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Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection (Review)

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[Intervention Review]

Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection

Zabeena Pandian[1], Jane Marjoribanks[2], Ozkan Ozturk[3], Gamal Serour[4], Siladitya Bhattacharya[1]

[1]Obstetrics and Gynaecology, Aberdeen Maternity Hospital, Aberdeen, UK. [2]Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand. [3]Academic Department of Obstetrics and Gynaecology, University College London Hospitals, London, UK. [4]The Egyptian IVF-ET Center, Al Azhar University, Cairo, Egypt

Contact address: Zabeena Pandian, Obstetrics and Gynaecology, Aberdeen Maternity Hospital, Foresterhill, Aberdeen, AB25 2ZD, UK. pandianzl@aol.com, ogy211@abdn.ac.uk.

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ABSTRACT

Background

Multiple embryo transfer during in vitro fertilisation (IVF) increases multiple pregnancy rates causing maternal and perinatal morbidity. Single embryo transfer is now being seriously considered as a means of minimising the risk of multiple pregnancy. However, this needs to be balanced against the risk of jeopardising the overall live birth rate.

Objectives

To evaluate the effectiveness and safety of different policies for the number of embryos transferred in couples who undergo assisted reproductive technology (ART).

Search methods

We searched the Cochrane Menstrual Disorders and Subfertility Group Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE and EMBASE, from inception to July 2013. We handsearched reference lists of articles, trial registers and relevant conference proceedings and contacted researchers in the field.

Selection criteria

We included randomised controlled trials (RCTs) comparing different policies for the number of embryos transferred following IVF or intra-cytoplasmic sperm injection (ICSI) in subfertile women. Studies of fresh or frozen and thawed transfer of one, two, three or four embryos at cleavage or blastocyst stage were eligible.

Data collection and analysis

Two review authors independently assessed trial eligibility and risk of bias and extracted the data. The overall quality of the evidence was graded in a summary of findings table.

Main results

Fourteen RCTs were included in the review (2165 women). Thirteen compared cleavage-stage transfers (2017 women) and two compared blastocyst transfers (148 women): one study compared both. No studies compared repeated single versus repeated multiple embryo transfer (SET).

Repeated SET versus DET

Repeated SET was compared with DET in three studies of cleavage-stage transfer. In these studies the SET group received either two cycles of fresh SET (one study) or one cycle of fresh SET followed by one frozen SET in a natural or hormone-stimulated cycle (two studies). When these three studies were pooled, the cumulative live birth rate after repeated SET was not significantly different from the rate after one cycle of DET (OR 0.82, 95% CI 0.62 to 1.09, three studies, n=811, I^2=0%, low quality evidence). This suggests that for a woman with a 42% chance of live birth following a single cycle of DET, the chance following repeated SET would be between 31% and 44%. The multiple pregnancy rate was significantly lower in the SET group (OR 0.03, 95% CI 0.01 to 0.13, three RCTs, n = 811, I^2 = 23%, low quality evidence), suggesting that for a woman with a 13% risk of multiple pregnancy following a single cycle of DET, the risk following repeated SET would be between 0% and 2%.

Single-cycle SET versus single-cycle DET

A single cycle of SET was compared with a single cycle of DET in 10 studies, nine comparing cleavage-stage transfers and two comparing blastocyst-stage transfers. When studies were pooled the live birth rate was significantly lower in the SET group (OR 0.48, 95% CI 0.39 to 0.60, nine studies, n = 1564, I^2 = 0%, high quality evidence). This suggests that for a woman with a 45% chance of live birth following a single cycle of DET, the chance following a single cycle of SET would be between 24% and 33%. The multiple pregnancy rate was also significantly lower in the SET group (OR 0.12, 95% CI 0.07 to 0.20, 10 studies, n = 1612, I^2 = 45%, high quality evidence), suggesting that for a woman with a 14% risk of multiple pregnancy following a single cycle of DET, the risk following a single cycle of SET would be between 1% and 3%. The heterogeneity for this analysis was attributable to a study with a high rate of cross-over between treatment arms.

Other comparisons

Other comparisons were evaluated in four studies which compared DET versus transfer of three or four embryos. Live birth rates did not differ significantly between the groups for any comparison, but there was a significantly lower multiple pregnancy rate in the DET group than in the three embryo transfer (TET) group (OR 0.36, 95% CI 0.13 to 0.99, two studies, n = 343, I^2 = 0%).

Authors' conclusions

In a single fresh IVF cycle, single embryo transfer is associated with a lower live birth rate than double embryo transfer. However, there is no evidence of a significant difference in the cumulative live birth rate when a single cycle of double embryo transfer is compared with repeated SET (either two cycles of fresh SET or one cycle of fresh SET followed by one frozen SET in a natural or hormone-stimulated cycle). Single embryo transfer is associated with much lower rates of multiple pregnancy than other embryo transfer policies. A policy of repeated SET may minimise the risk of multiple pregnancy in couples undergoing ART without substantially reducing the likelihood of achieving a live birth. Most of the evidence currently available concerns younger women with a good prognosis.

PLAIN LANGUAGE SUMMARY

Number of embryos for transfer in women undergoing assisted reproductive technology (ART)

Review question:

How many embryos should be transferred in couples undergoing ART?

Background:

Multiple pregnancy creates serious health risks for the mother (such as premature labour, diabetes and high blood pressure) and for the babies, who are at much higher risk than single babies of problems including premature birth, low birth weight, cerebral palsy and perinatal death. Single embryo transfer is now being seriously considered in order to reduce multiple pregnancies but this needs to be balanced against the risk of lowering the overall live birth rate. Researchers in The Cochrane Collaboration reviewed the evidence about the number of embryos transferred in women undergoing ART. The search is current to July 2013.

Study characteristics:

We found 14 randomised controlled trials with a total of 2165 participants. Most were not commercially funded.

Key findings:

Double versus repeated single embryo transfer

Based on low quality evidence, there was no indication that overall live birth rates differed substantially when *repeated* single embryo transfer (either two cycles of single embryo transfer or one cycle of single embryo transfer followed by transfer of a single frozen embryo in a natural or hormone-stimulated cycle) was compared with double embryo transfer. The evidence suggested that for a woman with a 42% chance of live birth following a single cycle of double embryo transfer, the chance following repeated single embryo transfer would be between 31% and 44%. The risk of multiple birth was very much lower in the single embryo transfer group: for a woman with a 13%

risk of multiple pregnancy following a single cycle of double embryo transfer, the estimated risk following a repeated single transfer was between 0% and 2%.

Double versus single embryo transfer

We found high quality evidence that the chances of live birth were lower after one cycle of fresh single embryo transfer than after one cycle of fresh double embryo transfer. For a woman with a 45% chance of live birth following a single cycle of double embryo transfer, the chance following a single cycle of single embryo transfer was between 24% and 33%. However, the risk of twins was about seven times higher after double embryo transfer.

Conclusion:

Repeated single embryo transfer appears the best option for most women undergoing ART. Most of the evidence currently available concerns younger women with a good prognosis.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Repeated single embryo transfer compared to double embryo transfer

Repeated single compared to mixed policies for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection

Population: women having embryo transfer following in vitro fertilisation or intra-cytoplasmic sperm injection
Settings: Assisted reproduction
Intervention: Repeated single embryo transfer (in one or more cycles)
Comparison: Double embryo transfer

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Double ET	Repeated single ET				
Cumulative live birth Repeated single embryo transfer versus double embryo transfer	420 per 1000	373 per 1000 (310 to 441)	OR 0.82 (0.62 to 1.09)	811 (3 Studies)	⊕⊕⊝⊝ LOW [1,2]	
Cumulative live birth - Single embryo transfer plus one cycle of frozen embryo transfer versus one cycle of double embryo transfer	422 per 1000	377 per 1000 (308 to 450)	OR 0.83 (0.61 to 1.12)	703 (2 Studies)	⊕⊕⊝⊝ LOW [1,2]	
Cumulative live birth - Two cycles of single embryo transfer SET (x2) versus one cycle of double embryo transfer	407 per 1000	352 per 1000 (198 to 542)	OR 0.79 (0.36 to 1.72)	108 (1 Studies)	⊕⊝⊝⊝ VERY LOW [1,2,3]	
Multiple pregnancy Repeated single embryo transfer versus double embryo transfer	133 per 1000	5 per 1000 (2 to 19)	OR 0.03 (0.01 to 0.13)	811 (3 Studies)	⊕⊕⊝⊝ LOW [1,2]	
Multiple pregnancy Single embryo transfer plus one cycle of frozen embryo transfer versus one cycle of double embryo transfer	136 per 1000	5 per 1000 (2 to 22)	OR 0.03 (0.01 to 0.14)	703 (2 Studies)	⊕⊕⊝⊝ LOW [1,2]	
Multiple pregnancy	111 per 1000	9 per 1000 (0 to 135)	OR 0.07 (0.00 to 1.25)	108 (1 Studies)	⊕⊝⊝⊝ VERY LOW [1,2,3]	

Two cycles of single embryo transfer SET (x2) versus one cycle of double embryo transfer	

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

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

[1]Methods of allocation concealment not described in enough detail
[2]Wide confidence intervals
[3]One small study

Summary of findings 2. Single embryo transfer compared to double embryo transfer (in a single cycle)

