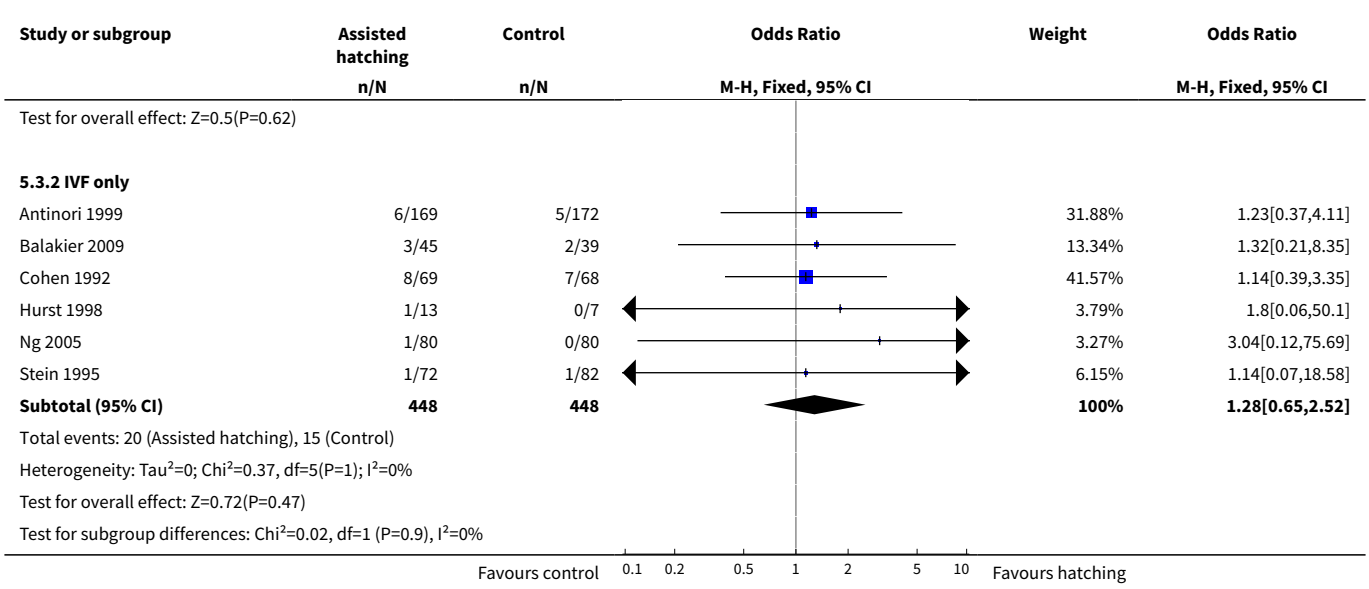


Analysis 5.2. Comparison 5 Miscarriage: Assisted hatching compared with no assisted hatching, Outcome 2 First or repeat attempt.

