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Brown J, Daya S, Matson P

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[Intervention Review]

Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection

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ABSTRACT

Background

Embryo transfer (ET) was traditionally performed two days after oocyte retrieval; however, developments in culture media have allowed embryos to be maintained in culture for longer periods. Delaying transfer from Day two to Day three would allow for further development of the embryo and might have a positive effect on pregnancy outcomes.

Objectives

To determine if there are any differences in live birth and pregnancy rates when embryo transfer is performed on day three after oocyte retrieval, compared with day two, in infertile couples undergoing treatment with in vitro fertilisation (IVF), including intracytoplasmic sperm injection (ICSI).

Search methods

We searched the Cochrane Gynaecology and Fertility Group Specialised Register of Controlled Trials, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid), Embase (Ovid), PsycINFO (Ovid) from the inception of the databases to 26th April 2016. We also searched ClinicalTrials.gov and the WHO portal for ongoing trials plus citation lists of relevant publications, review articles and included studies, as well as abstracts of appropriate scientific meetings.

Selection criteria

Randomised controlled trials that compared Day 3 versus Day 2 embryo transfer after oocyte retrieval during an IVF or ICSI treatment cycle in infertile couples.

Data collection and analysis

Two review authors independently assessed trial quality and extracted data. We contacted study authors for additional information. The primary outcome measures were live birth rate and ongoing pregnancy rate.

Main results

We included 15 studies. Fourteen studies reported data per woman (2894 women) and one study reported data per cycle (969 cycles). The quality of the evidence using the GRADE approach ranged from *moderate quality* to *very low quality.* The main reasons for downgrading evidence were poor methodological reporting, selective reporting, inconsistency and imprecision.

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Live birth per woman - Overall, there was no evidence of a difference in live birth rate between Day three and Day two embryo transfer (risk ratio (RR) 1.05, 95% confidence interval (CI) 0.89 to 1.23; three studies, n = 1200 women; I^2 = 63%; *very low quality evidence*). The data suggest that if 32% of women who underwent a Day two embryo transfer had a live birth, then between 28% to 39% of women undergoing a Day three embryo transfer would have a live birth.

Ongoing pregnancy per woman - There was no evidence of a difference between Day three and Day two embryo transfer for ongoing pregnancy (RR 0.98, 95% CI 0.85 to 1.12; six studies, n = 1740 women; I^2 = 52%; *very low quality of evidence*). The data suggest that if 33% of women undergoing a Day two embryo transfer had an ongoing pregnancy then between 28% to 37% of women undergoing a Day three embryo transfer would have an ongoing pregnancy.

Clinical pregnancy per woman - There was no evidence of a difference between Day three and Day two embryo transfer for the chance of a clinical pregnancy (RR 1.08, 95% CI 0.98 to 1.19; 12 studies, n = 2461, I^2 = 51%; *very low quality evidence*). The data suggest that if 39% of women undergoing Day two embryo transfer had a clinical pregnancy, then between 38% to 46% of women undergoing a Day three embryo transfer would have a clinical pregnancy.

Multiple pregnancy per woman - There was no evidence of a difference between Day three and Day two embryo transfer for the risk of a multiple pregnancy (RR 1.12, 95% CI 0.86 to 1.44; eight studies, n = 1837; I^2 = 0%; *moderate quality evidence*). The data suggest that if 11% of women undergoing Day two embryo transfer had a multiple pregnancy, then between 9% to 15% of women undergoing a Day three embryo transfer would have a multiple pregnancy.

Miscarriage rate per woman - There was no evidence of a difference between Day three and Day two embryo transfer for the risk of miscarriage (RR 1.16, 95% CI 0.84 to 1.60; nine studies, n = 2153 women, I^2 = 26%; *moderate quality evidence*). The data suggest that if 6% of women undergoing Day two embryo transfer had a miscarriage, then between 5% to 10% of women undergoing a Day three embryo transfer would have a miscarriage.

Ectopic pregnancy rate per woman - There was no evidence of a difference between Day three and Day two embryo transfer for the risk of ectopic pregnancy (RR 0.99, 95% CI 0.29 to 3.40; six studies, n = 1531 women, I^2 = 0%; *low quality evidence*). The data suggest that if 0.7% of women undergoing Day two embryo transfer have an ectopic pregnancy, then between 0.2% to 2% of women undergoing Day three embryo transfer would have an ectopic pregnancy.

Subgroup analysis for pregnancy outcomes did not identify any differential effect between IVF and ICSI.

None of the included studies prespecified complication rate (e.g. OHSS), fetal abnormality or women's evaluation of the procedure as outcomes in their studies.

Authors' conclusions

Twelve of 15 studies contributed data that could be included in meta-analyses. The quality of the evidence ranged from *moderate* to *very low.* Only three of the 15 studies reported data for live birth, although the data for ongoing pregnancy and clinical pregnancy are consistent with the live birth data, suggesting no difference between Day three and Day two embryo transfer for these outcomes. There was no evidence of a difference identified between Day three and Day two embryo transfer for multiple pregnancy, miscarriage or ectopic pregnancy per woman randomised. No data were reported for complication rate, fetal abnormality or woman's evaluation of the procedure. The current evidence has not identified any evidence of differences in pregnancy outcomes between Day two and Day three embryo transfers. Any further studies comparing these timings of embryo transfer are unlikely to alter the findings and we suggest that this review no longer be updated.

PLAIN LANGUAGE SUMMARY

Day three versus day two embryo transfer following in vitro fertilisation or intracytoplasmic sperm injection

Review question

Cochrane review authors investigated whether transferring an embryo on Day two or on Day three of development makes a difference to pregnancy outcomes in women having in vitro fertilisation or intracytoplasmic sperm injection.

Background

Embryo transfer has usually been performed two days after oocyte (egg) retrieval; however, developments in culture media and embryo culture methods have allowed embryos to be maintained in culture for longer periods. This means that more assessments can be undertaken to look at the implantation chances for each embryo. Delaying transfer from Day two to Day three would allow for further development of the embryo and might have a positive effect on pregnancy outcomes.

Study characteristics

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We identified 15 randomised trials meeting the review inclusion criteria. These include 14 trials reporting data from 2894 women; one trial reported data from 969 cycles so could not be included in meta-analysis. All of the included studies were parallel-design randomised controlled trials conducted in Brazil, Chile, Singapore, Argentina, Finland, Turkey, Spain, Israel, Canada, Greece, Japan, Italy, Norway and Belgium. The evidence is current to April 2016.

Key results

Only three of 15 studies reported on live birth as an outcome. We found that there was no clear evidence of a difference between Day three and Day two embryo transfer for rates of live birth, ongoing pregnancy, clinical pregnancy, multiple pregnancy or miscarriage. There were no data reported for complication rate, fetal abnormality or women's evaluation of the procedure.

Quality of the evidence

Allocation concealment was poorly reported in the included studies and blinding was not possible (although we feel this is unlikely to affect pregnancy outcomes). Blinding of outcome assessors was not reported. The quality of the evidence ranged from *moderate* to *very low.* The main reasons for downgrading the evidence were poor reporting of study methods (risk of bias), lack of agreement between studies (inconsistency), low event rates and lack of accuracy (imprecision) for some outcomes and poor reporting of live birth outcomes (selective reporting).

Any further studies comparing these timings of embryo transfer are unlikely to alter the findings and we do not plan to update this review again. Many of the trials included in this review have used outdated techniques that include stimulation, laboratory technology and transferring more than one embryo. We would direct the reader to the Glujovsky 2016 Cochrane review comparing Day 2/3 with day 5/6 embryo transfer.

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Summary of findings for the main comparison. Day 3 versus Day 2 embryo transfer following IVF or ICSI

Day 3 versus Day 2 embryo transfer following IVF or ICSI

Patient or population: Women undergoing *in vitro* fertilisation or intracytoplasmic sperm injection

Setting: Trials conducted in Brazil, Chile, Singapore, Argentina, Finland, Turkey, Spain, Israel, Canada, Greece, Japan, Italy, Norway and Belgium
Intervention: Day 3 embryo transfer
Comparison: Day 2 embryo transfer

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with Day 2 Embryo Transfer	Risk with Day 3				
Live birth rate per woman	315 per 1,000	331 per 1,000 (280 to 387)	RR 1.05 (0.89 to 1.23)	1200 (3 RCTs)	⊕⊖⊖⊖ VERY LOW [1, 2, 3]	
Ongoing pregnancy rate per woman	326 per 1,000	320 per 1,000 (277 to 365)	RR 0.98 (0.85 to 1.12)	1740 (6 RCTs)	⊕⊖⊖⊖ VERY LOW [2, 4, 5]	
Clinical pregnancy rate per woman	386 per 1,000	417 per 1,000 (378 to 459)	RR 1.08 (0.98 to 1.19)	2461 (12 RCTs)	⊕⊖⊖⊖ VERY LOW [2, 4, 6]	
Multiple pregnancy rate per woman	106 per 1,000	118 per 1,000 (91 to 152)	RR 1.12 (0.86 to 1.44)	1837 (8 RCTs)	⊕⊕⊕⊖ MODERATE [7]	
Miscarriage rate per woman	59 per 1,000	69 per 1,000 (50 to 95)	RR 1.16 (0.84 to 1.60)	2153 (9 RCTs)	⊕⊕⊕⊖ MODERATE [7]	
Ectopic pregnancy rate per woman	7 per 1,000	6 per 1,000 (2 to 22)	RR 0.99 (0.29 to 3.40)	1531 (6 RCTs)	⊕⊕⊖⊖ LOW [7, 8]	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

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Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1]One study had unclear allocation concealment and all studies reporting this outcome lacked details around blinding of participants, researchers and outcome assessors. Downgraded one level.
[2]Evidence of inconsistency - I^2 > 50% but < 70%. Downgraded one level.
[3]Only three of the 15 included studies reported live birth data. Downgraded one level.
[4]Poor methodological reporting of allocation concealment, blinding and high risk of selective reporting. Downgraded two levels.
[5]Only two of the six studies reporting ongoing pregnancy also reported on live birth. Downgraded one level.
[6]Poor reporting of live birth. Downgraded one level.
[7]Poor reporting of allocation concealment and blinding. Downgraded one level.
[8]Evidence of imprecision with wide confidence intervals and low event rates. Downgraded one level.

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BACKGROUND

Description of the condition

Infertility may be caused by male or female factors, or both, but in some couples no cause can be found (Cahill 2002). In vitro fertilisation (IVF) is considered beneficial for most couples who are unlikely to conceive without treatment, and for whom less invasive forms of treatment have failed or are unlikely to be effective (RCOG 1999).

Description of the intervention

IVF involves the use of hormones to stimulate the ovaries to produce many eggs (oocytes), followed by egg collection (oocyte retrieval), mixing of eggs and sperm, fertilisation, embryo culture and, lastly, the return of a selected embryo to the uterus (embryo transfer, ET). The aim of this review is to determine whether the number of days between oocyte retrieval and embryo transfer (i.e. the number of days the embryos are grown in vitro) has any effect on the success of IVF treatment, in particular, the live birth rate - the most important outcome for the couple (Steinberg 1998).

The question of optimal timing for embryo transfer arises when examining the differences between the IVF procedures and what happens naturally in vivo. Embryo transfer was performed traditionally two days after oocyte retrieval, when the embryos are at the cleavage stage (Coskun 2002), because it is thought the uterus provides the best environment for the survival of the embryo (Laverge 2001).

How the intervention might work

Early replacement in the uterine cavity may be advantageous for the embryo, by limiting the time spent in the in vitro environment of incubators in an embryology laboratory. Developments in culture media have enabled embryos to be maintained in culture for longer periods (Kovacic 2002), allowing for further development of the embryo in vitro. It has been suggested that the longer time in culture improves the accuracy of selection of the best quality embryos for transfer (Huisman 2000), because additional morphological features are available for assessment at this time (Desai 2000).

However, there remains a concern that extended culture of embryos may increase the risk of congenital abnormalities and pre-term births (Dar 2014; Källén 2010; Kalra 2012). When considering the best time for selecting and transferring early cleavage embryos, delaying transfer an extra day from Day two to Day three may increase the likelihood of successful implantation and also improve endometrial differentiation (Nikas 2000), taking a step closer to the natural situation in which the embryo arrives in the uterus four to five days after ovulation. In addition, the selection and deselection of embryos on Day three can be further improved using appropriate algorithms in conjunction with time-lapse videography (Conaghan 2013; Liu 2016).

The question of whether blastocyst stage embryos (Day 5/6) should be transferred compared to embryos at the cleavage stage (Day 2/3) is the subject of another Cochrane Review (Glujovsky 2016).

Why it is important to do this review

Assessing the development potential of embryos is an important determinant of its ability to implant after it has been transferred into the uterus. The improvement in culture media and in embryo culture methods has permitted embryos to be grown for longer periods of time, thereby enabling an assessment of their implantation potential. Consequently, the traditional approach of transferring embryos on Day two should be compared with that of Day three, whereby embryos that demonstrate optimal growth during the extra day of monitoring in culture can be preferentially selected.

OBJECTIVES

To determine if there are any differences in live birth and pregnancy rates when embryo transfer is performed on Day three after oocyte retrieval, compared with Day two, in infertile couples undergoing treatment with in vitro fertilisation (IVF), including intracytoplasmic sperm injection (ICSI).

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) comparing Day three embryo transfer with Day two embryo transfer in standard IVF or ICSI treatment for infertility.

Types of trials excluded were:

- trials that included only comparison with Day 4/5/6 embryo transfer
- trials that compared only cleavage stage versus blastocyst stage, because this comparison is the subject of another Cochrane Review (Glujovsky 2016)
- cross-over trials, (invalid trial design with pregnancy as the outcome), unless phase one data could be extracted
- quasi-randomised controlled trials (a method of randomisation that is not truly random, such as by hospital number or date of birth)
- trials in which individual women contributed more than one treatment cycle, unless data for the woman's first cycle within the trial or data per woman could be obtained.

Types of participants

Couples with infertility (from any cause or unexplained) undergoing an embryo transfer procedure during an IVF or ICSI cycle.