Single compared to multiple embryo transfer (in a single cycle) following in vitro fertilisation or intra-cytoplasmic sperm injection

Population: women having embryo transfer following in vitro fertilisation or intra-cytoplasmic sperm injection
Settings: Assisted reproduction
Intervention: Single embryo transfer
Comparison: Multiple embryo transfer (in a single cycle)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Multiple	Single				
Multiple pregnancy	144 per 1000	20 per 1000 (12 to 32)	OR 0.12 (0.07 to 0.20)	1612 (10 Studies)	⊕⊕⊕⊕ HIGH [2]	
Live birth	450 per 1000	282 per 1000 (242 to 329)	OR 0.48 (0.39 to 0.60)	1564 (9 Studies)	⊕⊕⊕⊕ HIGH [1]	

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

GRADE Working Group grades of evidence

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

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[1]36% of women noncompliant with treatment allocation in one study. However, no statistical heterogeneity detected (I^2=0%)
[2]Moderate heterogeneity attributable to 36% of women oncompliant with treatment allocation in one study (I^2=45%)

BACKGROUND

Description of the condition

Historically, in an effort to achieve 'acceptable' pregnancy rates, most women undergoing in vitro fertilisation (IVF) have received transfer of multiple embryos. However, this practice is being reassessed due to the high rates of multiple pregnancy that result from multiple embryo transfer and which commonly lead to poor clinical outcomes for the mother or her children, or both (ASRM 2012).

In the 1990s it was calculated that women undergoing IVF had an approximately 20-fold increased risk of twins and 400-fold increased risk of higher order pregnancies (Martin 1998). In 2006, twins accounted for nearly 20% of all live births resulting from IVF in Europe (De Mouzon 2010). Widespread concern about the medical, social and economic consequences of multiple pregnancy has prompted the development of strategies aimed at promoting birth of a single healthy baby following IVF.

Compared with singleton births, twins have a four-fold increased risk of mortality, and for triplets the risk is increased six-fold (ESHRE 2000). A recent study (ESHRE 2012) of 50,258 births following IVF and intra-cytoplasmic sperm injection (ICSI) pregnancies reported that twins accounted for half the total neonatal deaths and one-third of the perinatal deaths. Twins had a significantly higher perinatal mortality rate than singletons (27.8 per 1000 births and 12.4 per 1000 births, respectively). The relatively high congenital malformation rates observed in babies born after IVF and intracytoplasmic sperm injection (ICSI) are attributed to the high proportion of multiple births in this population compared to the general population (Sebire 2000; Wennerholm 2000). In babies with very low birth weight, twin gestation is an independent risk factor for neurodevelopmental impairment including cerebral palsy, severe bilateral hearing loss and bilateral blindness (Wadhawan 2009).

Twin pregnancy also increases the risk of obstetric complications, with a high incidence of miscarriage, pregnancy-induced hypertension, gestational diabetes, premature labour and abnormal delivery (FIVNAT 1995; ESHRE 2000). After the initial sense of achievement of parenthood, the care of children from a multiple gestation is often associated with practical difficulties and high stress levels (Garel 1992; Doyle 1996; Garel 1997). More hours per week are required to care for six-month old triplets and to carry out the necessary household tasks. Even in families with material resources and plenty of help, emotional stress is not uncommon and may necessitate psychiatric help (Garel 1997).

The economic impact of multiple pregnancies on health services is another consideration. In an Australian study, the average cost of an ART twin delivery was almost three times as high as for an ART singleton, while for higher order multiple births the cost was up to 11 times greater (Chambers 2007). It has been suggested that redeployment of money saved by reduction of multiple pregnancies could allow for increased provision of IVF treatment in the UK at no extra cost (Ledger 2006).

Description of the intervention

IVF or ICSI is followed by the transfer of one, two, three or four fresh or frozen and thawed embryos. Unused embryos can be frozen and transferred in a subsequent natural or hormone stimulated transfer cycle. Reduction of the number of embryos transferred is a strategy used to reduce rates of multiple pregnancy associated with ART.

There is a worldwide trend for an increase in the rates of elective single embryo transfer, defined as the transfer of a single embryo at cleavage or blastocyst stage, which is chosen from a larger number of available embryos. In Europe, in 2005, about 20% of all embryo transfers were of single embryos but much higher rates are reported in some countries (69% in Sweden in 2005, and 57% in Australia and New Zealand in 2006) (ASRM 2012).

Embryos are often transferred after culture for two or three days, when they comprise two to eight cells (cleavage stage). The rationale for cleavage-stage transfer is that the uterus is the best environment for the survival of the embryo (Laverge 2001). Over the past decade there has been a steady shift in practice to the transfer of embryos on day five or six, when they have developed into blastocysts with 64 cells. Blastocyst transfer has been shown to be successful (Papanikolaou 2006; Khalaf 2008) but requires laboratory expertise and experience in extended embryo culture. An advantage of blastocyst transfer is that embryos surviving five days are more likely to be viable than embryos at two or three days, and so the likelihood of implantation is higher. Disadvantages of blastocyst transfer include a higher risk of cycles being cancelled (Marek 1999) and fewer embryos being available for cryopreservation due to failed embryo development.

A Cochrane review comparing cleavage-stage versus blastocyst transfer (Glujovsky 2012) had mixed findings. There was evidence that blastocyst transfer was associated with a small but significant benefit in the live birth rate per couple but that cleavage-stage transfers were associated with higher cumulative clinical pregnancy rates. This finding was attributed to higher rates of frozen embryos and lower failure-to-transfer rates obtained from cleavage-stage protocols. Multiple birth rates did not differ between the two groups.

How the intervention might work

A strategy of reducing the risk of multiple pregnancy by limiting the number of embryos transferred needs to be balanced against the risk of jeopardising the overall pregnancy rate. An obvious solution is to consider an individualised embryo transfer policy based on identification of key clinical and laboratory parameters associated with a higher implantation rate. The above-mentioned ESHRE study (ESHRE 2012) of 50,258 births following IVF and ICSI pregnancies reported that double embryo transfer was associated with a 53% higher risk of perinatal mortality than single embryo transfer (19 per 1000 births compared with 13 per 1000 births). This difference was especially apparent when fresh (unfrozen) embryos were used. Births following the transfer of two fresh embryos had a 74% higher risk of perinatal mortality than those following fresh single embryo transfer.

Use of elective single embryo transfer at the cleavage stage (day two or three) has been limited in clinical practice for fear that the overall success rates of IVF would decline. This assumption has been supported by the published results of single embryo transfer where only one embryo was available. Because no opportunity for selection of more suitable embryos exists, the implantation potential of the only available embryo is usually poor, with clinical pregnancy rates of around 10% (FIVNAT 1995; Giorgetti 1995; Preutthipan 1996; Yaron 1997; Lieberman 1998; Westergaard 2000).

In a situation where the transferred embryos are the only available embryos, pregnancy rates are unfavourable even for multiple embryo transfer (Ludwig 2000).

A study from Finland reported a 20.2% pregnancy rate in 94 women who had only one embryo available for transfer compared with a rate of 29.7% in women who had multiple embryos available and from which a single high quality embryo was selected for transfer. The cumulative pregnancy rate after frozen and thawed embryo transfers in the elective single embryo transfer group was 47.3% per oocyte retrieval. By comparison, the pregnancy rate for double embryo transfers was 29.4% per transfer, of which 23.9% were twin pregnancies (Vilska 1999).

Another strategy for reducing multiple pregnancy is multifetal pregnancy reduction. However, this procedure is invasive, can have long term adverse psychological consequences for the potential parents (Berkowits 1996; McKinney 1996) and may be unacceptable to some couples given the attendant ethical and legal issues. Clinicians in Europe have generally accepted the desirability of reducing multiple births by limiting the number of embryos transferred, especially if this can be achieved without unduly reducing live birth rates (Roberts 2011).

Why it is important to do this review

It is important to find ways to limit the risk of multiple pregnancy without reducing the chance of achieving live birth in couples undergoing ART cycles. This systematic review evaluates the effectiveness and safety of different policies for the number of embryos transferred in couples who undergo assisted reproductive technology (ART).

OBJECTIVES

To evaluate the effectiveness and safety of different policies for the number of embryos transferred in couples undergoing assisted reproductive technology (ART) cycles.

METHODS

Criteria for considering studies for this review

Types of studies

Published and unpublished randomised controlled trials (RCTs) were eligible for inclusion. We excluded non-randomised studies (for example studies with evidence of inadequate sequence generation such as alternate days, chart numbers) as they are associated with a high risk of bias. Cross-over trials were eligible but it was planned that only data from the first phase would be included in the meta-analysis as the cross-over design is not valid in this context.

Types of participants

Trials of subfertile women who underwent embryo transfer following in vitro fertilisation or intra-cytoplasmic sperm injection treatment with their own gametes or as oocyte or embryo donation recipients were eligible for inclusion.

Types of interventions

We compared the following interventions.

1. Repeated single embryo transfer versus repeated multiple transfer.
2. Repeated single embryo transfer versus mixed policies
3. Single versus multiple embryo transfer in a single cycle.
4. Other fresh cycle comparisons.