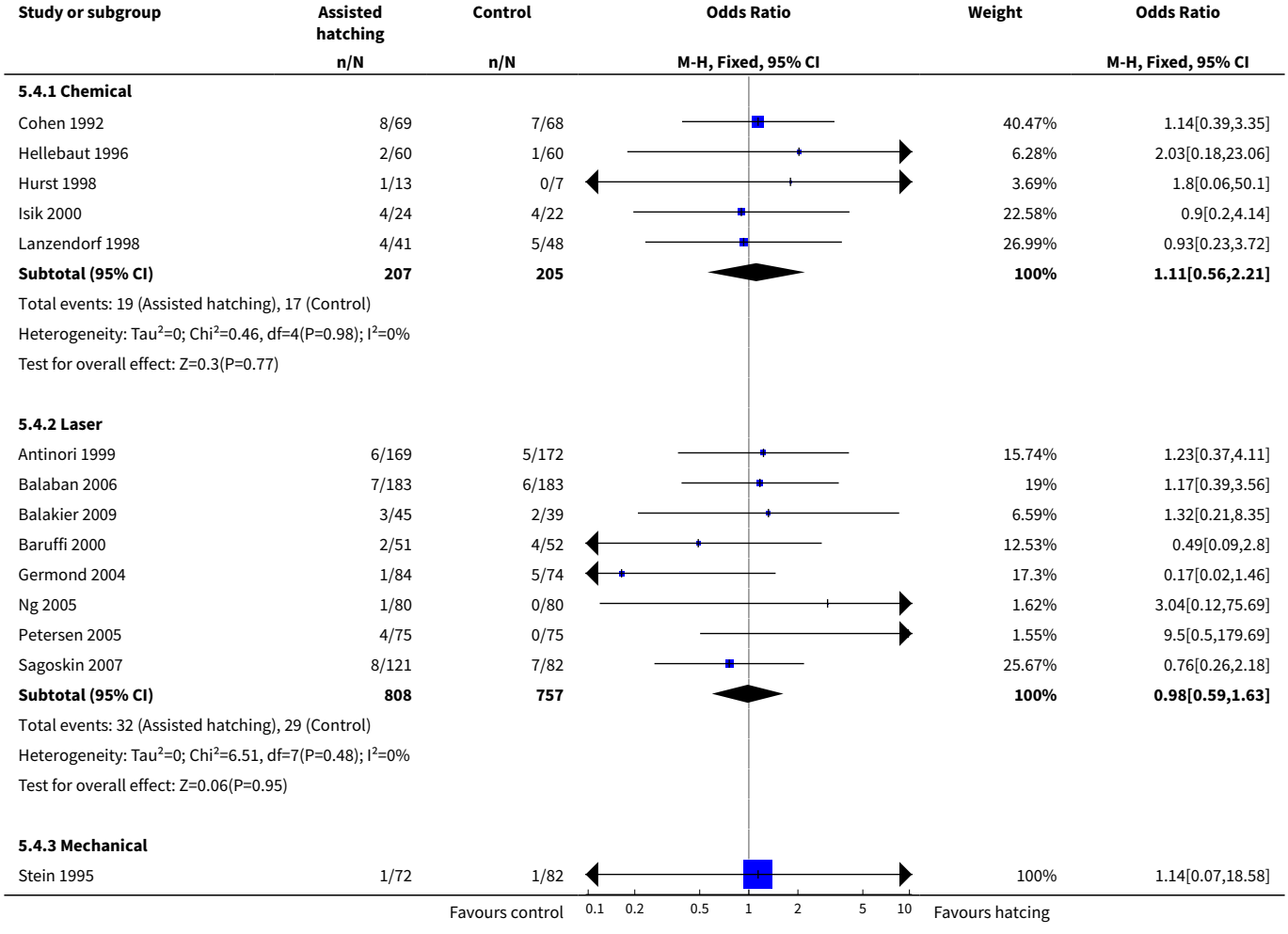


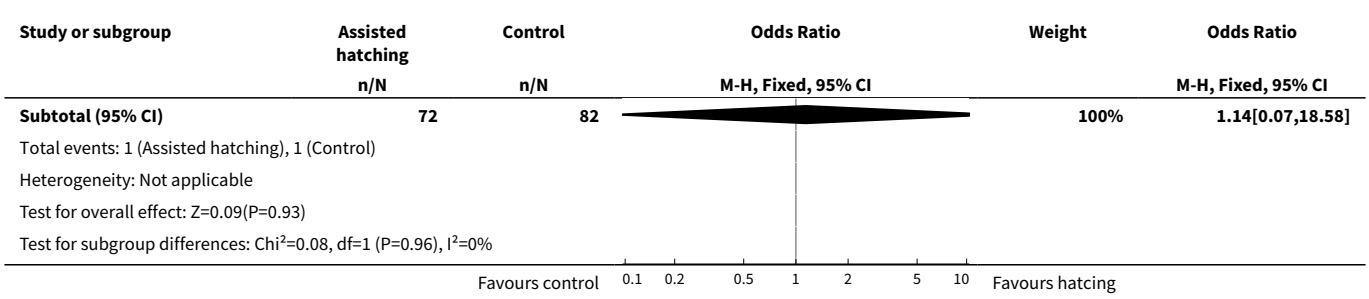
Analysis 5.3. Comparison 5 Miscarriage: Assisted hatching compared with no assisted hatching, Outcome 3 Conception mode.