Types of interventions

Embryo transfer on Day two or Day three after oocyte retrieval during an IVF or ICSI cycle.

Types of outcome measures

We recorded the following outcomes if the information was available:

Primary outcomes

• Live birth rate - live birth per woman
• Ongoing pregnancy rate - pregnancy continuing beyond 12 weeks' gestation per woman

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Secondary outcomes

• Clinical pregnancy rate - clinical pregnancy (pregnancy confirmed by ultrasound scan) per woman
• Complication rate - adverse events associated with treatment (e.g. ovarian hyperstimulation syndrome, OHSS) per woman
• Multiple pregnancy rate - multiple pregnancy (twins, triplets or higher order if specified) per woman
• Miscarriage rate - miscarriage of an intrauterine clinical pregnancy (confirmed by ultrasonography or by histology) per woman
• Ectopic pregnancy rate - ectopic gestation (confirmed by ultrasonography or by histology) per woman
• Fetal abnormality rate - fetal/neonatal abnormalities per woman
• Woman's evaluation of procedure

Search methods for identification of studies

We searched for all published and unpublished randomised controlled trials of Day three versus Day two embryo transfer, without language restriction, and in consultation with the Cochrane Gynaecology and Fertility Information Specialist.

Electronic searches

For the 2016 update of this review, we searched the following electronic databases and trials registries in April 2016:

The Cochrane Gynaecology and Fertility Group Specialised Register of Controlled Trials (Appendix 1), the Cochrane Central Register of Controlled Trials Online (CENTRAL) (Appendix 2), MEDLINE (Ovid) (Appendix 3), Embase (Ovid) (Appendix 4) and PsycINFO (Ovid) (Appendix 5). We combined the MEDLINE search with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials that appears in Section 6.4.11 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Version 5.1.0) (Higgins 2011). We combined the Embase and PsycINFO searches with the trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk/methodology/filters.html#random).

Other electronic sources of trials included:

- trials registers of registered and ongoing trials
  * clinicaltrials.gov (a service of the US National Institutes of Health);
  * who.int/trialsearch/Default.aspx (the World Health Organization International Trials Registry Platform search portal).

Searching other resources

For the 2016 update of this review we searched the citation lists of relevant publications and review articles to April 2016 and handsearched the references of the included studies.

Data collection and analysis

Selection of studies

For the 2016 update of this review, after an initial screen of the titles and abstracts retrieved in the searches (by JB), we retrieved the full texts of all potentially eligible studies. Two review authors (JB, SD) independently examined these full-text articles for compliance with the inclusion criteria and selected studies eligible for inclusion in the review. We corresponded with study investigators, as required, to confirm study eligibility. We resolved disagreements on study eligibility by consensus.

Data extraction and management

In earlier versions of this review CO and JG independently performed all assessments of the quality of trials and data extraction, using forms designed according to Cochrane guidelines, and resolving any discrepancies by consensus. In the 2016 update JB performed an initial screen for included studies. We sought additional information on trial methodology or original trial data or both from the authors of trials that appeared to meet the inclusion criteria but had aspects of methodology that were unclear or data that were in a form unsuitable for meta-analysis. We present details of each trial in the Characteristics of included studies tables.

Assessment of risk of bias in included studies

Two review authors independently assessed the included studies for risks of bias, using the Cochrane 'Risk of bias' assessment tool (Higgins 2011) to assess: selection (random selection and allocation concealment), performance (blinding of participants and personnel), detection (blinding of outcome assessors), attrition (incomplete outcome data), reporting (selective reporting), and other biases. We resolved disagreements by discussion or when necessary by recourse to a third author. We have described all aspects of risk of bias in detail in the 'Risk of bias' tables (Characteristics of included studies). For the purpose of this review we decided that in studies in which there was no evidence of blinding we would judge the risk of bias to be unclear, because it is not likely that blinding of participants would influence pregnancy outcomes. We made attempts to identify trial protocols and compare them to published papers. For studies that had not reported live birth but had reported other interim pregnancy outcomes, we undertook an informal assessment to determine if the interim data were similar to the data reported for live birth.

Measures of treatment effect

We have only reported on dichotomous data (e.g. live birth per woman randomised). We used the number of events in the control and intervention groups to calculate Mantel-Haenzel risk ratios (RRs); these data are presented with 95% confidence intervals (CIs).

Unit of analysis issues

The primary unit of analysis was per woman randomised. Data reporting outcomes per cycle were not included in the meta-analysis. We have counted multiple births as one birth event. We have not included cross-over trials in the current version of this review.

Dealing with missing data

As far as possible we have analysed data on an intention-to-treat basis and have made attempts to obtain missing data from primary authors of included trials. We have not imputed any data.

Assessment of heterogeneity

We have considered whether clinical and methodological characteristics of the included trials are sufficiently similar for meta-analysis to provide a clinically meaningful summary. We assessed heterogeneity using the I^2 statistic, taking a measurement > 50% to indicate substantial heterogeneity (Higgins 2003).

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Assessment of reporting biases

We attempted to minimise the risk of reporting biases by conducting a thorough and systematic search of the literature for published and unpublished literature without any restrictions by language. For 10 or more trials reporting an outcome, we explored reporting bias by visual examination of a funnel plot.

Data synthesis

For studies that were sufficiently similar, we combined the data using a fixed-effect model in the following comparison.

- Day three versus Day two embryo transfer

Subgroup analysis and investigation of heterogeneity

In studies in which data were available we conducted subgroup analysis to determine the separate evidence within the following subgroups:

- type of assisted reproductive technology (IVF versus ICSI)

When we detected substantial heterogeneity we explored possible explanations in subgroup or sensitivity analyses, or both.

Sensitivity analysis

We conducted sensitivity analyses for the primary outcomes of this review to determine whether the conclusions are robust to arbitrary decisions made regarding the eligibility and analysis. We considered whether the review conclusions would have changed if:

- Eligibility were restricted to studies without high risk of bias
- A random-effects model had been adopted

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

We have prepared a Summary of findings for the main comparison using GRADEpro GDT software. This table evaluates the overall quality of the body of evidence for the main review outcomes (live birth, ongoing pregnancy, clinical pregnancy, miscarriage) using GRADE criteria (study limitations, consistency, imprecision, indirectness and publication bias). Judgements about quality (high, moderate, low or very low) are justified and incorporated into the results for each outcome.

Periodic updates to the review

Although the optimal information size to test the hypothesis of no difference between Day two and Day three transfers has not yet been reached, we believe that any new studies are unlikely to change the findings of this review, and therefore consider the review to be stable. No further updates are planned.

RESULTS

Description of studies

Results of the search

In the previous version of this review there were 10 included studies, 18 excluded studies and one study awaiting classification.

In 2016 the search for the update identified 749 potential studies after duplicates had been removed. We included five new studies and added one to the Studies awaiting classification category. We excluded one study. See Figure 1 for flow diagram of study selection.

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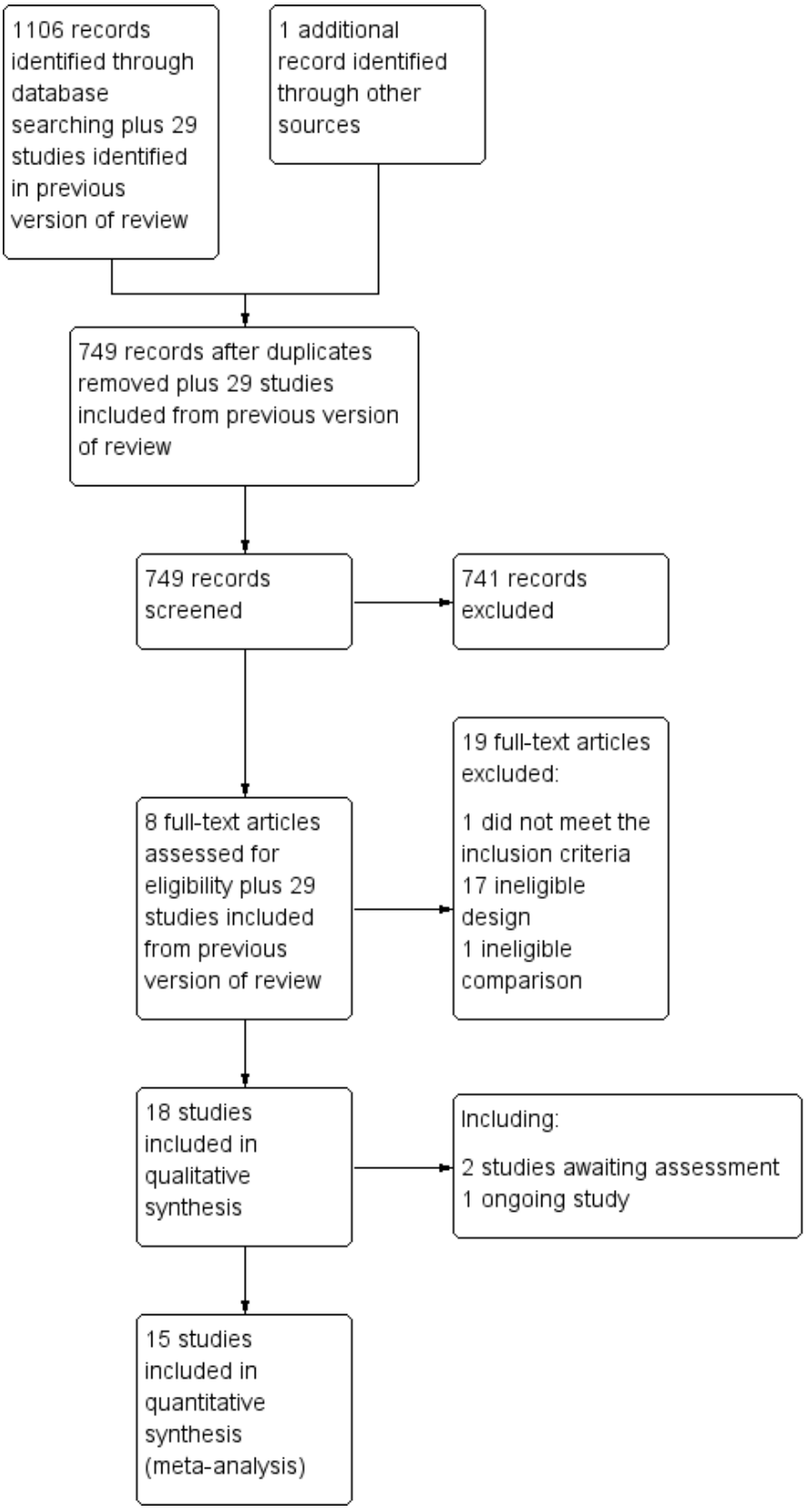
Figure 1. Study flow diagram.



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In the 2016 update of this review we have included 15 studies (Characteristics of included studies); we excluded 19 studies (Characteristics of excluded studies). Two studies are awaiting assessment (Characteristics of studies awaiting classification). In Amireh 1998, the inclusion criteria were met, because it was described as a randomised trial, but it was also described as a retrospective assessment. Furthermore, all the required data were not provided in the abstract. We have contacted the authors for additional information about the randomisation process and the numbers of women in each group. The second study awaiting classification is Shahine 2011. This study appears to be a randomised trial comparing Day two versus Day three embryo transfer in 251 women classified as poor responders undergoing IVF. The data in the table for clinical pregnancy and miscarriage do not tally, and we plan to contact the authors to provide information on the methods of randomisation and the correct numbers for these outcomes.

Included studies

See Characteristics of included studies

Study design and setting

All of the included studies were parallel-design randomised controlled trials. Six of the studies were only reported as conference abstracts and we could not identify the full publication (Chen 1999; DiBeradino 1998; Marsella 2005; Nodar 2002; Nordstrom 1995; Urman 1998). Studies were set in a number of different countries, including Brazil and Chile (Abdelmassih 1998), Brazil (Baruffi 2003), Singapore (Chen 1999), Argentina (Nodar 2002), Finland (Nordstrom 1995), Turkey (Urman 1998 and Bahceci 2006), Spain (De los Santos 2003), Israel (Caspi 1989), Canada (DiBeradino 1998), Greece (Pantos 2004), Japan (Suzuki 2004), Italy (Marsella 2005), Norway (Ertzeid 1999) and Belgium (Laverge 2001).

Participants

Two thousand eight hundred and ninety four women took part in the included trials; one study (De los Santos 2003) only reported on the number of cycles (969 cycles with 888 embryo transfers) and not the number of women. We have not included the data from this study in any meta-analyses.

Six hundred and ninety-eight women underwent IVF and 1601 women underwent ICSI. In four of the studies added in 2016 it was unclear how many women had undergone IVF or ICSI (De los Santos 2003; Marsella 2005; Pantos 2004; Suzuki 2004).

The age of participants was available for 11 studies. The mean female age ranged from 31 to 37 years and was comparable in the two study groups of each trial. Information about age was not provided in De los Santos 2003, DiBeradino 1998; Marsella 2005 or Nordstrom 1995. A maximum age limit was not stated in any trial.

Two studies only included women diagnosed as poor responders (Bahceci 2006; Pantos 2004).

Interventions

All studies compared the intervention of embryo transfer on Day three versus Day two. Details of the ovarian stimulation protocol, embryo culture methods, and embryo transfer procedure were noted, when available. In all trials that described the protocols and procedures used, they were similar for the two treatment groups. All trials used fresh embryos.

Outcomes

Live birth was reported in three of 15 studies (Chen 1999; Ertzeid 1999; Laverge 2001).

Data for ongoing pregnancy were available from seven of 15 studies (Bahceci 2006; Baruffi 2003; Chen 1999; De los Santos 2003 (per cycle data only); Ertzeid 1999; Laverge 2001; Pantos 2004). Additional information was obtained from Baruffi 2003, Chen 1999 and Laverge 2001 trials for this outcome. We obtained ongoing pregnancy to eight weeks from the authors of DiBeradino 1998, but information to 12 weeks was not available.