It was required that elective transfer of embryos followed an initial fresh IVF or ICSI treatment using standard protocols for controlled ovarian stimulation, oocyte retrieval under ultrasound guidance, insemination, embryo culture, and transcervical replacement of embryos (cleavage stage or blastocyst) using standard culture medium and catheters for the culture and transfer of embryos respectively.

Studies could (in addition) transfer one or more frozen thawed embryos in one or both arms using standard procedures in a natural or hormone-stimulated cycle.

Studies comparing cleavage-stage transfer versus blastocyst-stage transfer were excluded.

Types of outcome measures

Primary outcome

(1) Live birth rate per woman or couple, or cumulative live birth rate per woman or couple (in trials with multiple transfers or multiple cycles).

Live birth was defined as the delivery of one or more living infants. Cumulative live birth rate reflects the number of live births following fresh and frozen embryo transfers after a single IVF treatment leading to the harvesting of eggs, or (where stated) after multiple IVF cycles. It is calculated by dividing the total number of live births in each group by the total number of women randomised in each group. One IVF cycle is defined as a single treatment leading to the harvesting of eggs.

(2) Multiple pregnancy rate per woman or couple. The demonstration of more than one sac with a fetal pole on ultrasound scan defines a multiple pregnancy.

Secondary outcomes

(1) Pregnancy rate per woman or couple.

Pregnancy was defined as the presence of a gestational sac on ultrasound scan or confirmation of products of conception by pathological examination in the event of spontaneous abortion or ectopic pregnancy.

(2) Miscarriage rate per woman.

Search methods for identification of studies

We searched for all relevant published and unpublished RCTs without language restriction and in consultation with the Menstrual Disorders and Subfertility Group (MDSG) Trials Search Co-ordinator. For the search strategies, please see Appendix 1, Appendix 2, Appendix 3, Appendix 4, Appendix 5, Appendix 6.

Electronic searches

We searched the following electronic databases: the Menstrual Disorders and Subfertility Group (MDSG) Specialised Register of

controlled trials, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, PsycINFO and CINAHL. The last search date was July 17th 2013.

Other electronic sources of trials included the following.

- Trials registers for ongoing and registered trials:
  * www.clinicaltrials.gov/;
  * www.who.int/trialsearch/Default.aspx.
- OpenGrey for unpublished literature from Europe at www.opengrey.eu/.
- Citation index: Web of Science.

Searching other resources

We handsearched other resources as follows:

- conference proceedings - International Federation of Fertility Societies (IFFS), American Society for Reproductive Medicine (ASRM), British Fertility Society (BFS), European Society for Human Reproduction and Embryology (ESHRE) between 1997 and 2013;
- the bibliographies of the identified studies.

We personally communicated with experts and investigators in the field.

Data collection and analysis

Selection of studies

The selection of trials for inclusion in the review from those identified employing the search strategy was performed independently by at least two review authors. Disagreements about study eligibility were resolved by discussion.

Data extraction and management

Quality assessment and data extraction were independently performed by two review authors. Any discrepancies were resolved by discussion with senior review authors (GS, SB). Additional information on trial methodology or trial data was sought from the principal authors of trials which appeared to meet the eligibility criteria but were unclear in aspects of methodology, or where the data were in a form unsuitable for meta-analysis.

Assessment of risk of bias in included studies

The included studies were assessed for risk of bias using the Cochrane risk of bias tool to evaluate the following: random sequence generation; allocation concealment; blinding of participants, providers and outcome assessors; completeness of outcome data; selective outcome reporting; and other potential sources of bias (see Figure 1). At least two authors (ZP, SB, JM) assessed these six domains. Any disagreements were resolved by consensus or by discussion with another author. The assessments are presented in the 'Risk of bias' table (see Characteristics of included studies, Figure 1 and Figure 2).

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

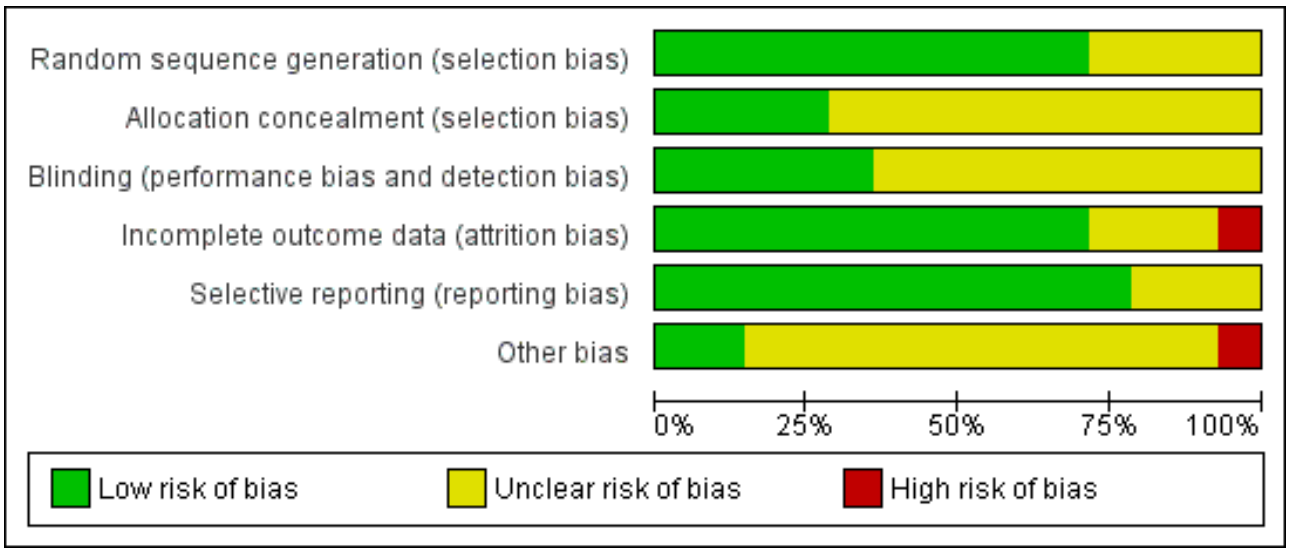


Figure 2. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.

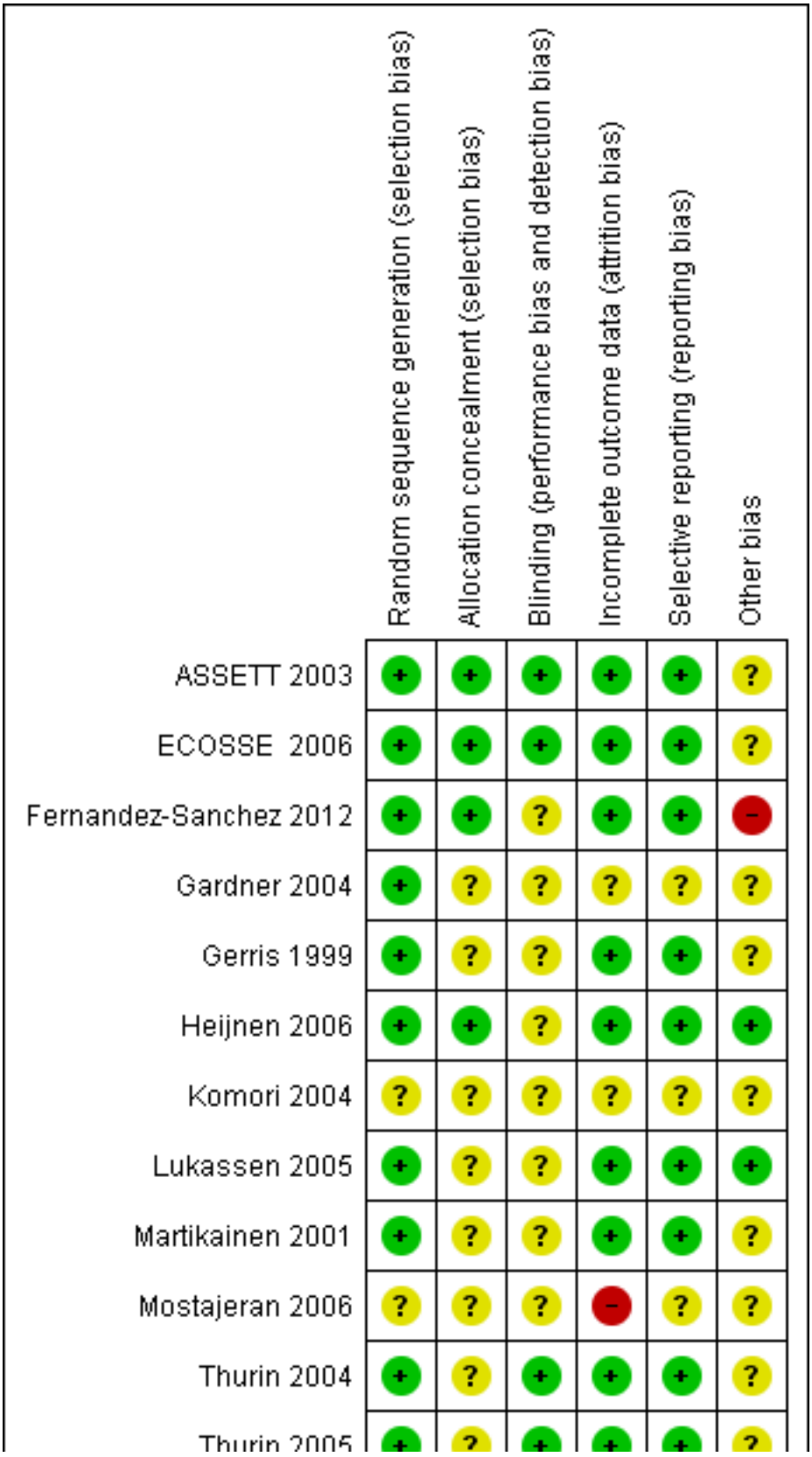
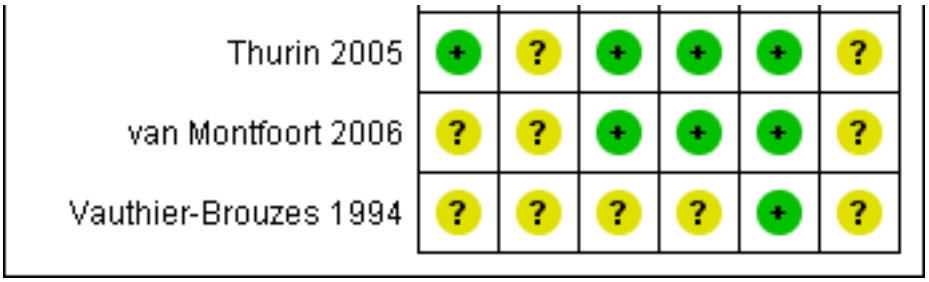