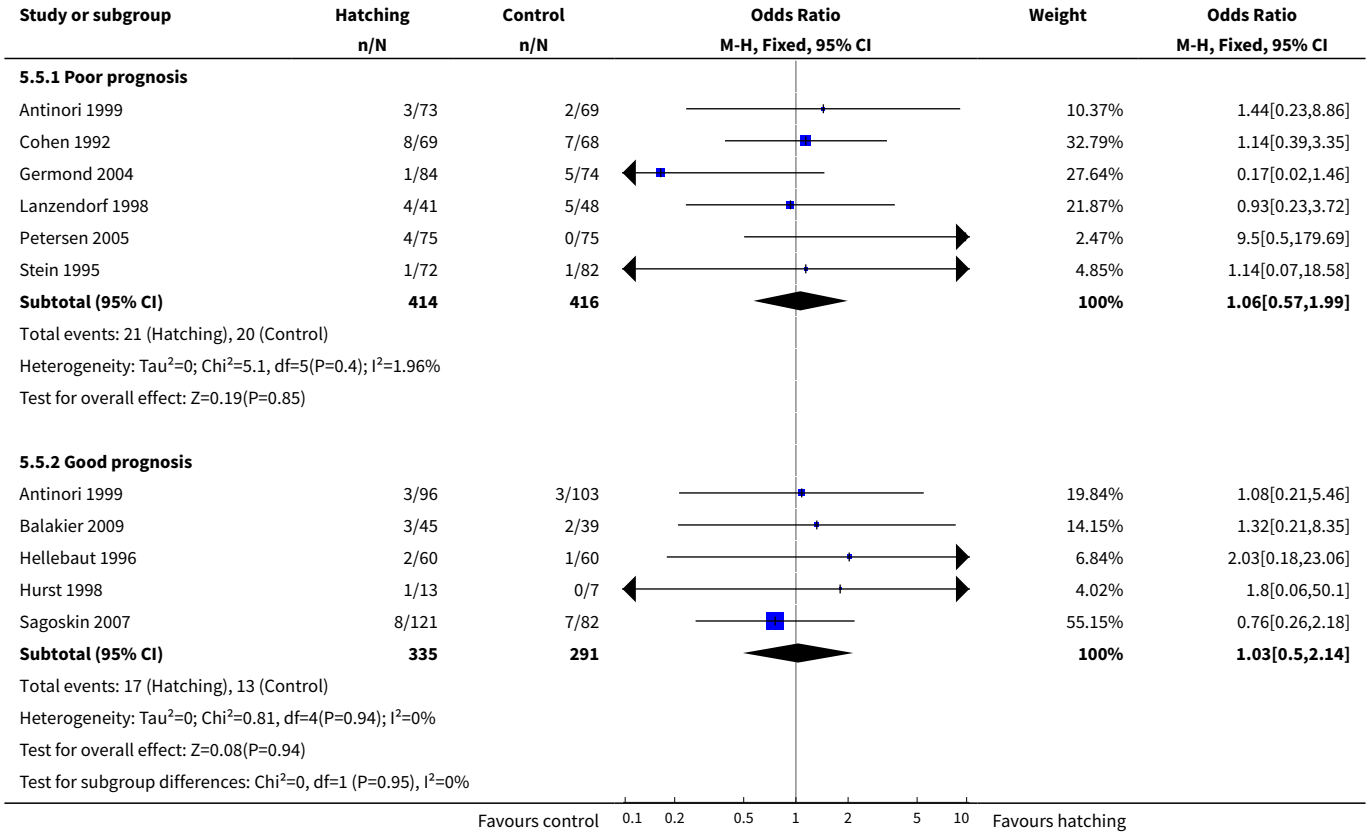


Analysis 5.4. Comparison 5 Miscarriage: Assisted hatching compared with no assisted hatching, Outcome 4 Hatching method.