Twelve of 15 studies reported usable data for clinical pregnancy rate (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; De los Santos 2003 (per cycle data only); DiBeradino 1998; Ertzeid 1999; Laverge 2001; Nodar 2002; Pantos 2004; Urman 1998).

Miscarriage rate was reported in nine of 15 studies (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; Ertzeid 1999; Laverge 2001; Nodar 2002; Pantos 2004).

Multiple pregnancy was reported by nine of 15 trials (Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; De los Santos 2003 (per cycle data only); DiBeradino 1998; Ertzeid 1999; Laverge 2001; Pantos 2004).

Ectopic pregnancy (or its absence) was reported by seven of 15 trials (Baruffi 2003; Chen 1999; De los Santos 2003 (per cycle data only); DiBeradino 1998; Ertzeid 1999; Laverge 2001; Pantos 2004).

No study reported data on complications, fetal abnormalities, or the women's evaluation of the procedure.

Number of cycles per woman

No study reported in its publication that any woman contributed more than one cycle to the study. Ertzeid 1999 reported only the number of cycles performed, but further information from the author confirmed that this information was consistent with the number of individual women randomised. De los Santos 2003 only reported on the number of cycles. It has not been established if this information is consistent with the number of women randomised. We therefore have not included these data in any meta-analysis.

Excluded studies

Excluded studies

See Characteristics of excluded studies
We excluded 19 studies from this review. Eighteen studies had been previously excluded:

One study did not meet the inclusion criteria (Pires 2000); nine of the studies were retrospective comparative studies (BarbarinoMonier 2002; Carrillo 1998; Dawson 1995; Galan 2001; Gonen 1999; Goto 1994; Racowsky 1998; Ramey 1997; Wilson 1996); six studies were excluded because women were quasi-randomised (Aboulghar 2003; Huisman 1994a; Huisman 1994b; Koo 1999; Marsella 2001; Van Os 1989), and in two studies women were not randomised (Cowan 1997; Fussell 1999).

For the 2016 update, we excluded one additional study because it had used different comparisons (Day one versus Day two and three versus Day four and five) (Margreiter 2003).

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Risk of bias in included studies

See Characteristics of included studies for 'Risk of bias' table and Figure 2; Figure 3.

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

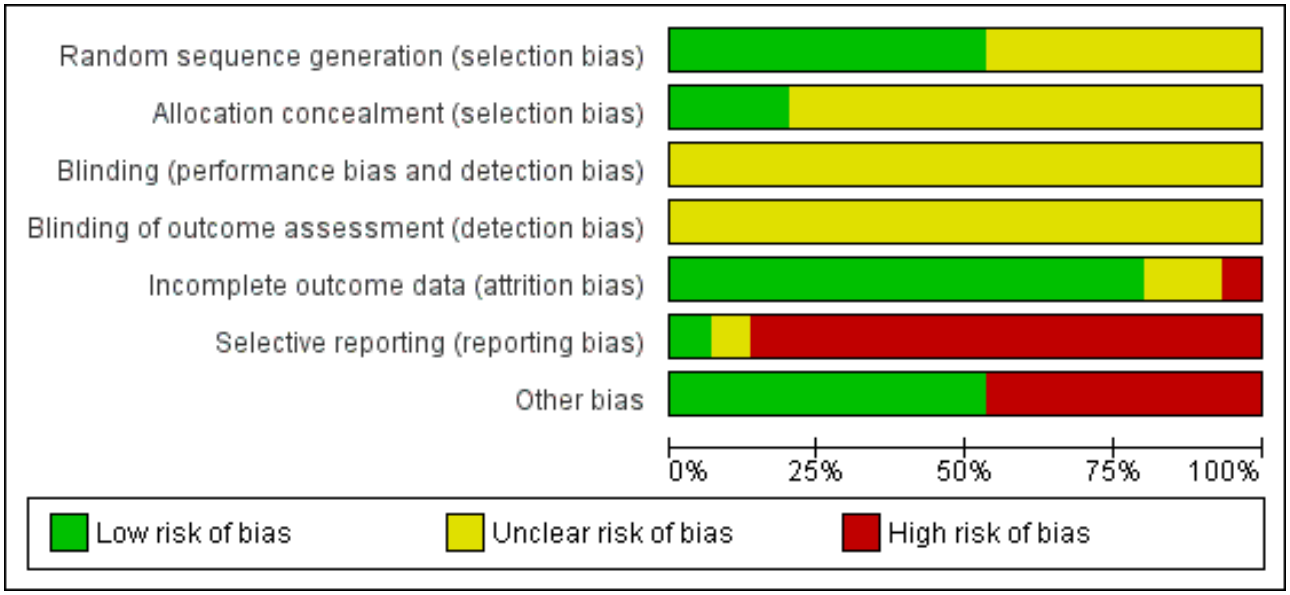

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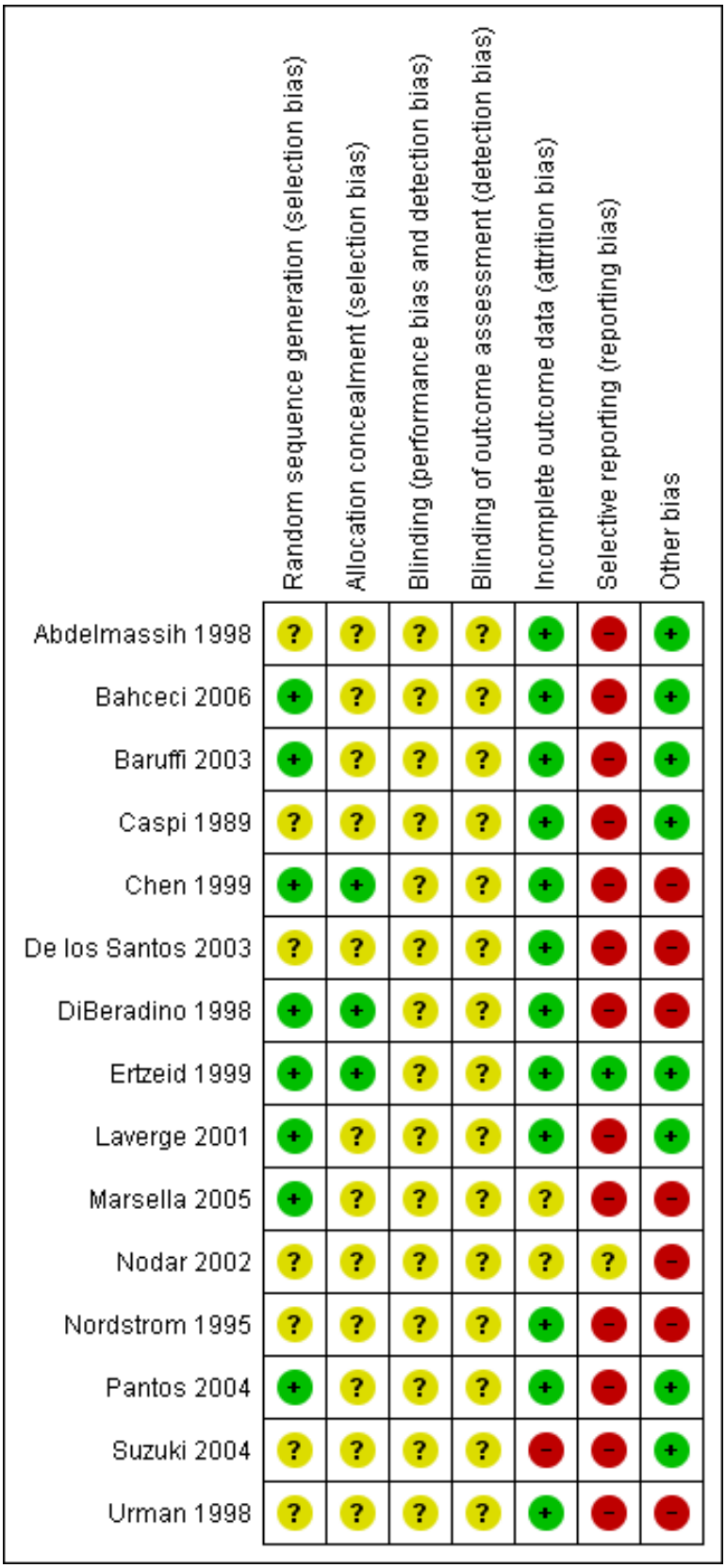
Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



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Allocation

Random sequence generation

We judged eight of 15 trials as having a low risk of bias for random sequence generation (Bahceci 2006; Baruffi 2003; Chen 1999; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Marsella 2005; Pantos 2004).

Baruffi 2003 randomised women using drawing of lots (additional information obtained from authors), seven studies reported computer randomisation methods (Bahceci 2006; Chen 1999; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Marsella 2005; Pantos 2004). Additional information was obtained for Chen 1999 and DiBeradino 1998. There were insufficient details in the remaining studies to judge how random sequence generation was performed, and we rate these studies at unclear risk of bias.

Allocation concealment

Three studies reported using sealed opaque envelopes (DiBeradino 1998; Ertzeid 1999; Chen 1999) and were considered to be at low risk of bias. There were insufficient details in the remaining studies to judge how allocation was concealed and we judge these studies to be at unclear risk of bias.

Blinding

Performance bias

None of the included studies reported on blinding of participants or researchers. Blinding is unlikely to have been possible due to the nature of the intervention being embryo transfer on two different days. Lack of blinding of participants is unlikely to have had an influence on pregnancy outcomes. We rated all included studies as having unclear risk of bias.

Detection bias

None of the included studies reported on blinding of outcome assessors and were therefore judged as having unclear risk of bias.

Incomplete outcome data

Twelve studies reported no losses to follow-up or all women randomised being analysed, or both (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; De los Santos 2003; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Nordstrom 1995; Pantos 2004; Urman 1998). We judged these studies to be at low risk of attrition bias. It was unclear from the Marsella 2005 study how many women had been randomised to each group and we rated the study as being at unclear risk of bias. Nodar 2002 was a conference abstract and it was unclear if this was the final trial report; we judged the study to be at unclear risk of attrition bias. The Suzuki 2004 study reported randomising 114 women, but because the post-randomisation exclusion rate was 68%, we judged the study to have a high risk of attrition bias.

Selective reporting

We judged only one of 15 studies to have a low risk of reporting bias (Ertzeid 1999). We rated one study (Nodar 2002) at an unclear risk of reporting bias; all outcomes specified in the abstract were reported on, but it was unclear if these were all of the outcomes of the study. The data are only presented as conference abstracts and we identified no full publication. The remaining studies did not prespecify outcomes or did not report on live births, or both.

Other potential sources of bias

We found no other sources of bias in eight studies (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Caspi 1989; Ertzeid 1999; Laverge 2001; Pantos 2004; Suzuki 2004), and we judged the remaining studies to be at high risk of bias. Six were conference abstracts only (Chen 1999; DiBeradino 1998; Marsella 2005; Nodar 2002; Nordstrom 1995; Urman 1998) and one study reported on cycles and not on women (De los Santos 2003).

Effects of interventions

See: **Summary of findings for the main comparison** Day 3 versus Day 2 embryo transfer following IVF or ICSI

Day 3 versus Day 2 embryo transfer

Primary outcomes

1.1 Live birth per woman

Three trials reported live birth per woman (Chen 1999; Ertzeid 1999; Laverge 2001). Overall, there was no evidence a difference in live birth rate between Day three and Day two embryo transfer (risk ratio (RR) 1.05, 95% confidence interval (CI) 0.89 to 1.23; three studies, n = 1200 women; I^2 = 63%; Analysis 1.1; *very low quality evidence*). We downgraded the evidence for unclear risk of bias, inconsistency and poor reporting of live birth outcomes from the 15 studies included in this review (Summary of findings for the main comparison). The data suggest that if 32% of women who underwent a Day two embryo transfer had a live birth, then between 28% to 39% of women undergoing a Day three embryo transfer would have a live birth.

We explored heterogeneity by performing a subgroup analysis. Ertzeid 1999 and Laverge 2001 reported data following IVF and Chen 1999 and Laverge 2001 reported data following ICSI. The test for subgroup analysis was not statistically significant (Chi^2 = 0.59, df = 1, P = 0.44, I^2 = 0%), indicating no differential effect between IVF and ICSI as the method of assisted reproductive technology (ART) used. We explored heterogeneity using sensitivity analysis by removing the Laverge 2001 study which had unclear risk of bias for allocation concealment. The removal of this study did not affect the overall results (RR 0.99, 95% CI 0.99 to 1.99, two studies, n = 454 women, I^2 = 0%; analysis not shown) and heterogeneity was reduced to I^2 = 0%.

1.2 Ongoing pregnancy per woman

Six trials reported ongoing pregnancy (Bahceci 2006; Baruffi 2003; Chen 1999; Ertzeid 1999; Laverge 2001; Pantos 2004). Overall there was no evidence of a difference between Day three and Day two embryo transfer for ongoing pregnancy (RR 0.98, 95% CI 0.85 to 1.12; six studies, n = 1740 women; I^2 = 52%; Analysis 1.2; *very low quality of evidence*). We downgraded the evidence for inconsistency, poor methodological reporting and poor reporting of live birth (selective reporting). The data suggest that if 33% of women undergoing a Day two embryo transfer had an ongoing pregnancy, then between 28% to 37% of women undergoing a Day three embryo transfer would have an ongoing pregnancy.

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We explored heterogeneity by performing a subgroup analysis. Three of the six studies (Ertzeid 1999; Laverge 2001; Pantos 2004) reported data following IVF and five of the six studies reported data following ICSI (Bahceci 2006; Baruffi 2003; Chen 1999; Laverge 2001; Pantos 2004). The hypothesis test for subgroup analysis was not significant (Chi^2 = 0.00, df = 1, P = 0.98, I^2 = 0%), indicating no differential effect between IVF and ICSI as the method of ART used. We explored heterogeneity using sensitivity analysis by removing studies with unclear risk of bias for allocation concealment (Bahceci 2006; Baruffi 2003; Laverge 2001; Pantos 2004). This approach left two studies for analysis (Chen 1999; Ertzeid 1999). The evidence suggested that Day three transfer was associated with an increased chance of an ongoing pregnancy (RR 1.44, 95% CI 1.02 to 2.03; two studies, n = 454 women; I^2 = 0%, analysis not shown).