Figure 2. (Continued)



Measures of treatment effect

All data were dichotomous. The numbers of events in the control and intervention groups of each study were used to calculate the Mantel-Haenszel odds ratios (ORs) with 95% confidence intervals (CIs).

Where outcome data were reported as a percentage of the total number of participants, they were included in the analyses by multiplying the percentage by the total number of participants (n) in that group and dividing by 100.

Unit of analysis issues

Multiple live births (for example twins or triplets) were counted as one live birth event. It was planned to include only first-phase data from cross-over trials. Per cycle data were not included in tables of comparison but were reported descriptively.

Dealing with missing data

The data were analysed on an intention-to-treat basis as far as possible and attempts were made to obtain missing data from the original investigators.

Assessment of heterogeneity

The authors considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a meaningful summary. Clinical heterogeneity in subfertility (such as variations in entry criteria, subtle differences in the treatment used and that are important from a clinical aspect) cannot be avoided because most centres use their own protocols which can vary in some aspects. When trials met the inclusion criteria and had performed the same intervention we considered it appropriate to pool their results. Statistical heterogeneity was assessed by inspecting the scatter in the data points and the overlap in their CIs and, more formally by checking the results of the I^2 statistic. An I^2 measurement greater than 50% was taken to indicate substantial heterogeneity (Higgins 2011). If substantial heterogeneity was detected, possible explanations were explored in sensitivity analyses. Even when included trials in a comparison group were statistically homogeneous, there were potentially considerable differences in clinical features (clinical heterogeneity). These differences were taken into account when analysing and interpreting the pooled results.

Assessment of reporting biases

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

Data synthesis

The data from primary studies were combined with RevMan software to calculate pooled Mantel-Haenszel ORs and 95% CIs, using a fixed-effect model, with the following comparisons.

1. Repeated single versus repeated multiple transfer.
2. Repeated single embryo transfer versus mixed policies
3. Single versus multiple embryo transfer in a single cycle
4. Other fresh cycle comparisons
5. Other fresh or frozen cycle comparisons

Data were stratified by the stage of embryo transfer (cleavage or blastocyst).

For the 2012 update, we reformatted the comparisons of interest, as above. The choice of repeated single versus repeated multiple embryo transfer as the first comparison of interest reflects the view that a policy of repeated SET may optimise the chance of live birth while minimising the risk of multiple pregnancy (Roberts 2011).

An increase in the odds of a particular outcome, which may be beneficial (for example live birth) or detrimental (for example multiple pregnancy), is displayed graphically in the meta-analyses to the right of the centre-line and a decrease in the odds of an outcome to the left of the centre-line.

Subgroup analysis and investigation of heterogeneity

If data were available, we planned to conduct subgroup analyses to determine the separate evidence within groups with different prognostic characteristics.

If we detected substantial heterogeneity, we planned to explore possible explanations in sensitivity analyses. We planned to take any statistical heterogeneity into account when interpreting the results.

Sensitivity analysis

We conducted sensitivity analyses for the primary outcomes to determine whether the conclusions were robust to arbitrary decisions made regarding study eligibility and statistical methods. We considered whether the review conclusions would have differed if:

1. eligibility was restricted to studies at lower risk of bias (i.e. with clearly reported methods of randomisation and allocation concealment and not at high risk of bias in any of the domains assessed);
2. a random-effects model had been adopted;
3. the summary effect measure had been relative risk rather than odds ratio (OR).

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

A 'Summary of findings' table was generated using the GRADEPro software. This table evaluated the overall quality of the body of evidence for the primary review outcomes for selected comparisons. Items assessed were study limitations (that is risk of bias), consistency of effect, imprecision, indirectness and publication bias. Judgements about evidence quality (high, moderate or low) were incorporated into the reporting of results.

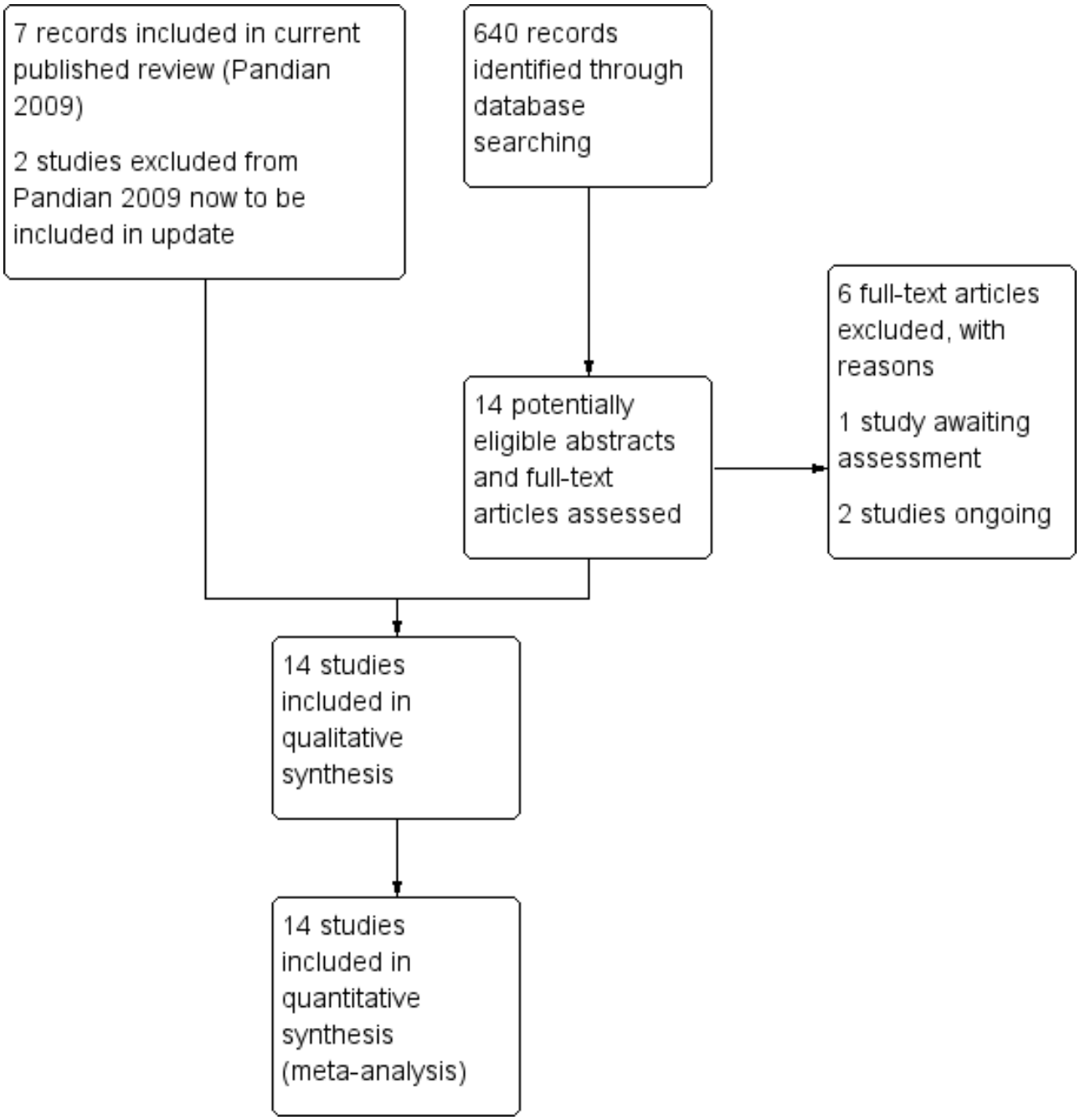
RESULTS

Description of studies

Results of the search

The search for the 2013 update identified 640 articles (including duplicates) of which 14 full text articles or online abstracts were retained for detailed appraisal. Five of the 14 were included in the review (ASSETT 2003; Gardner 2004; Thurin 2005; ECOSSE 2006; Fernandez-Sanchez 2012), six were excluded (Motta 1998 A & B; Livingstone 2001; Bowman 2004; Elgindy 2011; Guerif 2011; Forman 2012), one is awaiting assessment (Obrado 2012) and two are ongoing (Abuzeid 2012; Scott 2013). In addition, two studies excluded from the previous version of the review were included (Komori 2004; Mostajeran 2006). For details, see Figure 3.