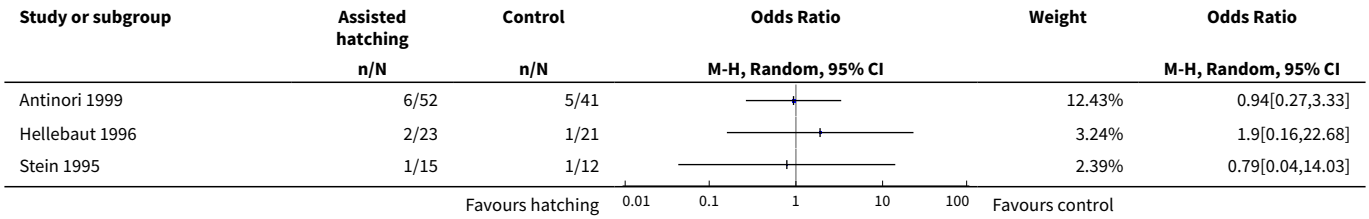


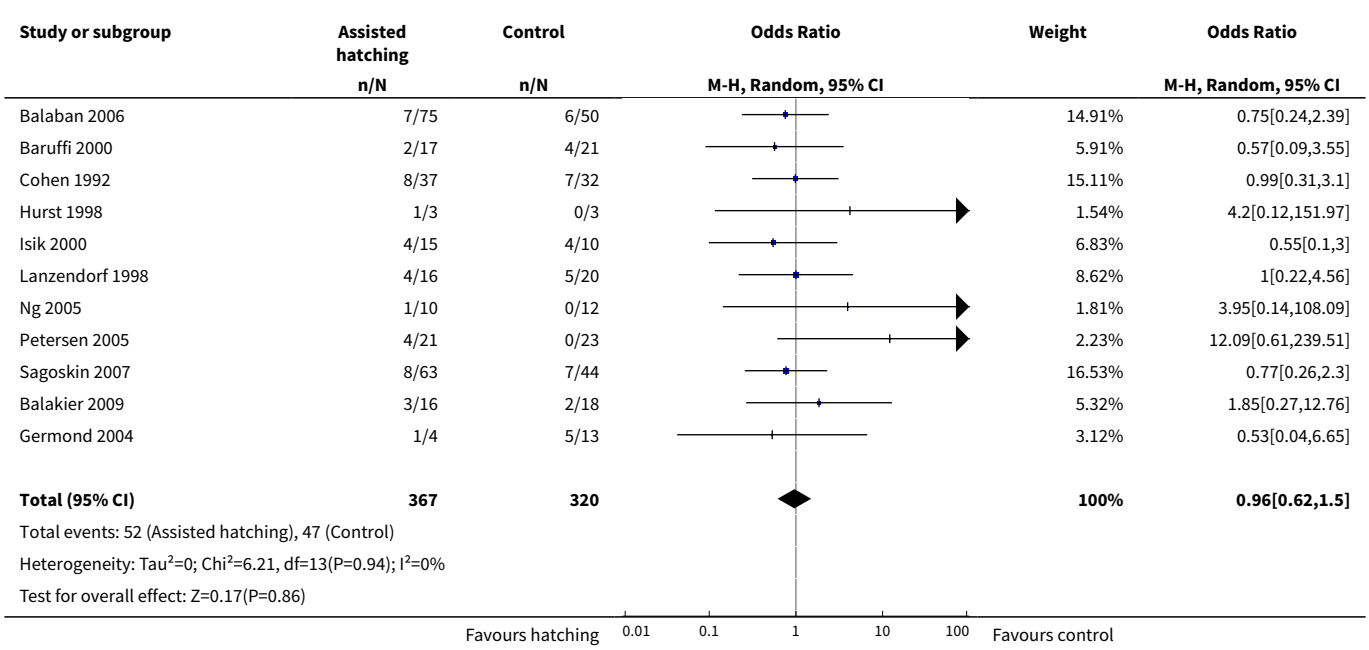


Analysis 5.5. Comparison 5 Miscarriage: Assisted hatching compared with no assisted hatching, Outcome 5 Prognosis.