Although not prespecified, we examined the data from two studies reporting outcomes in poor responders (Bahceci 2006; Pantos 2004). Day three embryo transfer was associated with a reduced risk of an ongoing pregnancy in poor responders (RR 0.63, 95% CI 0.41 to 0.97; two studies, n = 299 women; I^2 = 0%; analysis not shown).

Secondary outcomes

1.3 Clinical pregnancy per woman

Twelve studies reported clinical pregnancy rate per woman randomised (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Nodar 2002; Nordstrom 1995; Pantos 2004; Urman 1998). Overall there was no evidence of a difference between Day three and Day two embryo transfer for the chance of a clinical pregnancy (RR 1.08, 95% CI 0.98 to 1.19; 12 studies, n = 2461, I^2 = 51%; Analysis 1.3; *very low quality evidence*). We downgraded the evidence for inconsistency, poor methodological reporting and poor reporting of ongoing pregnancy or live birth. The data suggest that if 39% of women undergoing Day two embryo transfer had a clinical pregnancy, then between 38% to 46% of women undergoing a Day three embryo transfer would have a clinical pregnancy.

We explored heterogeneity by performing a subgroup analysis. Seven of the twelve studies reported data for clinical pregnancy per woman randomised following IVF (Caspi 1989; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Nodar 2002; Nordstrom 1995; Pantos 2004) and eight of the twelve studies reported data following ICSI (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Chen 1999; Laverge 2001; Nodar 2002; Pantos 2004; Urman 1998). The hypothesis test for subgroup analysis was not statistically significant (Chi^2 = 0.56, df = 1, P = 0.45, I^2 = 0%), suggesting no differential effect between IVF and ICSI as the method of ART used. We explored heterogeneity using sensitivity analysis by removing studies with an unclear risk of bias for allocation concealment. Among the three studies in this analysis (Chen 1999; DiBeradino 1998; Ertzeid 1999), there was no evidence of a difference between Day three and Day two embryo transfer for the outcome of clinical pregnancy (RR 1.28, 95% CI 0.99 to 1.66, three studies, n = 517 women, I^2 = 59% - analysis not shown).

For interest, we examined the data from two studies reporting outcomes in poor responders (Bahceci 2006; Pantos 2004). The data suggested that Day three embryo transfer was associated with a reduced clinical pregnancy rate compared with Day two embryo transfer (analysis not shown) (RR 0.60, 95% CI 0.42 to 0.86; two studies, n = 299 women, I^2 = 0%).

1.4 and 1.5 Multiple pregnancy

Eight studies reported multiple pregnancy (Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Pantos 2004). None of these trials transferred a single embryo. The mean number of embryos transferred per cycle ranged from two (Bahceci 2006) to four embryos (Caspi 1989; Pantos 2004).

Overall there was no evidence of a difference between Day three and Day two embryo transfer for the risk of a multiple pregnancy (RR 1.12, 95% CI 0.86 to 1.44; eight studies, n = 1837; I^2 = 0%; Analysis 1.4; *moderate quality evidence*). We downgraded the evidence for unclear risk of bias. The data suggest that if 11% of women undergoing Day two embryo transfer had a multiple pregnancy, then between 9% to 15% of women undergoing a Day three embryo transfer would have a multiple pregnancy.

The outcome of higher order multiple pregnancies (i.e. triplets) per woman randomised was reported in seven studies (Baruffi 2003; Caspi 1989; Chen 1999; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Pantos 2004). There was no evidence of a difference between Day three and Day two embryo transfer for the risk of a higher order multiple pregnancy (RR 0.88, 95% CI 0.38 to 2.07; seven studies, n = 1565 women, I^2 = 9%; Analysis 1.5). We advise caution in interpreting these results, due to wide confidence intervals that cross the line of no effect and the low event rates (8/784 Day three; 9/781 Day two) which may suggest imprecision.

For interest, we examined the data from two studies reporting data for poor responders (Bahceci 2006; Pantos 2004). There was no evidence of a difference for multiple pregnancy between Day three and Day two embryo transfer (RR 0.79, 95% CI 0.30 to 2.06; two studies, n = 299 women; analysis not shown). Caution is advised in interpreting these results, due to wide confidence intervals crossing the line of no effect and low event rates (7/146 Day three; 9/153 Day two), suggesting imprecision. There were no events of multiple pregnancy reported by Pantos 2004.

1.6 Miscarriage rate per woman randomised

Nine studies reported miscarriage rate per woman randomised (Abdelmassih 1998; Bahceci 2006; Baruffi 2003; Caspi 1989; Chen 1999; Ertzeid 1999; Laverge 2001; Nodar 2002; Pantos 2004). There was no evidence of a difference between Day three and Day two embryo transfer for the risk of miscarriage (RR 1.16, 95% CI 0.84 to 1.60; nine studies, n = 2153 women, I^2 = 26%; Analysis 1.6; *moderate quality evidence*). We downgraded the evidence or unclear risk of bias. The data suggest that if 6% of women undergoing Day two embryo transfer had a miscarriage, then between 5% to 10% of women undergoing a Day three embryo transfer would have a miscarriage.

For interest, we examined the data from two studies reporting outcomes in poor responders (Bahceci 2006; Pantos 2004). There was no evidence of a difference for the risk of miscarriage between Day 3 and Day 2 embryo transfer (RR 0.62, 95% CI 0.30 to 1.28; two studies, n = 434 women; analysis not shown).

1.7 Ectopic pregnancy per woman

Six studies reported data for ectopic pregnancy per woman randomised (Baruffi 2003; Chen 1999; DiBeradino 1998; Ertzeid 1999; Laverge 2001; Pantos 2004). There was no evidence of a difference between Day three and Day two embryo transfer for the risk of ectopic pregnancy (RR 0.99, 95% CI 0.29 to 3.40; six

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studies, n = 1531 women, I^2 = 0%; Analysis 1.7; *low quality evidence*). We downgraded the evidence because of unclear risk of bias and imprecision. The data suggest that if 0.7% of women undergoing Day two embryo transfer have an ectopic pregnancy, then between 0.2% to 2% of women undergoing Day three embryo transfer would have an ectopic pregnancy.

Caution is advised in interpreting these results, due to wide confidence intervals crossing the line of no effect and low event rates (5/767 Day three; 5/764 Day two), suggesting imprecision. No events in either the Day three or the Day two groups were reported in two studies (Baruffi 2003; Chen 1999).

Other outcomes for this review

None of the included studies prespecified complication rate (e.g. OHSS), fetal abnormality or women's evaluation of the procedure as outcomes in their studies.

DISCUSSION

Summary of main results

We found no evidence of differences in live birth, ongoing pregnancy, clinical pregnancy, multiple pregnancy, miscarriage or ectopic pregnancy when delaying embryo transfer from Day two to Day three. The evidence is based on data from 15 studies, of which 14 reported data per woman (2894 women) and were analysed, while one study reported data per cycle (969 cycles) and was not included. The quality of the evidence ranged from moderate to very low quality (Summary of findings for the main comparison) .

Overall completeness and applicability of evidence

The data for most pregnancy outcomes were similar enough to make meaningful comparisons. The heterogeneity observed for ongoing pregnancy and clinical pregnancy could not be explained by the subgroup analysis examining the method of assisted reproductive technology used (IVF or ICSI). Live birth outcomes were poorly reported, with only three of 15 included studies reporting this outcome. We calculated that to detect a clinically significant difference of five percentage points in live birth rate, using the control rate of 32% that was observed in the Day two group, with an alpha of 5% and a power of 90%, would require an optimal information size of 1900 participants per group (Daya 2002). The current meta-analysis for live birth includes three studies comprising 1200 women (Analysis 1.1). However, the data for ongoing and clinical pregnancy rates per woman randomised are consistent and show no evidence of a difference between Day three and Day two embryo transfer.

Caution is required in interpreting some of the data, especially for ectopic pregnancy, because the reported event rates are low with wide confidence intervals crossing the line of no effect, demonstrating imprecision. There is poor reporting for the number of cryopreserved embryos and the cumulative pregnancy rate.

All of the included trials transferred more than one embryo in the Day three and the Day two intervention groups. Of the eight trials reporting the outcome of multiple pregnancy, between two to four embryos were transferred per cycle. The evidence in this review is therefore not readily transferable to current practice in most fertility settings.

Although it was not a prespecified analysis, we identified that Day three embryo transfer was associated with a reduced clinical pregnancy rate compared with Day two embryo transfer in women who were poor responders. This may warrant further investigation.

Quality of the evidence

The included studies could be compromised by the low level of methodological quality. Allocation concealment was poorly reported and blinding was not possible (although this criterion is unlikely to affect pregnancy outcomes). Blinding of outcome assessors was not reported. Only three of 15 included studies reported live births. Using the GRADE approach, we judged that the quality of the evidence ranged from moderate to very low (Summary of findings for the main comparison), with the main reasons for downgrading the evidence being unclear risk of bias, inconsistency, imprecision and publication bias.

Potential biases in the review process

We believe that we have conducted a comprehensive and systematic search of the literature. There have been no date or language restrictions and we have searched for unpublished as well as published evidence. The funnel plot (Figure 4) indicates that there were three studies that appeared to be outliers (Abdelmassih 1998; Bahceci 2006; Chen 1999). These studies all reported on embryo transfer following ICSI. Subgroup analysis looking at the method of assisted reproductive technology used did not identify any differential effect between ICSI and IVF.

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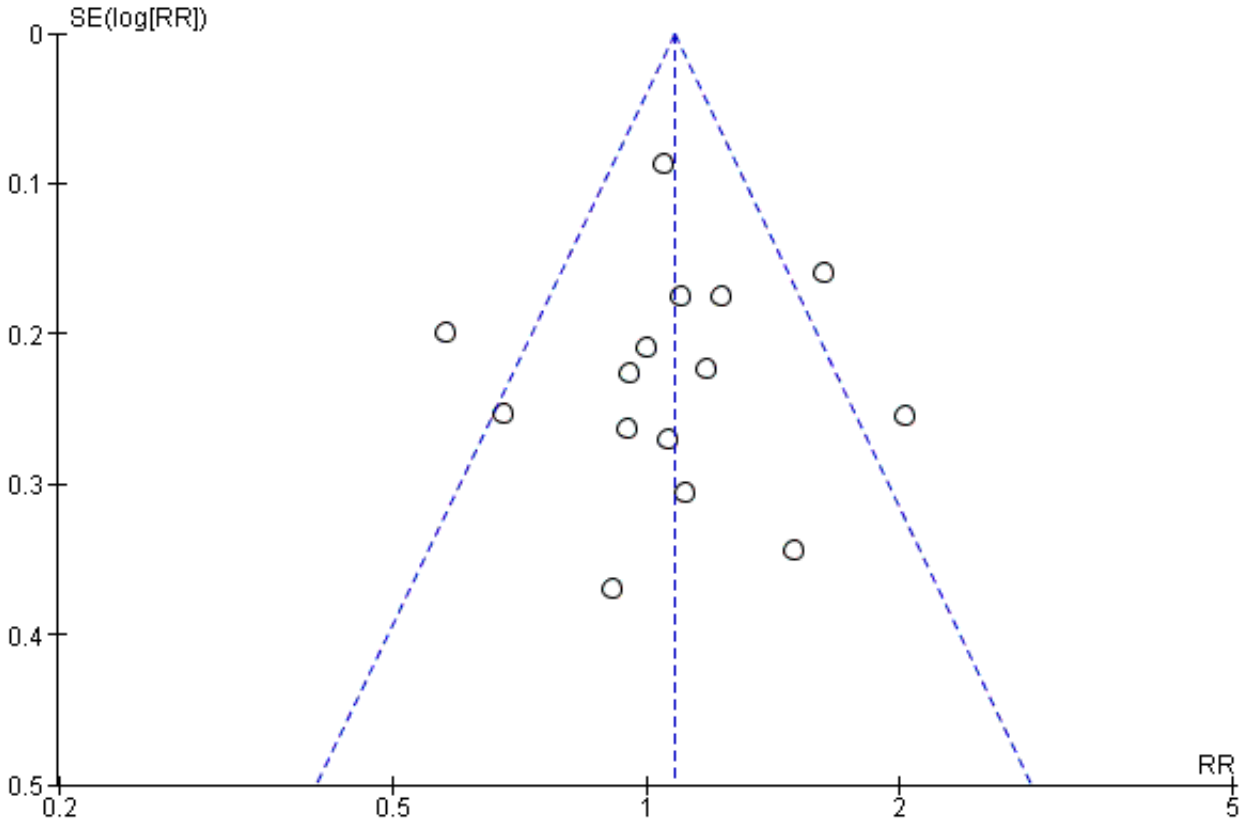
Figure 4. Funnel plot of comparison: 1 Day 3 versus Day 2 Embryo Transfer, outcome: 1.3 Clinical pregnancy rate per woman.



Agreements and disagreements with other studies or reviews

We could not identify any other systematic reviews that have evaluated the comparison of Day three versus Day two embryo transfer.

AUTHORS' CONCLUSIONS

Implications for practice

There is insufficient evidence to demonstrate an increase in pregnancy success with Day three embryo transfer. Any further trials comparing these timings of embryo transfer (Day three versus Day two) are unlikely to alter the findings and we suggest that this review no longer be updated. Many of the trials included in this review have used outdated techniques that include stimulation, laboratory technology and number of embryos transferred. We would direct the reader to the Glujovsky 2016 Cochrane Review comparing Day 2/3 with day 5/6 embryo transfer.