Figure 3. Study flow diagram.



Included studies

For this update, seven studies were added to the seven included in the original review, making a total of 14 included studies. Five new studies (ASSETT 2003; Gardner 2004; Thurin 2005; ECOSSE 2006; Fernandez-Sanchez 2012) were added. Two previously excluded studies (Komori 2004; Mostajeran 2006) were also added. These two studies had been excluded from the previous version of the review for failure to report full details of randomisation and allocation concealment. They were added to this update after discussion between the review authors, who noted that poor reporting was not a review exclusion criterion. Additional information was sought from authors of all the new trials and replies were received from four (ASSETT 2003; Thurin 2005; ECOSSE 2006; Fernandez-Sanchez 2012). See the 'Characteristics of included studies' table.

Study design and setting

Fourteen studies with a total of 2165 participants were included in the review (Vauthier-Brouzes 1994; Gerris 1999; Martikainen 2001; ASSETT 2003; Gardner 2004; Komori 2004; Thurin 2004; Lukassen 2005; Thurin 2005; ECOSSE 2006; Heijnen 2006; Mostajeran 2006; van Montfoort 2006; Fernandez-Sanchez 2012). All were randomised parallel-group trials. Six were multicentred (Martikainen 2001; ASSETT 2003; Thurin 2004; Thurin 2005; ECOSSE 2006; Heijnen 2006). Sample sizes ranged from 23 to 661 women.

Of the four unpublished studies that have been added to this update, one was a pilot trial published as part of a PhD dissertation (Thurin 2005). Another, the 'Australian study of single embryo transfer' (ASSETT 2003) was stopped early because its implementation immediately and substantially altered consumer decision making. This had the effect of more than tripling rates of elective single embryo transfer during the study period and reducing study participation rates (M Davies, University of Adelaide, personal communication). A UK trial, known as the 'Efficacy and cost effectiveness of selective single embryo transfer' (ECOSSE) study, was also stopped early due to poor recruitment (ECOSSE 2006). The fourth unpublished study (Fernandez-Sanchez 2012) was in press.

Nine studies reported their funding sources. Six reported non-commercial funding (Gerris 1999; ASSETT 2003; ECOSSE 2006; Mostajeran 2006; van Montfoort 2006; Fernandez-Sanchez 2012) and three reported pharmaceutical company funding (Gardner 2004; Thurin 2004; Thurin 2005).

Participants

Study inclusion criteria differed with regard to participant age. Most studies had a maximum age threshold. This varied across studies and included 34 years (Gerris 1999), 35 years (Vauthier-Brouzes 1994; Lukassen 2005), 36 years (Thurin 2004), 38 years (ECOSSE 2006; Fernandez-Sanchez 2012), and 40 years (ASSETT 2003). One study included women aged between 38 and 45 years (Heijnen 2006) while another required them to be at least 36 years old (Thurin 2005). Other studies used a variety of age limits (Martikainen 2001; van Montfoort 2006).

Two studies only included women in their first treatment cycle (Gerris 1999; van Montfoort 2006) while three included women with an indication for IVF or ICSI either for the first time or after a previous successful treatment (Vauthier-Brouzes 1994; Lukassen 2005; Heijnen 2006). Three studies included women in their first or second IVF or ICSI treatment cycle (ASSETT 2003; Thurin 2004; Thurin 2005). In a multicentre study, one centre included women in their first treatment cycle only and another centre included women in their first or second cycle (Martikainen 2001). One study included all women undergoing IVF and embryo transfer (Gardner 2004) who agreed to participate.

The duration of infertility was mentioned in six studies (Gerris 1999; Thurin 2004; Lukassen 2005; Thurin 2005; Heijnen 2006; van Montfoort 2006) and seven mentioned the indication(s) for treatment (Martikainen 2001; Thurin 2004; Lukassen 2005; Thurin 2005; Heijnen 2006; Mostajeran 2006; van Montfoort 2006). See 'Prognostic factors' in Table 1.

Two studies did not provide details of participant characteristics (Komori 2004; Mostajeran 2006).

Interventions

All the studies included embryo transfer after fresh IVF or ICSI cycles and two studies included frozen cycles administered to one or both groups (Thurin 2004; Thurin 2005). Several other studies also administered frozen cycles during follow-up but not as part of the randomised comparison (Vauthier-Brouzes 1994; Martikainen 2001; ECOSSE 2006; Fernandez-Sanchez 2012).

Interventions in the included studies were as follows:

- one fresh single embryo transfer (SET) plus one frozen embryo transfer (1FZET) in a natural or hormone-stimulated cycle compared with one fresh cycle of double embryo transfer (DET) (Thurin 2004; Thurin 2005);
- two fresh cycles of SET compared with one fresh cycle of DET (Lukassen 2005);
- one fresh cycle of SET plus multiple cycles of frozen DET compared with one cycle of fresh DET plus multiple cycles of frozen DET (ECOSSE 2006)
- one fresh cycle of SET compared with one fresh cycle of DET (Gerris 1999; Martikainen 2001; Gardner 2004; ASSETT 2003; van Montfoort 2006; Fernandez-Sanchez 2012);
- one fresh cycle of DET compared with one fresh cycle of triple embryo transfer (TET) (Heijnen 2006);
- fresh or frozen DET compared with fresh or frozen TET, multiple cycles (Komori 2004)
- two fresh cycles of DET compared to two fresh cycles of TET (Heijnen 2006);
- three fresh cycles of DET compared to three fresh cycles of TET (Heijnen 2006);
- fresh DET compared with fresh TET where the number of cycles used was unclear (Mostajeran 2006);
- one fresh cycle of DET compared with one fresh cycle of four embryo transfer (FET) (Vauthier-Brouzes 1994).

One study (Komori 2004) reported only per-cycle data. There a large disparity between the number of women (169) and the number of cycles (212), and it was unclear how many women were included in each group. The data from this study were therefore unusable.

Four studies that randomised women to more than one embryo transfer cycle reported interim data after the first fresh cycle of SET versus DET (Thurin 2004; Thurin 2005; Lukassen 2005; ECOSSE 2006). In the case of ECOSSE 2006, these were the only data available, as the trial was stopped due to poor recruitment and data were only available for the first cycle (i.e. fresh DET versus fresh SET).

Protocols for ovarian stimulation, oocyte recovery and embryo transfer were clearly described in nine studies (Vauthier-Brouzes 1994; Gerris 1999; Martikainen 2001; Thurin 2004; Lukassen 2005; Thurin 2005; Heijnen 2006; van Montfoort 2006; Fernandez-Sanchez 2012). Good quality embryos were transferred in all studies, usually at cleavage stage. However, in four studies all or some women had embryos transferred at blastocyst rather than cleavage stage; this applied to a small number of women in two studies (Thurin 2004; Thurin 2005), half the women in one study (Fernandez-Sanchez 2012) and all women in another study (Gardner 2004). The stage of embryo transfer was not mentioned in one study (Mostajeran 2006).

Natural progesterone was used for luteal phase support in most cases (Gerris 1999; Martikainen 2001; Gardner 2004; Thurin 2004; Lukassen 2005; Thurin 2005; Heijnen 2006; van Montfoort 2006; Fernandez-Sanchez 2012). One study used both human chorionic gonadotropin (HCG) and natural progesterone for luteal phase support (Vauthier-Brouzes 1994).

Outcomes

Primary outcomes

1. Live birth rate and cumulative live birth rate

Eleven studies reported live birth rate per couple (Vauthier-Brouzes 1994; Gerris 1999; Martikainen 2001; ASSETT 2003; Thurin 2004; Lukassen 2005; Thurin 2005; ECOSSE 2006; Heijnen 2006; van Montfoort 2006; Fernandez-Sanchez 2012). One reported 'take home baby' per cycle only (Komori 2004).

Five studies reported cumulative live birth rates (ASSETT 2003; Thurin 2004; Lukassen 2005; Thurin 2005; Heijnen 2006).

2. Multiple pregnancy rate per woman or couple

All but one study reported multiple pregnancy rate per couple. One reported multiple pregnancy per cycle only (Komori 2004).

Secondary outcomes

1. Clinical pregnancy rate

Ten studies reported pregnancy rate per couple (Vauthier-Brouzes 1994; Gerris 1999; Martikainen 2001; Gardner 2004; Thurin 2004; Lukassen 2005; Heijnen 2006; Mostajeran 2006; van Montfoort 2006; Fernandez-Sanchez 2012).

2. Miscarriage rate per woman

Three studies reported miscarriage rate (Martikainen 2001; Lukassen 2005; van Montfoort 2006).

Excluded studies

See Characteristics of excluded studies.