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

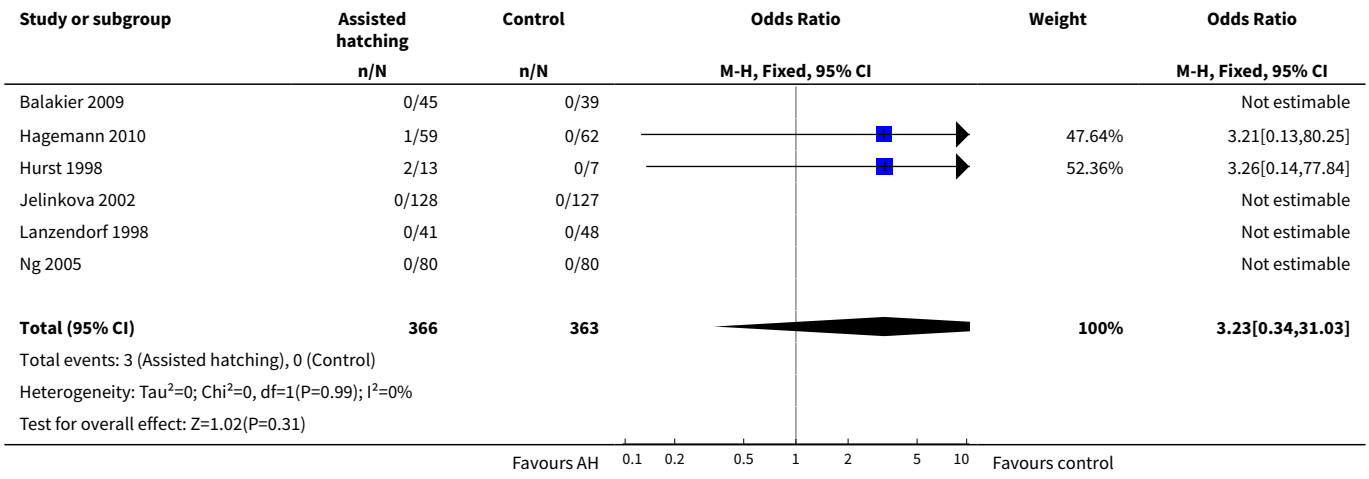




Comparison 6. Monozygotic twinning: Assisted hatching compared with no assisted hatching

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

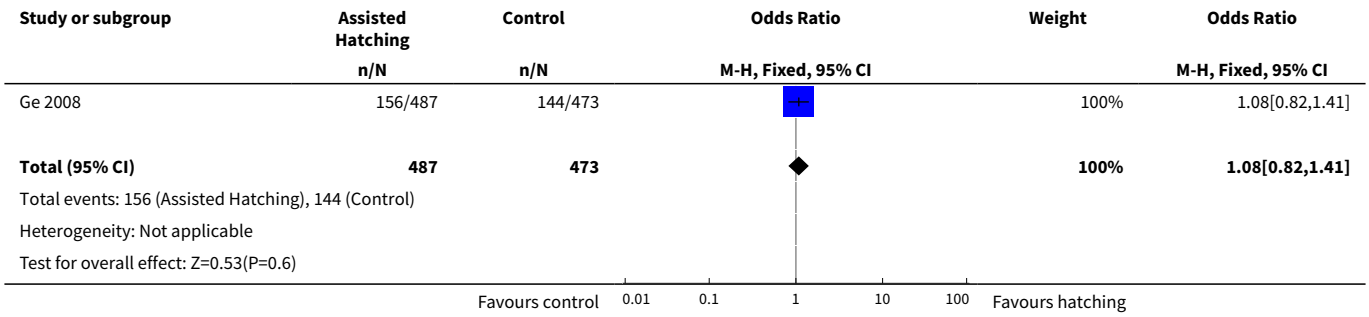
Analysis 6.1. Comparison 6 Monozygotic twinning: Assisted hatching compared with no assisted hatching, Outcome 1 Monozygotic twinning per woman randomised.