Implications for research

To date, the cumulative data from the trials selected for review have not reached the optimal information size required to adequately test the null hypothesis. Given the estimates of the treatment effect observed in the selected trials, it is unlikely that the magnitude of the summary treatment effect observed (of no significant difference) will be altered by adding more trials to this review; only the precision of this estimate would be improved. We do not believe that any further trials comparing Day three with Day two embryo transfer would alter the results of this review. If further trials should be conducted they should examine the comparison between Day 2/3 transfer with Day five transfer in poor responders or in couples with poor embryo development. These future trials should report on live birth as a primary outcome. We suggest that there is no further need to continue updating this review.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Abdelmassih 1998

Methods	Multicentre (2) RCT
Participants	Number of women randomised: 205 (cycles) all women analysed Inclusion criteria: women undergoing ET following ICSI Exclusion criteria: none Mean age: Day 3 - 35.6, Day 2 - 35.3 years Infertility diagnosis: n/s Duration of infertility: n/s No. previous treatment cycles: n/s Timing: 1997 Location: Brazil and Chile
Interventions	Treatment groups: Day 3 - 72-hour post-OR (n = 103), Day 2 - 48-hour post-OR (n = 102) Ovarian stimulation protocol: n/s Proportion IVF/ICSI: all ICSI Embryo culture media: n/s Mean no. embryos transferred per cycle: 4.0 both groups Fresh embryo transfer method: n/s
Outcomes	Clinical pregnancy (fetal heart beat at 6 - 8 weeks), Miscarriage
Notes	ITT analysis: yes Sample size calculation: no

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	No details but unlikely to have occurred. Would probably not influence pregnancy outcomes
Blinding of outcome assessment (detection bias)	Unclear risk	No details

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Abdelmassih 1998 *(Continued)*

All outcomes		
Incomplete outcome data (attrition bias) All outcomes	Low risk	All women were analysed
Selective reporting (reporting bias)	High risk	Limited outcomes. Did not include ongoing pregnancy or live birth
Other bias	Low risk	Groups appear balanced, no evidence of other bias

Bahceci 2006

Methods	Parallel randomised controlled trial
Participants	Number of women randomised and analysed: 272 Inclusion criteria: women with ≤ 5 follicles (size > 13 mm) at the end of ovarian hyperstimulation (poor responders), and in whom only fresh ejaculated sperm was used for insemination Exclusion criteria: failed oocyte retrieval Mean (± SD) age : 36.5 ± 0.8 (Day 2), 36.6 ± 0.8 (Day 3) Infertility diagnosis: female factor (45%), male factor (24%), co-existing (11%), unexplained (19%) Duration of infertility: n/s No. previous cycles: both groups comparable but data not specified Location: Turkey Timing of trial: June – November 2004
Interventions	Treatment groups: Day 2 (n = 137), Day 3 (n = 135) Ovarian stimulation protocol: long protocol using GnRH agonist (Lucrin) and recombinant FSH (Gonal F); or microdose flare-up protocol using low dose oral contraceptive (Desolett, on previous menstrual cycle) followed by GnRH agonist (Lucrin, on Day 2) and gonadotrophin (on Day 3) Proportion IVF/ICSI: all ICSI Embryo culture media: Quinn's Cleavage media Mean number of embryos transferred per cycle: 2.0 (Day 2), 1.7 (Day 3) Fresh embryo transfer method: using Edwards-Wallace catheter under ultrasound guidance Frozen-thawed transfer: none Number of cycles/woman: 1
Outcomes	Implantation rates, Clinical pregnancy rates (sacs seen on ultrasound scan with rising serum bHCG, per oocyte retrieval), Ongoing pregnancy (beyond 12 weeks, per oocyte retrieval), Multiple pregnancy rates (per clinical pregnancy), Miscarriage rates (per clinical pregnancy)
Notes	ITT analysis: yes Sample size calculation: yes (n ≥ 133)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"table of random numbers"
Allocation concealment (selection bias)	Unclear risk	No details

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Bahceci 2006 *(Continued)*

Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up. Intention-to-treat analysis performed
Selective reporting (reporting bias)	High risk	No outcomes were prespecified
Other bias	Low risk	Groups were similar at baseline. No other risk of bias identified

Baruffi 2003

Methods	Parallel randomised controlled trial
Participants	Number of women randomised and analysed: 106 Inclusion criteria: women undergoing ET following ICSI with at least 1 excess embryo for freezing. Mean age: Day 3 - 32.7 ± 4.4, Day 2 - 33.1 ± 4.5 years Infertility diagnosis: n/s Duration of infertility: n/s No. previous cycles: first ICSI cycle for 44% of women Exclusion criteria: none Setting: Brazil Timing: Not stated
Interventions	Treatment groups: Day 3 (n = 53), Day 2 (n = 53) Ovarian stimulation protocol: GnRH agonist in the long luteal protocol plus recombinant FSH Proportion IVF/ICSI: all ICSI Embryo culture media: IVF-50 medium to Day 2, G1.2 medium to Day 3 Mean number of embryos transferred per cycle: Day 3 - 2.6 ± 0.8, Day 2 - 2.8 ± 0.7 (Maximum 4) Fresh embryo transfer method: n/s Number of cycles/woman: 1
Outcomes	Ongoing pregnancy (to 12 and 36 weeks), Clinical pregnancy (fetal heart beat at 6 - 8 wks), multiple pregnancy, miscarriage, ectopic pregnancy
Notes	Additional information obtained from author ITT analysis: yes Sample size calculation: no

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	List prepared by drawing lots (additional information from authors). Open randomisation list

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Baruffi 2003 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Open randomisation. Lack of binding is unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up reported. All women randomised are analysed
Selective reporting (reporting bias)	High risk	Outcomes were not clearly prespecified
Other bias	Low risk	Groups appear balanced at baseline

Caspi 1989

Methods	RCT
Participants	Number of women randomised and analysed: 34 Inclusion criteria: ≥ 4 embryos at 44 - 48 hours Mean age: Day 3 - 34 ± 4.0, Day 2 - 33 ± 5.2 years Infertility diagnosis: 44% tubal, 47% unexplained, 9% other Duration of infertility: Day 3 - 5.5 ± 3.1, Day 2 - 5.5 ± 2.8 years No. previous treatment cycles: n/s Exclusion criteria: none Location: Israel Timing: Not reported.
Interventions	Treatment groups: Day 3 - 68 - 72-hour post-insemination (n = 17), Day 2 - 44 - 48 hour post-insemination (n = 17) Ovarian stimulation protocol: long protocol GnRH agonist plus hMG, except hMG only in 4 cycles Proportion IVF/ICSI: all IVF Embryo culture media: n/s Number of embryos transferred per cycle: 4 in each cycle in both groups Fresh embryo transfer method: in 30 µl medium using Wallace catheter No. cycles per woman: 1
Outcomes	Clinical pregnancy (not defined), multiple pregnancy, miscarriage
Notes	ITT analysis: yes Sample size calculation: no

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"randomly and equally allocated" no other details provided

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Caspi 1989 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up. All data accounted for
Selective reporting (reporting bias)	High risk	Outcomes not prespecified
Other bias	Low risk	No evidence of other bias.

Chen 1999

Methods	RCT
Participants	Number of women randomised and analysed: 129 Inclusion criteria: women undergoing ET following ICSI Exclusion criteria: none Mean age: 35.1 ± 3.9 years Infertility diagnosis: n/s Duration of infertility: 2.9 years No. previous treatment cycles: none Location: Singapore
Interventions	Treatment groups: Day 3 (n = 68), Day 2 (n = 61) Ovarian stimulation protocol: GnRH agonist in the long luteal protocol plus urinary or recombinant FSH Proportion IVF/ICSI: all ICSI Embryo culture media: Medicult IVF medium to Day 2, then Medicult IVF or M3 medium to Day 3 No. embryos transferred per cycle: 3 in each cycle in both groups Fresh embryo transfer method: n/s No. cycles per woman: 1
Outcomes	Live birth, ongoing pregnancy (to 12 weeks), clinical pregnancy (gestational sac), multiple pregnancy, miscarriage, ectopic pregnancy
Notes	Additional information obtained from author ITT analysis: yes Sample size calculation: no

Risk of bias

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

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Chen 1999 *(Continued)*

Random sequence generation (selection bias)	Low risk	Random numbers table (additional information from authors)
Allocation concealment (selection bias)	Low risk	Sealed opaque envelopes (additional information from authors)
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up. All women randomised were analysed
Selective reporting (reporting bias)	High risk	No outcomes are prespecified, these data are presented in abstract form only
Other bias	High risk	Publication as a conference abstract only. No full publication could be found

De los Santos 2003

Methods	RCT
Participants	Number of women randomised: 969 cycles with 888 ET randomised Inclusion criteria: donor oocyte recipient Exclusion criteria: No details Mean age: n/s Infertility diagnosis: n/s Duration of infertility: n/s No. previous cycles: n/s Setting: Spain Timing of trial: July 1999 – May 2002
Interventions	Treatment groups: Day 2 vs Day 3 (prospective); Day 3 vs Day 6 (retrospective) Ovarian stimulation protocol: HRT protocol – leuproreline (Finecrine Depot) 3.75 mg im for pituitary desensitisation, followed by daily E2 valerate (Progynova) in increasing dosage from 2 mg (Day 1 - 8), 4 mg (Day 9 - 11), to 6 mg (Day 12 onwards), until donor oocytes became available. Micronised intravaginal progesterone (Progeffik) 800 mg/d given from Day 2 after egg donation until day of pregnancy test Proportion IVF/ICSI: n/s Embryo culture media: G1.2 (Vitrolife) under paraffin oil (Medicult). On Day 2, co-cultured with endometrial epithelial cells (1 ml IVF: CCM in 1:1 ratio) Mean number of embryos transferred per cycle: 2.6 (Day 2) & 2.7 (Day 3) Mean number of embryos transferred per transfer: 2.9 Fresh embryo transfer method: yes Frozen-thawed transfer: no
Outcomes	Mean no. oocytes, Implantation rate, Pregnancy rates, ongoing pregnancy, multiple pregnancy, ectopic pregnancy

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De los Santos 2003 *(Continued)*

Notes	969 cycles, 888 embryo transfers. No reporting of number of women studied ITT analysis: no Sample size calculation: no

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	No blinding but unlikely to affect pregnancy outcome
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	969 cycles with 888 randomised
Selective reporting (reporting bias)	High risk	Outcomes are not prespecified in the methods section.
Other bias	High risk	Data reported by cycles and not women randomised

DiBeradino 1998

Methods	RCT
Participants	Number of women randomised and analysed: 63 (cycles) Inclusion criteria: > 5 zygotes Exclusion criteria: none Mean age: n/s Infertility diagnosis: n/s Duration of infertility: n/s No. previous treatment cycles: n/s Location: Canada
Interventions	Treatment groups: Day 3 - 72-hour post-OR (n = 33), Day 2 - 48-hour post-OR (n = 30) Ovarian stimulation protocol: n/s Proportion IVF/ICSI: all IVF Embryo culture media: n/s Mean no. embryos transferred per cycle: Day 3 - 2.5, Day 2 - 3.0 Fresh embryo transfer method: n/s
Outcomes	Clinical pregnancy (gestational sac), multiple pregnancy, ectopic pregnancy
Notes	Additional information obtained from author

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DiBeradino 1998 *(Continued)*

ITT analysis: yes
Sample size calculation: no

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	computer-generated random numbers table (additional information from author)
Allocation concealment (selection bias)	Low risk	sealed opaque envelopes (additional information from author)
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No blinding but unlikely to affect pregnancy outcome
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up. All women randomised were analysed
Selective reporting (reporting bias)	High risk	Outcomes were not prespecified in this conference abstract
Other bias	High risk	Publication as a conference abstract only. No full publication could be found

Ertzeid 1999

Methods	RCT
Participants	Number of women randomised: 325 (cycles), analysed 321 Inclusion criteria: ≥ 1 zygote Exclusion criteria: male factor Mean age: Day 3 - 33.7 ± 3.9, Day 2 - 33.6 ± 3.9 years Infertility diagnosis: 62% tubal, 18% unexplained, 8% endometriosis, 13% other, including ovulatory disorders Primary infertility: 86% vs 84% Duration of infertility: n/s No. previous treatment cycles: n/s Location: Norway Timing: 1993 - 94
Interventions	Treatment groups: Day 3 (n = 160), Day 2 (n = 165) Ovarian stimulation protocol: GnRH agonist in the long luteal protocol plus hMG Proportion IVF/ICSI: all IVF Embryo culture media: universal IVF medium to Day 2, M3 medium to Day 3 Mean no. embryos transferred per cycle: Day 3: 2.39 ± 0.57, Day 2: 2.19 ± 0.68 (maximum 3) Fresh embryo transfer method: n/s

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Ertzeid 1999 *(Continued)*

Outcomes	Live birth, ongoing pregnancy (to 12 wks), clinical pregnancy (gestational sac), multiple birth, miscarriage (before and after 12 wks), ectopic pregnancy
Notes	Additional information obtained from author ITT analysis: no, but data on exclusions available Sample size calculation: yes, but based on implantation, not pregnancy

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"computer-based block randomization"
Allocation concealment (selection bias)	Low risk	sealed opaque envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No blinding but unlikely to affect pregnancy outcome
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	Excluded post-randomisation: 4 - single fertilised oocyte, but no further embryo development. No losses to follow-up
Selective reporting (reporting bias)	Low risk	No evidence of reporting bias
Other bias	Low risk	Groups were balanced at baseline. No other evidence of risk of bias

Laverge 2001

Methods	RCT
Participants	Number of women randomised and analysed: 746 Inclusion criteria: ≥ 7 normally fertilised oocytes. ICSI in those with previous failed IVF/oligoasthenoteratozoospermia Mean age: Day 3 - 31.3 ± 4.2, Day 2 - 31.4 ± 4.1 years Infertility diagnosis: 61% male factor, 16% female factor, 18% combined male and female factors, 5% unexplained Duration of infertility: 3.8 years No. previous treatment cycles: 58% of women in first cycle Exclusion criteria: none Location: Belgium Timing of trial: 1995 - 97
Interventions	Treatment groups: IVF: Day 3 (n = 59), Day 2 (n = 61); ICSI: Day 3 (n = 313), Day 2 (n = 313) Ovarian stimulation protocol: short protocol GnRH analogue plus hMG Proportion IVF/ICSI: 16%/84% Embryo culture medium: Earle's balanced salt solution plus 0.4% human serum albumin