Fourteen studies were excluded from the review for the following reasons:

- four studies were not randomised (Bowman 2004; van Montfoort 2005; Moustafa 2008; Guerif 2011);
- 10 studies did not report a comparison of interest (Staessen 1993; Gardner 1998; Motta 1998 A & B; Livingstone 2001; Frattarelli 2003; Levitas 2004; Pantos 2004; Heijnen 2007; Elgindy 2011; Forman 2012).

Risk of bias in included studies

See Characteristics of included studies; Figure 1; Figure 2.

Allocation

Generation of random sequence

Ten studies were at low risk of bias related to random sequence generation as they used computer-generated methods. Four studies did not describe their randomisation methods and were therefore at unclear risk of this bias.

Allocation concealment

Four studies were at low risk of bias related to allocation concealment. They used sealed opaque envelopes (ASSETT 2003) or remote allocation (ECOSSE 2006; Heijnen 2006; Fernandez-Sanchez 2012). In the other ten studies a satisfactory method of allocation concealment was not described clearly enough or no information was given, and the risk of this bias was therefore rated as unclear.

Blinding

Five trials were rated as at low risk of bias related to blinding (ASSETT 2003; Thurin 2004; Thurin 2005; ECOSSE 2006; van Montfoort 2006) as neither the patient nor physician knew whether one embryo or two embryos had been transferred. Two studies were unblinded (Lukassen 2005; Fernandez-Sanchez 2012) and the others did not mention blinding. These nine studies were rated as at unclear risk of bias as it was unclear whether lack of blinding would be likely to influence the outcomes of this review.

Incomplete outcome data

Ten studies were rated as at low risk of this bias as they included all randomised women in the analysis. Three studies were rated as at unclear risk of this bias because it was unclear how many women were included in the analysis (Gardner 2004; Komori 2004; Vauthier-Brouzes 1994). One study (Mostajeran 2006) was rated as at high risk of this bias because it was unclear how many women were randomised: women non-compliant with the drug regimen or who had ovarian hyperstimulation syndrome (numbers not stated) were excluded and three women with ectopic pregnancy were also excluded from the analysis.

Selective reporting

Eleven studies were deemed to be at low risk of this bias. Two studies (Gardner 2004; Mostajeran 2006) that did not report live birth and one study which only reported per cycle data (Komori 2004) were deemed to be at unclear risk of this bias.

Other potential sources of bias

Two studies were judged to be at low risk of other potential biases and 11 were at unclear risk. One study (Fernandez-Sanchez 2012) gave women the option of changing the number of embryos transferred or the day of transfer if they were unhappy with the group to which they were randomised. A large number of participants (21%) chose to change, including 36% of women in the SET groups who changed to DET. Although the study was analysed by intention to treat, the results were deemed to be at high risk of bias due to the high level of non-compliance and the fact that nearly all the changes were in the same direction.

Effects of interventions

See: **Summary of findings for the main comparison** Repeated single embryo transfer compared to double embryo transfer; **Summary of findings 2** Single embryo transfer compared to double embryo transfer (in a single cycle)

The results below are formatted by type of comparison, as follows.

1. Repeated single embryo transfer versus repeated multiple transfer
2. Repeated single embryo transfer versus mixed policies
3. Single versus multiple embryo transfer in a single cycle
4. Other fresh cycle comparisons.

1. Repeated single embryo transfer versus repeated multiple transfer.

No studies compared repeated single embryo transfer versus repeated multiple transfer.

2. Repeated single embryo embryo transfer versus mixed policies

Three studies, all of cleavage-stage transfer, made this comparison (Thurin 2004; Lukassen 2005; Thurin 2005).

Specific interventions were as follows (with the number of cycles in brackets).

- Single embryo transfer (x 2) versus double embryo transfer (x 1) (SET (x2) versus DET (X1))(Lukassen 2005).
- Single embryo transfer (x 1) plus transfer of one frozen thawed embryo in a natural or hormone-stimulated cycle versus double embryo transfer (x 1) (SET + 1 FZET versus DET (X1)) (Thurin 2004; Thurin 2005).

Primary outcomes

2.1 Cumulative live birth rate

When the three studies (Thurin 2004; Lukassen 2005; Thurin 2005) were pooled, the cumulative live birth rate after repeated single embryo transfer was not significantly different from the rate after one cycle of DET (OR 0.82, 95% CI 0.62, to 1.09, three studies, n=811, I^2=0%). This suggests that for a woman with a 40% chance of live birth following a single cycle of DET, the chance following repeated SET would be between 31% and 44%.

2.1.1 SET + 1 FZET versus DET (x1)

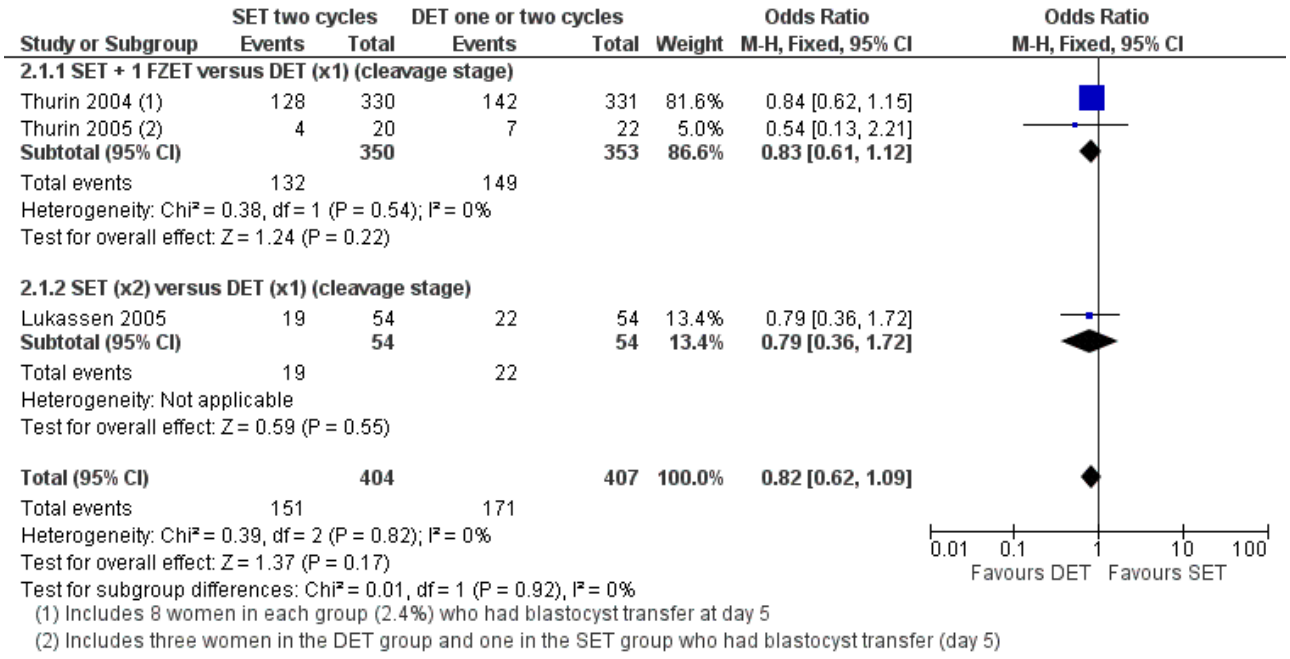
Two studies reported cumulative live birth rates after SET followed by 1 FZET versus DET in a single cycle (Thurin 2004; Thurin 2005). The difference in cumulative live birth rate between SET + 1 FZET and DET was not statistically significant (OR 0.83, 95% CI 0.61 to 1.12, two studies, n = 703, I^2 = 0%).

2.1.2 SET (x 2) versus DET (x1)

A single study compared cumulative live birth rate after two fresh cycles of SET versus a single fresh cycle of DET (Lukassen 2005). It did not find a significant difference between the two groups (OR 0.79, 95% CI 0.36 to 1.72, one study, n = 108).

See Analysis 2.1; Figure 4

Figure 4. Forest plot of comparison: 2 Repeated single versus mixed policies, outcome: 2.1 Cumulative live birth.



2.2 Multiple pregnancy rate

When the three studies (Thurin 2004; Lukassen 2005; Thurin 2005) were pooled, the multiple pregnancy rate after repeated single embryo transfer was significantly lower than after a single cycle of DET (OR 0.03, 95% CI 0.01 to 0.13, three studies, n=811, I^2 = 23%). This suggests that for a woman with a 13% risk of multiple pregnancy following a single cycle of DET, the risk following repeated SET would be between 0% and 2%.