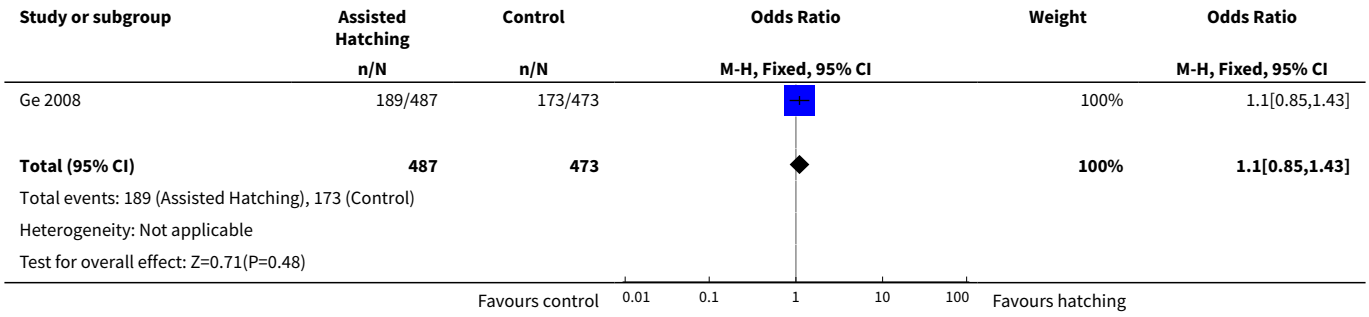
Comparison 7. Robust studies (randomisation method and allocation concealment stated & live birth reported): Assisted hatching compared with no assisted hatching

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live Births	1	960	Odds Ratio (M-H, Fixed, 95% CI)	1.08 [0.82, 1.41]
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 & live birth reported): Assisted hatching compared with no assisted hatching, Outcome 1 Live Births.



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



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
Antinori 1999: First IVF	73, 37.5	69, 36.0	1.27 (0.70, 2.32)
Antinori 1999: Repeat IVF	96, 27.5	103, 27	1.86 (0.81, 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)
Ciray 2005	60, 33.1 (4.2)	30, 34.0 (3.7)	0.62 (0.26 to 1.49)
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)
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)
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)
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)
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, 2.08)
Kutlu 2010:Poor prognosis	58, 38.0 (2.3)	55, 37.4 (2.4)	1.23 (0.58, 2.60)
Lanzendorf 1998	41, 38.30 (0.31)	48, 38.50 (0.26)	0.90 (0.38 to 2.10)
Mansour 2000	30, 37.30 (5.60)	41, 36.30 (5.20)	3.86 (0.91 to 16.41)
Nagy 1999	20, 32.0 (4.0)	20, 31.4 (3.7)	8.0 (1.44 to 44.3)
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 one previous implantation failure	35, 34.6 (4.6)	35, 34.1 (5.3)	1.15 (0.41 to 3.19)
Petersen 2005 several previous implanatation failures	40, 35.7 (3.8)	40, 35.3 (5.1)	4.11 (1.04 to 16.29)
Sagoskin 2007	118, 34.0 (3.3)	81, 34.0 (3.2)	0.94 (0.53 to1.65)

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

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
Valojerdi 2010	200, 30.86 (5.82)	200, 29.85 (5.14)	0.53 (0.35 to 0.80)

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
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
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, in subgroups
Hagemann 2010	Mean age data only given for combined cycles 1 and 2	Yes	under 38 years, >2 previous failed cycles, ZP thickness >13micrometers	No data	No	20micrometer diameter opening	Fresh
Hellebaut 1996	Yes	Yes	Good	No data	No	Complete hole	Fresh
Hurst 1998	Yes	Yes	Good	< 10	No	Complete hole	Fresh

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

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 Sub-groups	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
Nagy 1999	Yes	Yes	Unstated	No data	No	Thinning	Frozen-thaw cycles
Ng 2005	Yes	Higher proportion of controls received 3 embryos	Unstated	< 11	No	Thinning	Frozen-thaw cycles
Petersen 2005	Yes	Yes	Poor	No data	No	Thinning	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
Sagoskin 2007	Yes	Yes	Good	< 10	No	Hole	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
Valojerdi 2010	Yes	Yes	Not stated	No data	No	Partially thinned	Vitrified-warmed embryo

AH = assisted hatching
ET = embryo transfer

FSH = follicle-stimulating hormone

APPENDICES

Appendix 1. MEDLINE

MEDLINE (1966 to April 2012)