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Laverge 2001 *(Continued)*

	Mean no. embryos transferred per cycle: IVF: Day 3 - 2.41 ± 0.75, Day 2 - 2.33 ± 0.60; ICSI: Day 3 - 2.50 ± 0.86, Day 2 - 2.50 ± 0.85. Usually 2 embryos transferred, but maximum 3 embryos if age > 38 yrs, 2 previous unsuccessful cycles, or embryos poor quality Fresh embryo transfer method: n/s No. cycles per woman: 1
Outcomes	Live birth, ongoing pregnancy (not defined), clinical pregnancy (gestational sac at 6 weeks), multiple pregnancy, miscarriage, ectopic pregnancy
Notes	Additional information obtained from author ITT analysis: yes Sample size calculation: yes

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Computer programme"
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details but unlikely due to timing of the intervention. Unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up
Selective reporting (reporting bias)	High risk	Outcomes were not clearly prespecified
Other bias	Low risk	None identified

Marsella 2005

Methods	RCT
Participants	Number of women randomised and analysed: 400 Inclusion criteria: age ≤ 40, single cycle treatment - IVF/ICSI, number of fertilised oocytes > 5 Mean age: n/s Infertility diagnosis: n/s Duration of infertility: n/s No. previous cycles: n/s Exclusion criteria: n/s Location: Italy Timing of trial: 2002 - 2004

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Marsella 2005 *(Continued)*

Interventions	Treatment groups: Day 2 vs Day 3 Ovarian stimulation protocol: pharmacological – not clearly described Proportion IVF/ICSI: n/s Embryo culture media: n/s Mean number of embryos transferred per cycle: n/s Fresh embryo transfer method: n/s Frozen-thawed transfer: none Number of cycles/woman: 1
Outcomes	Percentage of good quality cleaved embryos, Implantation rate, pregnancy rate
Notes	ITT analysis: no Sample size calculation: yes Unable to determine how many women were randomised to each group

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Random numbers' table"
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding and unlikely to have occurred. Unlikely to influence pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	It is unclear how many women were randomised to each group and if there are any missing data as no denominators are provided
Selective reporting (reporting bias)	High risk	This is a conference abstract only, it is not clear if other outcomes were recorded. Live birth and ongoing pregnancy are not reported
Other bias	High risk	This is a conference abstract only. No full publication has been identified

Nodar 2002

Methods	RCT
Participants	Number of women randomised and analysed: 174 Inclusion criteria: those willing to freeze excess embryos Mean age: IVF: Day 3 - 33.3 ± 0.8, Day 2 - 32.3 ± 0.8; ICSI: Day 3 - 31.8 ± 0.6, Day 2 - 31.7 ± 0.5 years Infertility diagnosis: n/s Duration of infertility: n/s No. previous treatment cycles: n/s Exclusion criteria: none Location: Argentina

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Nodar 2002 *(Continued)*

	Timing of trial: 1999
Interventions	Treatment groups: IVF: Day 3 - 72-hour post-insemination (n = 37), Day 2 - 48-hour post-insemination (n = 35); ICSI: Day 3 (n = 48), Day 2 (n = 54) Ovarian stimulation protocol: n/s Proportion IVF/ICSI: 41%/59% Embryo culture media: n/s Mean no. embryos transferred per cycle: IVF: Day 3 - 3.3 ± 0.6, Day 2 - 3.2 ± 0.6; ICSI: Day 3 - 3.0 ± 0.6, Day 2 - 3.3 ± 0.6 Fresh embryo transfer method: n/s No. cycles per woman: 1
Outcomes	Pregnancy (not defined), miscarriage
Notes	ITT analysis: yes Sample size calculation: no

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"randomized"; no other details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect the pregnancy outcome
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No losses to follow-up in abstract but not clear if these are the full trial data
Selective reporting (reporting bias)	Unclear risk	Report the outcomes specified in the abstract but not clear if this is all outcomes associated with the trial
Other bias	High risk	Conference abstract only. No full paper publication identified

Nordstrom 1995

Methods	RCT
Participants	Number of women randomised and analysed: 107 Inclusion criteria: n/s Age: n/s Infertility diagnosis: n/s Duration of infertility: n/s No. previous treatment cycles: n/s Exclusion criteria: none

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Nordstrom 1995 *(Continued)*

	Location: Finland
Interventions	Treatment groups: Day 3 (n = 48), Day 2 (n = 36) Ovarian stimulation protocol: long protocol GnRH analogue plus hMG Proportion IVF/ICSI: probably all IVF Embryo culture media: conventional IVF medium to Day 2, M3 to Day 3 Mean no. embryos transferred per cycle: n/s Fresh embryo transfer method: n/s No. cycles per woman: 1
Outcomes	Pregnancy (not defined)
Notes	ITT analysis: yes Sample size calculation: no

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"randomly"; no other details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up. Women randomised were analysed
Selective reporting (reporting bias)	High risk	Outcomes were not prespecified
Other bias	High risk	Publication as a conference abstract only. No full publication could be found

Pantos 2004

Methods	RCT
Participants	Number of women randomised: 243; analysed: 81 (Day 2), 81 (Day 3), 81 (Day 6) Inclusion criteria: primary infertility, maternal age ≤ 40 years, previous unsuccessful ART attempts ≤ 3, available embryos for transfer Mean (± SD) age: 32.4 ± 6.3 (Day 2), 31.3 ± 5.2 (Day 3) Infertility diagnosis: male factor (52%), combined factor (8%), tubal factor (7%), anovulation (14%), endometriosis (8%), unexplained (12%) Duration of infertility: 1.45 ± 0.44 (Day 2), 1.52 ± 0.42 (Day 3) No. previous cycles: 0.84 ± 0.89 (Day 2), 0.90 ± 1.02 (Day 3) Exclusion criteria: consistent with inclusion criteria

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Pantos 2004 *(Continued)*

	Location: Greece Timing of trial: June to December 2002
Interventions	Treatment groups: Day 2 (n = 81), Day 3 (n = 81) Ovarian stimulation protocol: long or short protocol, using GnRH agonist and recombinant FSH Proportion IVF/ICSI: 42% (Day 2), 40% (Day 3) Embryo culture media: sequential media from Vitrolife (IVF-20, G1.2, G2.2) Mean (± SD) number of embryos transferred per cycle: 4 ± 1.51 (Day 2), 4.01 ± 1.51 (Day 3) Fresh embryo transfer method: n/s. no donated oocytes used Frozen-thawed transfer: none Number of cycles/woman: 1
Outcomes	Embryos cryopreserved: Proportion: 49.38% (Day 2), 48.14% (Day 3) Mean (± SD) number of frozen embryos: 7.42 ± 3.99 (Day 2), 6.38 ± 3.87 (Day 3) Implantation rates (sacs per transferred embryos): 15.74% (Day 2), 16% (Day 3) (P ≥ 0.9) Clinical pregnancy rates (as detected by ultrasound per ET): 46.91% (Day 2), 48.14% (Day 3) (P > 0.15) Ongoing pregnancy (beyond 1st trimester per ET): 40.74% (Day 2), 43.2% (Day 3) Multiple pregnancy rates (per clinical pregnancies): 28.94% (Day 2), 30.76% (Day 3) Miscarriage rates (per clinical pregnancies): 13.15% (Day 2), 10.25% (Day 3) OHSS: 1.2% (Day 2), 0% (Day 3) No other complications observed other than OHSS and miscarriage
Notes	ITT analysis: no Sample size calculation: yes – α = 0.05, power 80%, difference in clinical pregnancy rates predetermined at 14% Additional information obtained

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random numbers table
Allocation concealment (selection bias)	Unclear risk	Sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up were reported
Selective reporting (reporting bias)	High risk	Outcomes were not prespecified
Other bias	Low risk	Groups appeared to be balanced at baseline, no other risk of bias identified

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Suzuki 2004

Methods	RCT
Participants	Number of women randomised: 114; analysed: 36 (31.6%) Inclusion criteria: age < 40, 1st treatment cycle Mean (± SD) age: 32.8 ± 3.4 (Day 2), 32.5 ± 2.7 (Day 3) Infertility diagnosis: primary (64%); tubal (39%), male (22%), immunological (5.5%), unexplained (33%) Duration (± SD) of infertility: 3.8 ± 2.7 (Day 2), 4.2 ± 2.3 (Day 3) No. previous cycles: 0 Exclusion criteria: good-quality embryos ≤ 3 (post-randomisation), no consent for elective transfer of 2 good-quality embryos Location: Japan Timing of trial: August 1999 to August 2002
Interventions	Treatment groups: Day 2 (n = 75 women, 24 ET), Day 3 (n = 39 women, 12 ET) Ovarian stimulation protocol: long protocol with GnRH agonist plus 3 days of 300 IU/d of FSH (Fertinom P), followed by 150 IU/d of hMG (Humegon); hCG (Mochida) for ovulation trigger Proportion IVF/ICSI: n/s Embryo culture media: n/s Mean number of embryos transferred per cycle: 2 Fresh embryo transfer method: n/s Frozen-thawed transfer: none Number of cycles/woman: 1
Outcomes	Cancellation rate, embryos cryopreserved, implantation rate, clinical pregnancy rates, multiple pregnancy
Notes	ITT analysis: no Sample size calculation: no

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"randomized", no other details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	High risk	Number of women randomised: 114; analysed: 36 (31.6%). High post-randomisation exclusion rate: 68.4%
Selective reporting (reporting bias)	High risk	Outcomes were not prespecified.

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Suzuki 2004 *(Continued)*

Other bias	Low risk	Groups appeared to be balanced at baseline. No other sources of risk of bias identified

Urman 1998

Methods	RCT
Participants	Number of women randomised and analysed: 161 Inclusion criteria: women having ET after ICSI Exclusion criteria: none Mean age: Day 3 - 32.8 ± 3.8, Day 2 - 32.2 ± 4.8 years Infertility diagnosis: n/s Duration of infertility: Day 3 - 10.3 ± 5.4, Day 2 - 8.4 ± 5.1 years No. previous treatment cycles: n/s Location: Turkey
Interventions	Treatment groups: Day 3 (n = 80), Day 2 (n = 81) Ovarian stimulation protocol: long protocol GnRH agonist plus hMG and FSH Proportion of cycles using IVF/ICSI: all ICSI Embryo culture medium: S2 Mean no. embryos transferred per cycle: Day 3 - 3.8 ± 0.6, Day 2 - 4.0 ± 0.4 (maximum 4) Fresh embryo transfer method: Wallace or difficult Frydman catheter according to previous trial transfer No. cycles per woman: 1
Outcomes	Clinical pregnancy (not defined)
Notes	ITT analysis: yes Sample size calculation: no

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"randomized"; no other details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No evidence of blinding but unlikely to affect pregnancy outcomes
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up, women randomised were analysed

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Urman 1998 *(Continued)*

Selective reporting (reporting bias)	High risk	No outcomes were prespecified in this conference abstract
Other bias	High risk	Publication as a conference abstract only. No full publication could be found

ET: embryo transfer
FSH: follicle stimulating hormone
ICSI: intracytoplasmic sperm injection
ITT: intention-to-treat
IVF: in vitro fertilisation
GnRH: gonadotrophin releasing hormone
hMG: human menopausal gonadotrophins
n/s: not stated
OR: ooycte retrieval
RCT: randomised controlled trial

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

Study	Reason for exclusion
Aboulghar 2003	Quasi-randomised by day of the week
BarbarinoMonier 2002	Retrospective comparative study
Carrillo 1998	Retrospective comparative study
Cowan 1997	Not stated as randomised
Dawson 1995	Retrospective comparative study
Fussell 1999	Not stated as randomised
Galan 2001	Retrospective comparative study
Gonen 1999	Retrospective comparative study
Goto 1994	Retrospective comparative study
Huisman 1994a	Quasi-randomised by day of the week
Huisman 1994b	Quasi-randomised by day of the week
Koo 1999	Quasi-randomised by day of the week
Margreiter 2003	Trial compares Day 1 versus Day 2 and 3 versus Day 4 and 5
Marsella 2001	Quasi-randomised by date of birth
Pires 2000	Participants had more than 1 cycle of treatment in the trial, and we were unable to obtain the results from only the first cycles
Racowsky 1998	Retrospective comparative study
Ramey 1997	Retrospective comparative study

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Study	Reason for exclusion
Van Os 1989	Quasi-randomised by day of the week
Wilson 1996	Retrospective comparative study

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

Amireh 1998

Methods	Randomised trial but also states retrospective
Participants	Couples undergoing ICSI
Interventions	Day 3 versus Day 2 embryo transfer
Outcomes	No data in abstract. Outcomes are unclear.
Notes	Authors of trial were contacted but no response.