2.2.1 SET + 1 FZET versus DET (x 1)

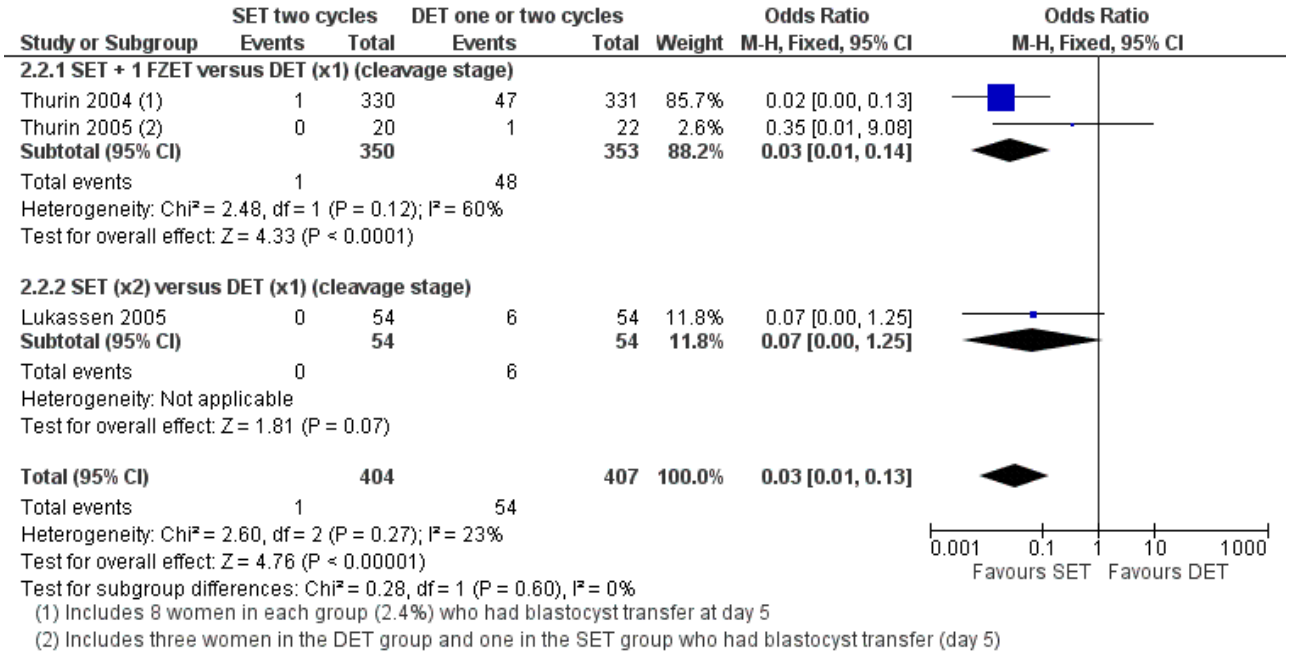
Two studies reported multiple pregnancy rates after SET plus 1 FZET versus DET in a single cycle (Thurin 2004; Thurin 2005). There was a significantly lower multiple pregnancy rate in the SET group, with substantial heterogeneity (OR 0.03, 95% CI 0.01 to 0.14, two studies, n = 703, I^2 = 60%). There was no obvious explanation for the heterogeneity.

2.2.2 SET (x 2) versus DET (x 1)

A single study compared the multiple pregnancy rate after two fresh cycles of SET versus a single fresh cycle of DET (Lukassen 2005) and did not find a significant difference between the two groups (OR 0.07, 95% CI 0.00 to 1.25, one study, n = 108).

See Analysis 2.2; Figure 5

Figure 5. Forest plot of comparison: 2 Repeated single versus mixed policies, outcome: 2.2 Multiple pregnancy.



(1) Includes 8 women in each group (2.4%) who had blastocyst transfer at day 5
(2) Includes three women in the DET group and one in the SET group who had blastocyst transfer (day 5)

Secondary outcomes

2.3 Clinical pregnancy rate

When two studies reporting this outcome (Lukassen 2005; Thurin 2004) were pooled, the clinical pregnancy rate after repeated single embryo transfer was not significantly different from the rate after one cycle of DET (OR 0.81, 95% CI 0.61 to 1.08, two studies, n=768, I^2=0%)

2.3.1 SET + 1 FZET versus DET (x 1)

A single study reported the clinical pregnancy rate after SET followed by 1 FZET versus DET in a single cycle (Thurin 2004). No significant difference was found between the groups (OR 0.83 95% CI 0.61 to 1.12, one study, n = 661).

2.3.2 Fresh SET (x 2) versus DET (x 1)

A single study compared the clinical pregnancy rate after two fresh cycles of SET versus a single fresh cycle of DET (Lukassen 2005) and did not find a significant difference between the two groups (OR 0.71, 95% CI 0.33 to 1.53, one study, n= 107).

See Analysis 2.3

2.4 Miscarriage rate

A single study reported the miscarriage rate after two fresh cycles of SET versus a single fresh cycle of DET (Lukassen 2005). No significant difference was found between the two groups (OR 0.60, 95% CI 0.18 to 1.97, one study, n = 107).

See Analysis 2.4

3. Single versus multiple embryo transfer in a single cycle

Nine studies of cleavage-stage transfer (Gerris 1999; Martikainen 2001; ASSETT 2003; Thurin 2004; Lukassen 2005; Thurin 2005; ECOSSE 2006; van Montfoort 2006; Fernandez-Sanchez 2012) and two of blastocyst-stage transfer (Gardner 2004; Fernandez-Sanchez 2012) made this comparison. One reported both (Fernandez-Sanchez 2012).

All compared one cycle of single versus one cycle of double embryo transfer (SET (x 1) versus DET (x 1)). As noted above, for four of these studies (Thurin 2004; Thurin 2005; Lukassen 2005; ECOSSE 2006) the data for this comparison derive from an interim analysis, as women in one or both arms were randomised to undergo further transfer cycles if the first cycle did not result in pregnancy.

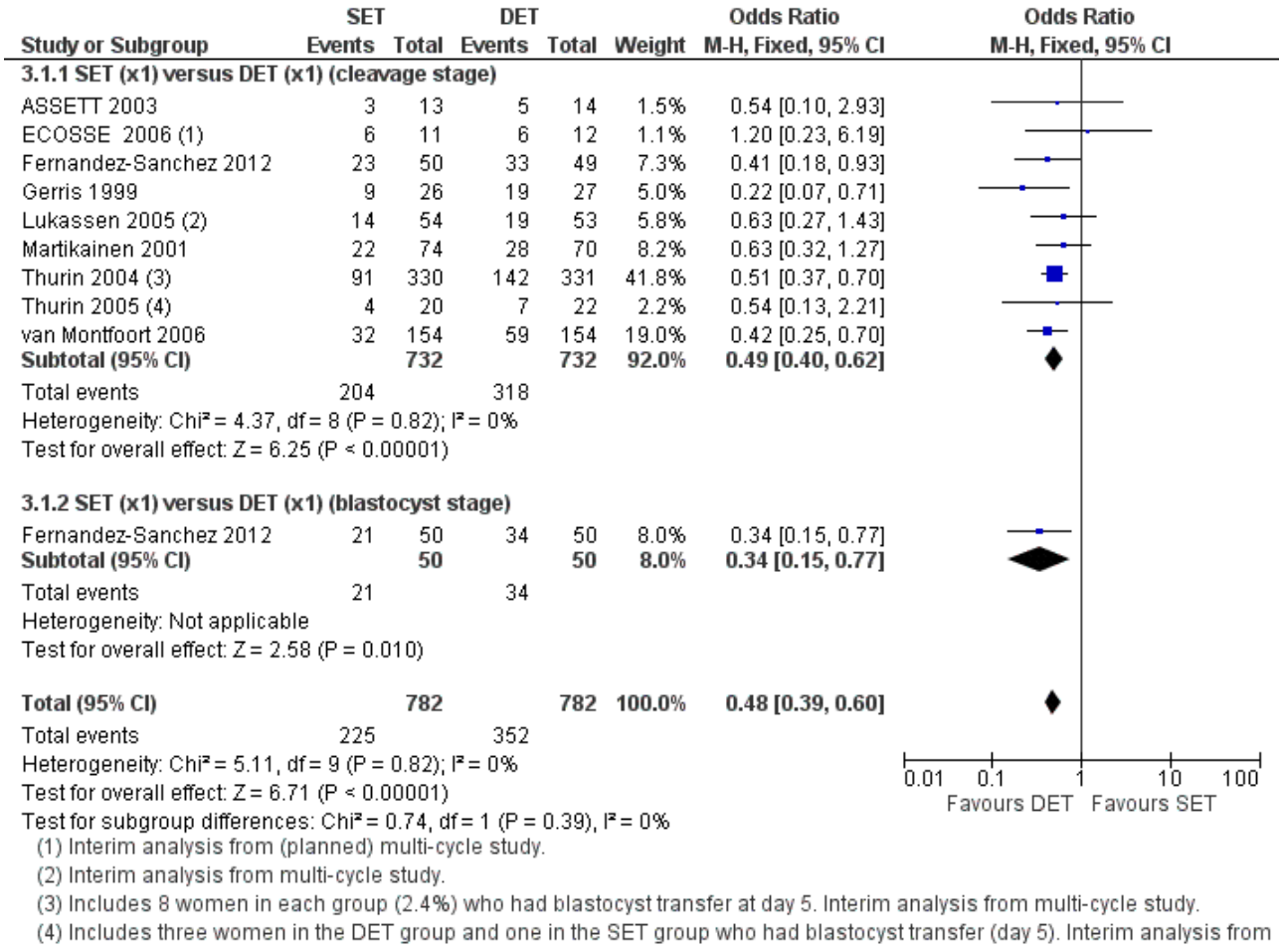
Primary outcomes

3.1 Live birth rate

3.1.1 SET (x 1) versus DET (x 1)

Nine studies of cleavage-stage transfer and one of blastocyst transfer reported this outcome. See Analysis 3.1; Figure 6

Figure 6. Forest plot of comparison: 3 Single versus multiple (in a single cycle), outcome: 3.1 Live birth.