1 randomised controlled trial.pt. (201065)
2 controlled clinical trial.pt. (68353)
3 Randomised controlled trials/ (37180)
4 random allocation/ (53076)
5 double-blind method/ (81524)
6 single-blind method/ (8937)
7 or/1-6 (341829)
8 clinical trial.pt. (405523)
9 exp clinical trials/ (164946)
10 (clin$ adj25 trial$).ti,ab,sh. (109338)
11 ((singl$ or doubl$ or tripl$ or trebl$) adj25 (blind$ or mask$)).ti,ab,sh. (80778)
12 placebos/ (23682)
13 placebo$.ti,ab,sh. (100239)
14 random$.ti,ab,sh. (355156)
15 Research design/ (40564)
16 or/8-15 (742407)
17 animal/ not (human/ and animal/) (2870499)
18 7 or 16 (746072)
19 18 not 17 (684366)
20 (assist$ adj5 hatch$).ti,ab,sh. (160)
21 (zona$ adj5 (pellucid$ or manipulat$ or disrupt$ or thin$ or drill$)).ti,ab,sh. (3190)
22 (zona$ adj5 (dissect$ or tyrode$ or proteinase$ or piezon$ or krypton$ or yag$)).ti,ab,sh. (166)
23 pzd.tw. (57)
24 or/20-23 (3346)
25 7 and 16 and 19 and 24 (75)

Appendix 2. EMBASE

EMBASE (1980 to April 2012)

1 Controlled study/ or randomised controlled trial/ (1972522)
2 double blind procedure/ (55961)
3 single blind procedure/ (5342)
4 crossover procedure/ (16269)
5 drug comparison/ (81243)
6 placebo/ (77846)
7 random$.ti,ab,hw,tn,mf. (303696)
8 latin square.ti,ab,hw,tn,mf. (976)
9 crossover.ti,ab,hw,tn,mf. (28767)
10 cross-over.ti,ab,hw,tn,mf. (10308)
11 placebo$.ti,ab,hw,tn,mf. (122744)
12 ((doubl$ or singl$ or tripl$ or trebl$) adj5 (blind$ or mask$)).ti,ab,hw,tn,mf. (94676)
13 (comparative adj5 trial$).ti,ab,hw,tn,mf. (5072)
14 (clinical adj5 trial$).ti,ab,hw,tn,mf. (392129)
15 or/1-14 (2384221)
16 nonhuman/ (2564036)
17 animal/ not (human/ and animal/) (12787)
18 or/16-17 (2567625)
19 15 not 18 (1389301)
20 (assist$ adj5 hatch$).ti,ab,hw. (168)
21 (zona$ adj5 (pellucid$ or manipulat$ or disrupt$ or thin$ or drill$)).ti,ab,hw. (3135)
22 (zona$ adj5 (dissect$ or tyrode$ or proteinase$ or piezon$ or krypton$ or yag$)).ti,ab,hw. (143)
23 pzd.tw. (53)
24 or/20-23 (3236)
25 15 and 19 and 24 (420)

Appendix 3. Data Extraction Proforma

Trial quality characteristics

Refer to Figure 1 and Figure 2.

(1) Method of randomisation:

(a) randomised allocation - method of randomisation clearly stated and correct;

(b) randomised allocation - method of randomisation not stated or unclear.

(2) Allocation concealment:

(a) randomisation sequence adequately concealed;

(b) allocation concealment unclear;

(c) allocation concealment inadequate.

(3) Blinding:

(a) presence or absence of blinding of participants;

(b) presence or absence of blinding of outcome assessors.

(4) Power calculation reported.

(5) Intention-to-treat analysis stated or implied.

(6) Publication as full paper or abstract only.

Trial design and flow

(7) Trial flow:

(a) numbers of women recruited;

(b) numbers of women randomised;

(c) numbers of women excluded;

(d) numbers of women analysed;

(e) numbers of women lost to follow up.

(8) Study setting:

(a) single- or multi-centre;

(b) location;

(c) timing.

(9) Indication for zona manipulation:

(a) both diagnostic and therapeutic;

(b) therapeutic.

Study participants

(10) Baseline characteristics:

(a) age (mean and standard deviation in each study arm);

(b) primary or secondary infertility;

(c) cause and duration of infertility;

(d) previous treatment.