Shahine 2011

Methods	Parallel, randomised controlled trial
Participants	386 women eligible and 251 randomised Mean age (SD) 39.9 ± 3.0 (Day 2), 39.2 ± 4.0 (Day 3) Inclusion criteria: Undergoing fresh, autologous IVF treatment, at least 1 fertilised oocyte and an intent to transfer all available embryos. Poor responder Exclusion criteria: Planning pre-implantation genetic screening, not consented, no oocytes retrieved or no fertilisation Timing: January 2007 to March 2009 Setting: University IVF program, California, USA
Interventions	1 cycle of treatment only Mean number of embryos transferred 2.1 ± 2.8 (Day 2), 2.4 ± 2.6 (Day 3) Ovarian stimulation protocol based on physician preference Trans-abdominal ultrasound guided embryo transfer Day 2 (n = 123) versus trans-abdominal ultrasound guided embryo transfer Day 3 (n = 128) Tefcat or Echotip Softpass catheter used
Outcomes	Biochemical pregnancy, clinical pregnancy, spontaneous pregnancy loss, ongoing pregnancy, live birth, implantation rate
Notes	Numbers for clinical pregnancy and miscarriage do not add up and may be entered wrongly in the published table. Authors to be contacted for confirmation

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DATA AND ANALYSES

Comparison 1. Day 3 versus Day 2 Embryo Transfer

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth rate per woman	3	1200	Risk Ratio (M-H, Fixed, 95% CI)	1.05 [0.89, 1.23]
1.1 IVF	2	445	Risk Ratio (M-H, Fixed, 95% CI)	0.94 [0.68, 1.31]
1.2 ICSI	2	755	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.90, 1.31]
2 Ongoing pregnancy rate per woman	6	1740	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.85, 1.12]
2.1 IVF	3	511	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.73, 1.30]
2.2 ICSI	5	1229	Risk Ratio (M-H, Fixed, 95% CI)	0.97 [0.84, 1.14]
3 Clinical pregnancy rate per woman	12	2461	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.98, 1.19]
3.1 IVF	7	764	Risk Ratio (M-H, Fixed, 95% CI)	1.01 [0.83, 1.23]
3.2 ICSI	8	1697	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.99, 1.23]
4 Multiple pregnancy rate per woman	8	1837	Risk Ratio (M-H, Fixed, 95% CI)	1.12 [0.86, 1.44]
4.1 IVF	5	608	Risk Ratio (M-H, Fixed, 95% CI)	0.85 [0.51, 1.40]
4.2 ICSI	5	1229	Risk Ratio (M-H, Fixed, 95% CI)	1.24 [0.92, 1.67]
5 High order multiple pregnancy per woman (triplets)	7	1565	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.38, 2.07]
6 Miscarriage rate per woman	9	2153	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [0.84, 1.60]
7 Ectopic pregnancy rate per woman	6	1531	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.29, 3.40]

Analysis 1.1. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 1 Live birth rate per woman.

Study or subgroup	Day 3 n/N	Day 2 n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 IVF					
Ertzeid 1999	36/160	30/165		15.65%	1.24[0.8,1.91]
		Favours Day 2	0.5 0.7 1 1.5 2	Favours Day 3	

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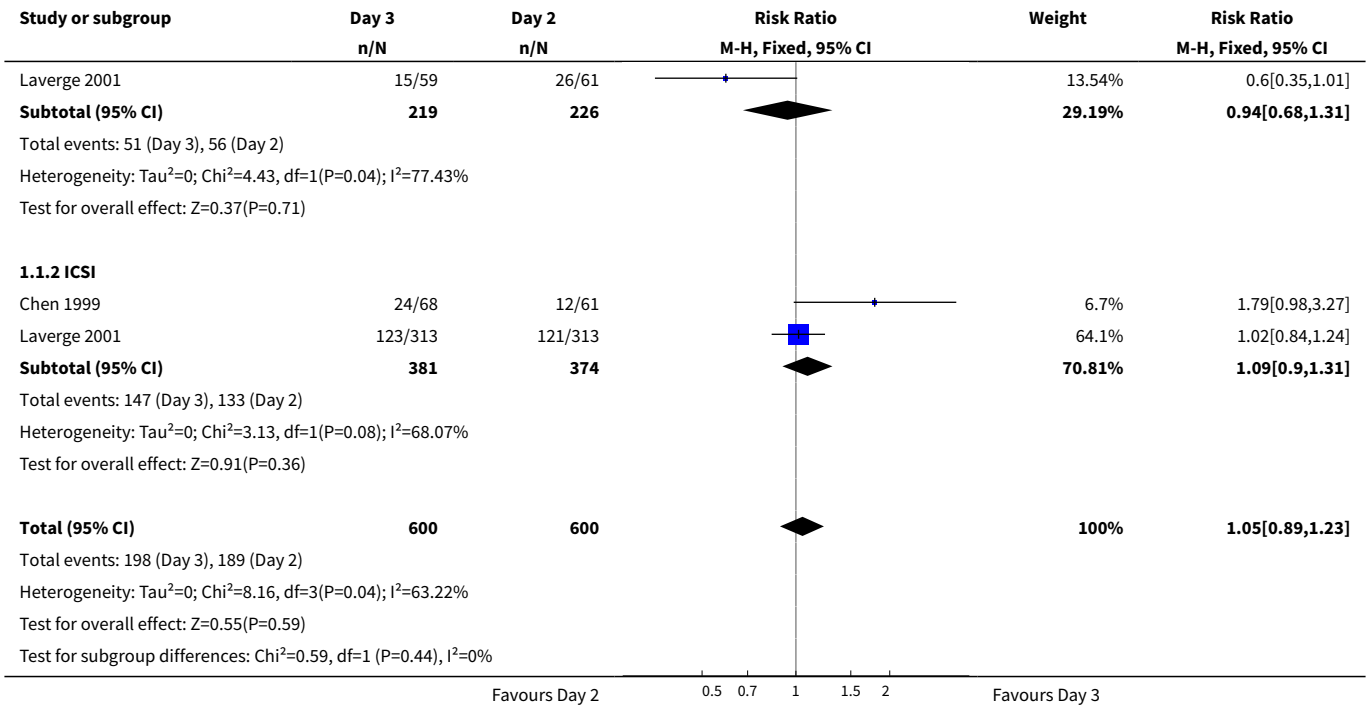
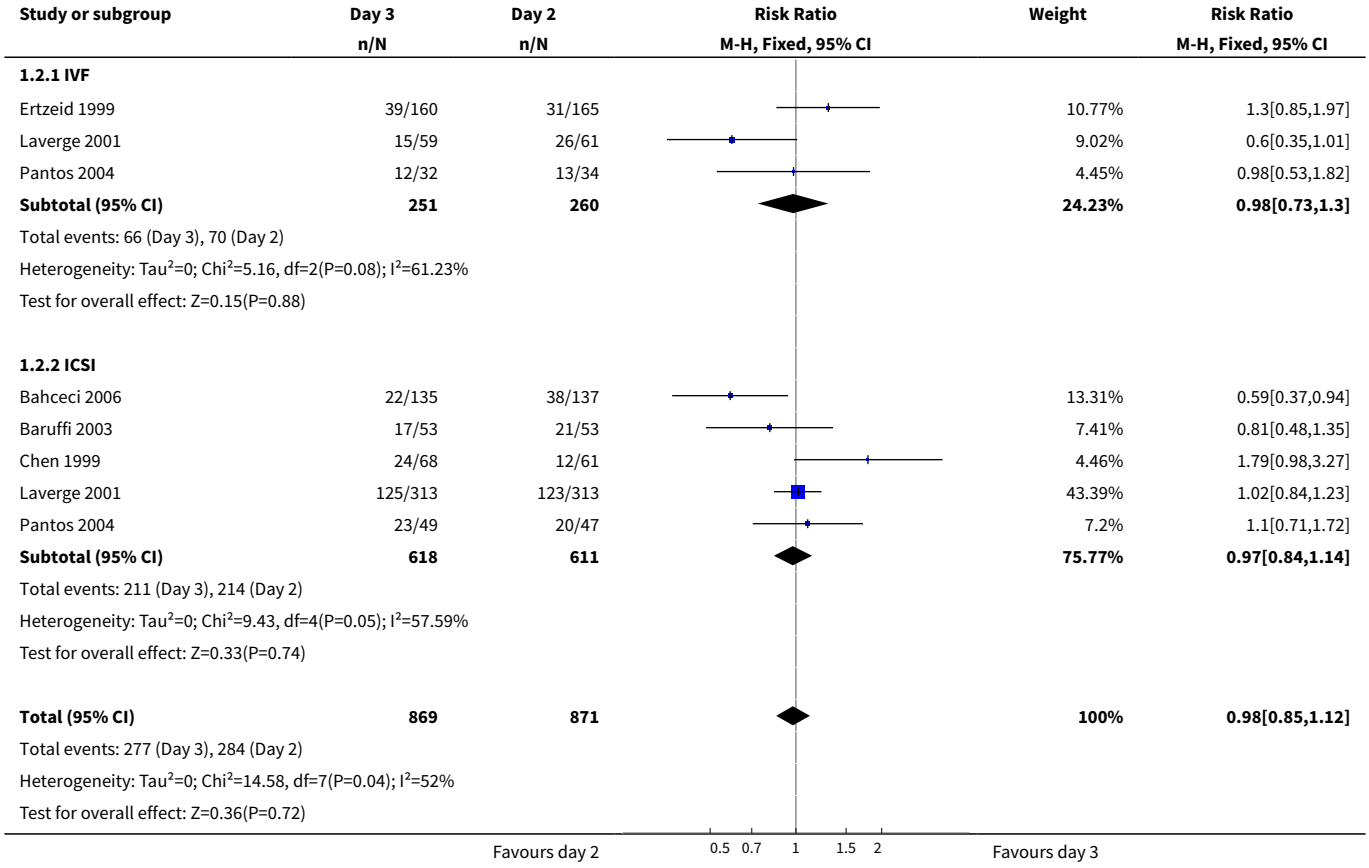


Analysis 1.2. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 2 Ongoing pregnancy rate per woman.



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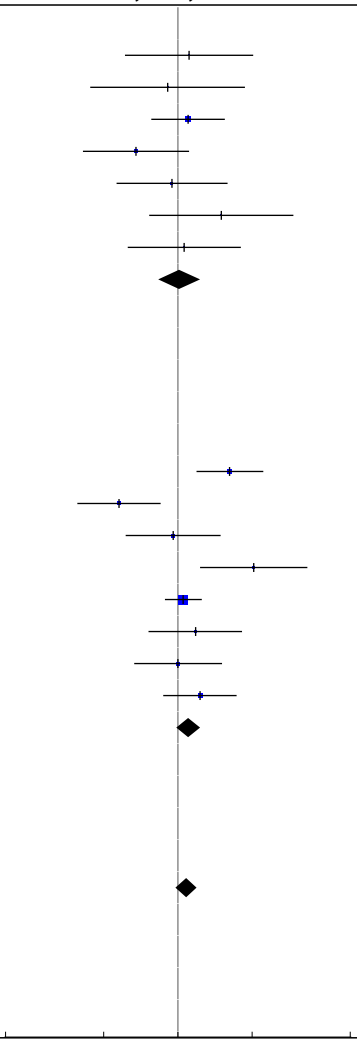
Study or subgroup	Day 3 n/N	Day 2 n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Test for subgroup differences: Chi²=0, df=1 (P=0.98), I²=0%					

Favours day 2 | Favours day 3

Analysis 1.3. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 3 Clinical pregnancy rate per woman.

Study or subgroup	Day 3 n/N	Day 2 n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.3.1 IVF					
Caspi 1989	10/17	9/17		1.9%	1.11[0.61,2.02]
DiBeradino 1998	10/33	10/30		2.21%	0.91[0.44,1.87]
Ertzeid 1999	48/160	45/165		9.36%	1.1[0.78,1.55]
Laverge 2001	17/59	26/61		5.4%	0.68[0.41,1.11]
Nodar 2002	16/37	16/35		3.48%	0.95[0.56,1.59]
Nordstrom 1995	18/48	9/36		2.17%	1.5[0.76,2.94]
Pantos 2004	15/32	15/34		3.07%	1.06[0.63,1.8]
Subtotal (95% CI)	**386**	**378**		**27.6%**	**1.01[0.83,1.23]**
Total events: 134 (Day 3), 130 (Day 2)					
Heterogeneity: Tau²=0; Chi²=4.36, df=6(P=0.63); I²=0%					
Test for overall effect: Z=0.1(P=0.92)					
1.3.2 ICSI					
Abdelmassih 1998	59/103	36/102		7.64%	1.62[1.19,2.22]
Bahceci 2006	29/135	51/137		10.7%	0.58[0.39,0.85]
Baruffi 2003	22/53	23/53		4.86%	0.96[0.61,1.49]
Chen 1999	34/68	15/61		3.34%	2.03[1.23,3.35]
Laverge 2001	147/313	140/313		29.58%	1.05[0.89,1.25]
Nodar 2002	23/48	22/54		4.38%	1.18[0.76,1.82]
Pantos 2004	24/49	23/47		4.96%	1[0.67,1.51]
Urman 1998	40/80	33/81		6.93%	1.23[0.87,1.73]
Subtotal (95% CI)	**849**	**848**		**72.4%**	**1.1[0.99,1.23]**
Total events: 378 (Day 3), 343 (Day 2)					
Heterogeneity: Tau²=0; Chi²=23.73, df=7(P=0); I²=70.5%					
Test for overall effect: Z=1.7(P=0.09)					
Total (95% CI)	**1235**	**1226**		**100%**	**1.08[0.98,1.19]**
Total events: 512 (Day 3), 473 (Day 2)					
Heterogeneity: Tau²=0; Chi²=28.78, df=14(P=0.01); I²=51.35%					
Test for overall effect: Z=1.49(P=0.14)					
Test for subgroup differences: Chi²=0.56, df=1 (P=0.45), I²=0%					



Favours Day 2 | Favours Day 3

Analysis 1.4. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 4 Multiple pregnancy rate per woman.

Study or subgroup	Day 3 n/N	Day 2 n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.4.1 IVF					
Caspi 1989	3/17	2/17		2.06%	1.5[0.29,7.87]

Favours Day 3 | Favours Day 2

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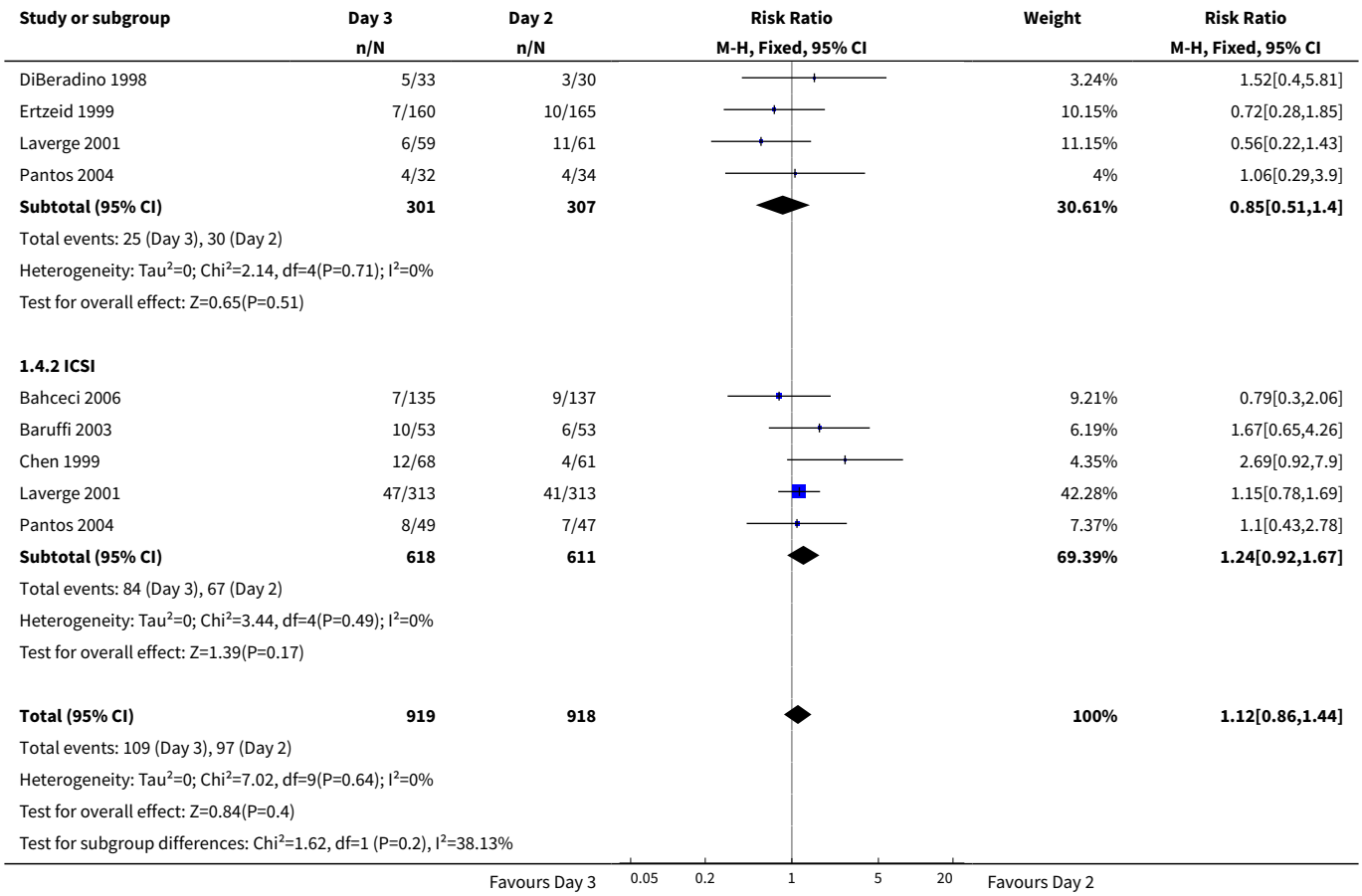
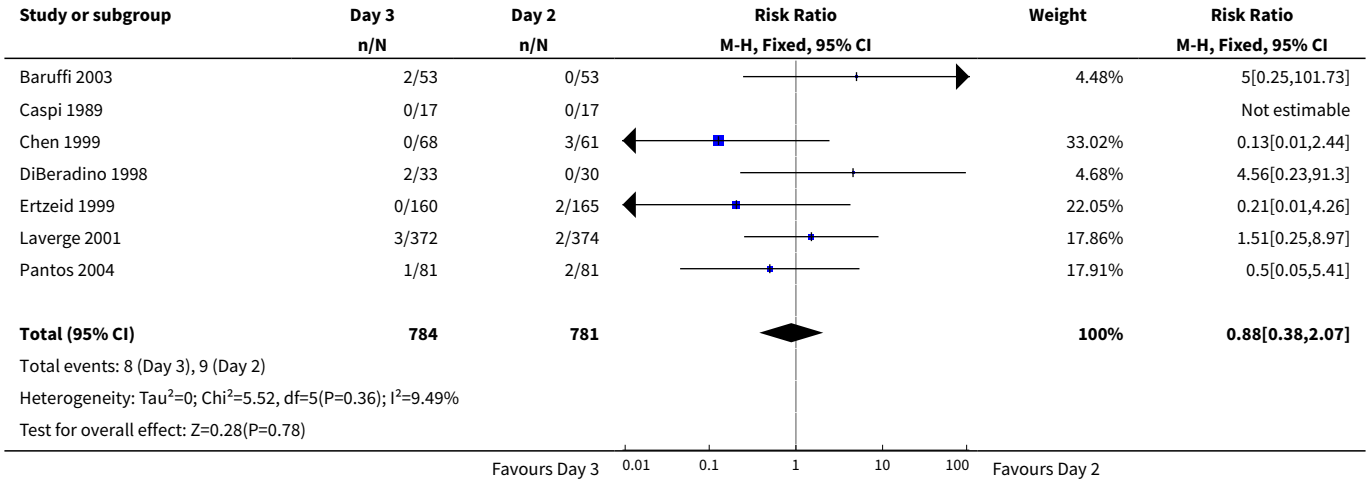


Analysis 1.5. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 5 High order multiple pregnancy per woman (triplets).



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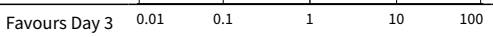
Analysis 1.6. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 6 Miscarriage rate per woman.

Study or subgroup	Day 3 n/N	Day 2 n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Abdelmassih 1998	8/103	2/102		3.16%	3.96[0.86,18.2]
Bahceci 2006	7/135	13/137		20.27%	0.55[0.22,1.33]
Baruffi 2003	5/53	2/53		3.14%	2.5[0.51,12.32]
Caspi 1989	3/17	3/17		4.71%	1[0.23,4.27]
Chen 1999	10/68	3/61		4.97%	2.99[0.86,10.36]
Ertzeid 1999	11/160	13/165		20.1%	0.87[0.4,1.89]
Laverge 2001	22/372	16/374		25.06%	1.38[0.74,2.59]
Nodar 2002	4/85	7/89		10.74%	0.6[0.18,1.97]
Pantos 2004	4/81	5/81		7.85%	0.8[0.22,2.87]
Total (95% CI)	**1074**	**1079**		**100%**	**1.16[0.84,1.6]**
Total events: 74 (Day 3), 64 (Day 2)					
Heterogeneity: Tau^2=0; Chi^2=10.74, df=8(P=0.22); I^2=25.53%					
Test for overall effect: Z=0.89(P=0.37)					

Analysis 1.7. Comparison 1 Day 3 versus Day 2 Embryo Transfer, Outcome 7 Ectopic pregnancy rate per woman.

Study or subgroup	Day 3 n/N	Day 2 n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Baruffi 2003	0/53	0/53			Not estimable
Chen 1999	0/68	0/61			Not estimable
DiBeradino 1998	1/33	1/30		20.89%	0.91[0.06,13.9]
Ertzeid 1999	1/160	2/165		39.27%	0.52[0.05,5.63]
Laverge 2001	2/372	1/374		19.89%	2.01[0.18,22.08]
Pantos 2004	1/81	1/81		19.94%	1[0.06,15.72]
Total (95% CI)	**767**	**764**		**100%**	**0.99[0.29,3.4]**
Total events: 5 (Day 3), 5 (Day 2)					
Heterogeneity: Tau^2=0; Chi^2=0.63, df=3(P=0.89); I^2=0%					
Test for overall effect: Z=0.01(P=0.99)					



APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility specialised register search strategy

Procite platform

from inception until 26 April 2016

Keywords CONTAINS "Embryo Transfer" or "Embryo Transfer-uterine" or "ET" or Title CONTAINS "Embryo Transfer" or "Embryo Transfer-uterine" or "ET"

AND

Keywords CONTAINS "day 2" or "day 3 embryo transfer" or "day of transfer" or Title CONTAINS "day 2" or "day 3 embryo transfer" or "day of transfer" (173 hits)

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Appendix 2. CENTRAL CRSO search strategy

CRSO Online web platform

from inception until 26 April 2016

#1 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES 886
#2 (Embryo* adj5 Transfer*):TI,AB,KY 1925
#3 #1 OR #2 1925
#4 (day* adj3 "2"):TI,AB,KY 14417
#5 (day* adj3 two):TI,AB,KY 3199
#6 (day* adj3 three):TI,AB,KY 2515
#7 (day* adj3 "3"):TI,AB,KY 15119
#8 #4 OR #5 OR #6 OR #7 30427
#9 #3 AND #8 390

Appendix 3. MEDLINE search strategy

Ovid platform

from inception until 26 April 2016

1 Embryo Transfer/ (13410)
2 (Embryo$ adj5 Transfer$).tw. (15178)
3 ET.tw. (188604)
4 or/1-3 (206607)
5 (day$ adj3 "2").tw. (143512)
6 (day$ adj3 two).tw. (39556)
7 (day$ adj3 "3").tw. (179633)
8 (day$ adj3 three).tw. (42820)
9 or/5-8 (356937)
10 4 and 9 (4916)
11 randomized controlled trial.pt. (414265)
12 controlled clinical trial.pt. (90584)
13 randomized.ab. (344222)
14 placebo.tw. (173750)
15 clinical trials as topic.sh. (176260)
16 randomly.ab. (247924)
17 trial.ti. (149665)
18 (crossover or cross-over or cross over).tw. (67109)
19 or/11-18 (1035983)
20 exp animals/ not humans.sh. (4230784)
21 19 not 20 (952660)
22 10 and 21 (612)

Appendix 4. Embase search strategy

Ovid platform

from inception until 26 April 2016

1 Embryo Transfer/ (23851)
2 (Embryo$ adj5 Transfer$).tw. (21815)
3 (blastocyst$ adj5 transfer$).tw. (3190)
4 or/1-3 (31468)
5 (day$ adj "2").tw. (39647)
6 (day$ adj two).tw. (5270)
7 (day$ adj "3").tw. (48193)
8 (day$ adj three).tw. (4358)
9 or/5-8 (91273)
10 4 and 9 (3368)
11 Clinical Trial/ (857207)
12 Randomized Controlled Trial/ (399695)
13 exp randomization/ (70353)

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14 Single Blind Procedure/ (21974)
15 Double Blind Procedure/ (127872)
16 Crossover Procedure/ (46851)
17 Placebo/ (273628)
18 Randomi?ed controlled trial$.tw. (134137)
19 Rct.tw. (20060)
20 random allocation.tw. (1514)
21 randomly allocated.tw. (24482)
22 allocated randomly.tw. (2100)
23 (allocated adj2 random).tw. (752)
24 Single blind$.tw. (17226)
25 Double blind$.tw. (160903)
26 ((treble or triple) adj blind$).tw. (541)
27 placebo$.tw. (231060)
28 prospective study/ (330844)
29 or/11-28 (1563394)
30 case study/ (37464)
31 case report.tw. (303937)
32 abstract report/ or letter/ (956873)
33 or/30-32 (1291336)
34 29 not 33 (1522524)
35 10 and 34 (643)

Appendix 5. PsycINFO search strategy

Ovid platform

from inception until 26 April 2016

1 exp reproductive technology/ (1554)
2 (Embryo$ adj5 Transfer$).tw. (148)
3 ET.tw. (108746)
4 or/1-3 (110313)
5 (day$ adj "2").tw. (2398)
6 (day$ adj two).tw. (353)
7 (day$ adj "3").tw. (2005)
8 (day$ adj three).tw. (228)
9 or/5-8 (4699)
10 4 and 9 (78)

WHAT'S NEW

Date	Event	Description
13 January 2017	Review declared as stable	We do not expect there to be further evidence published on this topic.

HISTORY

Protocol first published: Issue 3, 2003
Review first published: Issue 2, 2004

Date	Event	Description
17 November 2016	New citation required but conclusions have not changed	The inclusion of 5 new studies has not led to any change in the conclusions of this review. We will no longer update this review.

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Date	Event	Description
17 November 2016	New search has been performed	In 2016 we updated this review, adding 5 new studies (Bahceci 2006; De los Santos 2003; Marsella 2005; Pantos 2004; Suzuki 2004) and one study awaiting classification (Shahine 2011).
10 November 2008	Amended	Converted to new review format.
15 December 2003	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

In 2016 Julie Brown took over the lead authorship of the review update, amending the text in line with updated Cochrane requirements and restructured the analyses. She also completed all of the updated 'Risk of bias' tables and Summary of findings for the main comparison, which were previously not included in other published versions of this review.

Salim Daya and Phill Matson commented on and approved the final draft for the 2016 update.

Previous published versions:

C. Oatway: Took the lead in writing previous published versions, developed the background, objectives, selection criteria, search strategy and methods, description of studies and methodological quality sections, the discussion and conclusions. Extracted data and assessed included studies for methodological quality.

J. Gunby: Initiated and conceptualised the original protocol, commented on drafts and contributed to the initial objectives, selection criteria, methods, description of studies and methodological quality sections. Extracted data and assessed included studies for methodological quality.

S. Daya: Initiated and conceptualised the original protocol, and commented on final draft.

DECLARATIONS OF INTEREST

JB has no conflicts of interest to declare.

SD has no conflicts of interest to declare.

PM has no conflicts of interest to declare.

SOURCES OF SUPPORT

Internal sources

- New Source of support, Other.

  There has been no support for the preparation of this review

External sources

- None, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the 2016 update we removed the outcome 'Complication rate'. We also removed analyses that related to outcomes per embryo transfer rather than per woman randomised, because of the increased risk of bias from inaccurate units of analysis.

Due to the suggestion that Day three and Day two transfer may be relevant for specific groups of women undergoing fertility treatment, we included, where data were available, an analysis that had not been prespecified, reporting on ongoing pregnancy, clinical pregnancy and multiple pregnancy in poor responders.

Also for the 2016 update, we analysed the data using Mantel-Haenszel risk ratios.

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INDEX TERMS

Medical Subject Headings (MeSH)

*Embryo Transfer; *Fertilization in Vitro; *Sperm Injections, Intracytoplasmic; Abortion, Spontaneous [epidemiology]; Embryonic Development; Live Birth [epidemiology]; Oocyte Retrieval; Pregnancy Outcome; Pregnancy Rate; Randomized Controlled Trials as Topic; Time Factors

MeSH check words

Female; Humans; Pregnancy

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