(11) Other subgroup criteria:

(a) women undergoing IVF only;

(b) women undergoing ICSI only;

(c) women over the age of 35 undergoing IVF, ICSI or both;

(d) women with high early proliferative phase FSH levels undergoing IVF, ICSI or both;

(e) women with repeated implantation failure undergoing IVF, ICSI or both;

(f) women with a higher risk of zona hardening undergoing IVF, ICSI or both.

Interventions

(12) Embryo manipulation:

(a) mechanical zona disruption - zona dissection, piezon vibrator, micro-manipulator;

(b) chemical zona disruption - acid tyrodes, proteinases;

(c) laser zona manipulation - krypton, ND-Yag, carbon dioxide.

(13) Complete (holes), partial (thinning) zona breach, complete removal.

Outcomes

(14) Primary:

(a) live birth (per woman randomised).

(15) Secondary:

(a) clinical pregnancy (per woman randomised);

(b) miscarriage (per woman randomised);

(c) multiple pregnancy (per woman randomised);

(d) ectopic pregnancy;

(e) monozygotic twinning;

(f) congenital and chromosomal abnormalities;

(g) embryo damage (per embryo generated).

WHAT'S NEW

Date	Event	Description
28 February 2013	Amended	Minor correction to review title (format only)

HISTORY

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

Date	Event	Description
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; and Valojerdi 2010).
8 August 2012	New citation required but conclusions have not changed	Seven new studies added; no change to conclusions.
17 June 2008	New search has been performed	New search identified four 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 amendment

CONTRIBUTIONS OF AUTHORS

Mourad Seif contributed to conceiving the review, designing the review, publishing the protocol, co-ordinating the review, data collection for the review, developing a search strategy, undertaking searches, screening search results, organising retrieval of papers, screening retrieved papers against inclusion criteria, arbitration on quality and data extraction of papers, interpretation of 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.

Cindy Farquhar updated the review in July 2005 by completing the new searches, selecting the studies, restructuring the table of comparisons, extracting the data, and rewriting parts of the review. She assisted with the updating of the review in 2007 by identifying the studies and assisting with data extraction and writing.

Debbie Blake was involved in the 2005 update by cross-checking newly extracted data, assessing inclusion criteria, subgroup analysis, editing, and providing a scientific perspective to the updated text.

Sangeeta Das was involved in the 2007 update by completing the new searches, retrieval of papers, screening retrieved papers against inclusion criteria, extracting data, and rewriting the review.

Sarah-Kate Carney was involved in the 2012 update by completing the new searches, retrieval of papers, screening retrieved papers against inclusion criteria, extracting data, and rewriting the review.

Linsey Nelson was involved in the 2012 updated by completing the new searches, retrieval of papers, screening retrieved papers against inclusion criteria, extracting data, and editing the review.

Andy Vail assisted the authors by performing the meta-regression for maternal age and pregnancy outcomes. Catherine Fullwood provided a statistical overview.

DECLARATIONS OF INTEREST

None known

SOURCES OF SUPPORT

Internal sources

- Central Manchester and Manchester Children's University Trust, UK.
- University of Manchester, UK.
- University of Auckland, New Zealand.

External sources

- Ministry of Health, New Zealand.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

1. In the 2005 update the following subgroups were investigated:

- age (where 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.

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

No new subgroups were added in the 2012 update

2. For the 2012 update the review was reformatted in line with current recommended Cochrane methods. Current Cochrane methodological standards require one identification name per paper. Papers that had one method but separated results into subgroups had these results pooled for the overall individual outcomes (i.e. live birth, clinical pregnancy, etc.), however the subgroups results were separated accordingly in the subgroup analysis (i.e. fresh versus frozen, first versus repeat attempt, etc.).

NOTES

Carter 2003 was included after information regarding the number of couples was provided by the authors.

INDEX TERMS

Medical Subject Headings (MeSH)

Embryo Implantation [*physiology]; Fertilization in Vitro [*methods]; Pregnancy Outcome; Pregnancy Rate; Randomized Controlled Trials as Topic; Sperm Injections, Intracytoplasmic [methods]; Zona Pellucida [*physiology]

MeSH check words

Female; Humans; Pregnancy