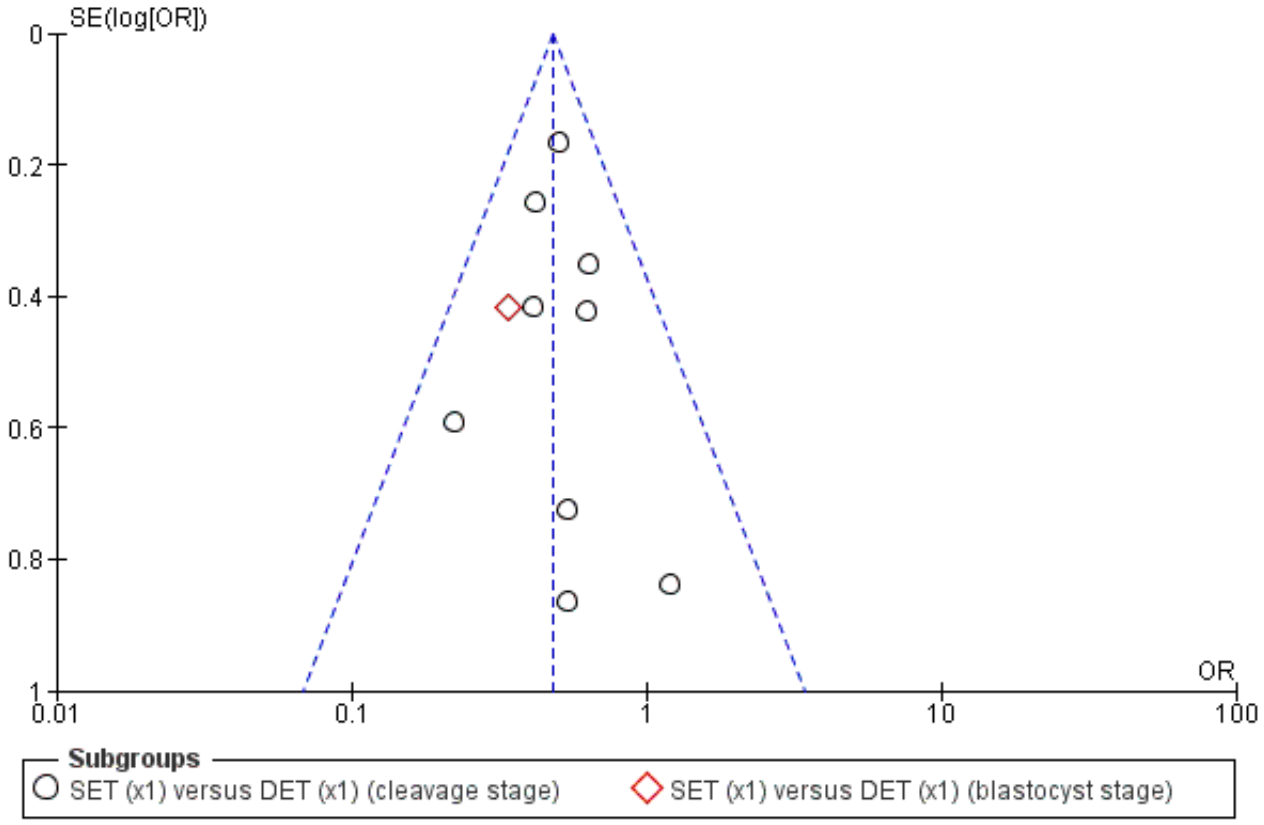
(1) Interim analysis from (planned) multi-cycle study.
(2) Interim analysis from multi-cycle study.
(3) Includes 8 women in each group (2.4%) who had blastocyst transfer at day 5. Interim analysis from multi-cycle study.
(4) Includes three women in the DET group and one in the SET group who had blastocyst transfer (day 5). Interim analysis from

When all studies were pooled, the live birth rate per woman was significantly lower in women who had SET than those who had DET (OR 0.48, 95% CI 0.39 to 0.60, nine studies, n = 1564, I^2 = 0%). This suggests that for a woman with a 45% chance of live birth following a single cycle of DET, the chance following a single cycle of SET would be between 24% and 33%.

These findings applied in comparisons of cleavage-stage transfer (OR 0.49, 95% CI 0.40 to 0.62, nine studies, n = 1464, I^2 = 0%) and also in the single comparison of blastocyst transfer (OR 0.34, 95% CI 0.15 to 0.77, one study, n = 100).

A funnel plot for this outcome was not suggestive of publication bias. See Figure 7

Figure 7. Funnel plot of comparison: 3 Single versus multiple (in a single cycle), outcome: 3.1 Live birth.

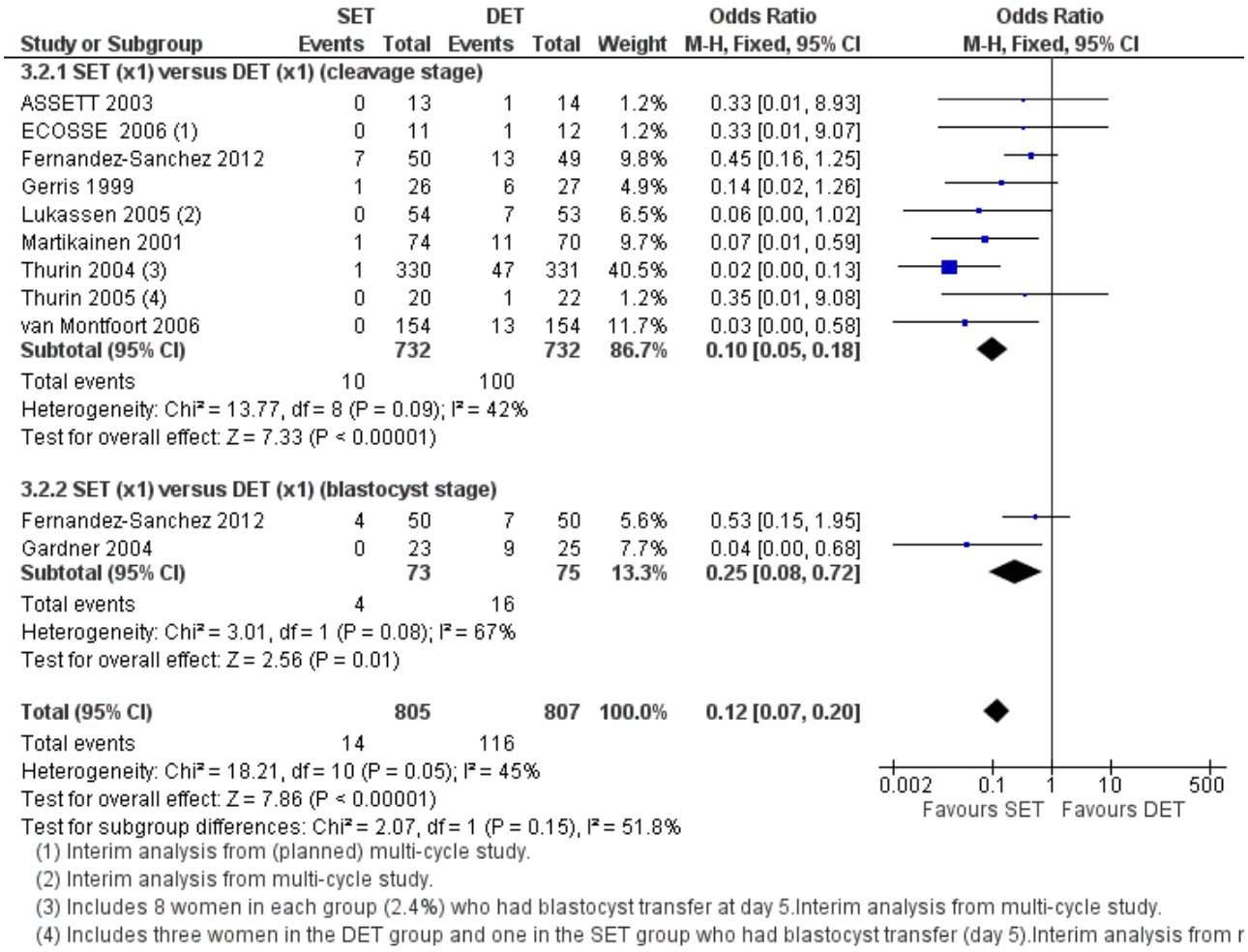


3.2 Multiple pregnancy rate

3.2.1 SET (x 1) versus DET (x 1)

Nine studies of cleavage-stage transfer and two of blastocyst transfer reported this outcome. See Analysis 3.2; Figure 8

Figure 8. Forest plot of comparison: 3 Single versus multiple (in a single cycle), outcome: 3.2 Multiple pregnancy.



When all studies were pooled, the multiple pregnancy rate per woman was significantly lower in those who had SET than those who had DET (OR 0.12, 95% CI 0.07 to 0.20, 10 studies, n = 1612, I^2 = 45%). This suggests that for a woman with a 14% risk of multiple pregnancy following a single cycle of DET, the risk following a single cycle of SET would be between 1% and 3%

These findings applied in comparisons of cleavage-stage transfer (OR 0.10, 95% CI 0.05 to 0.18, nine studies, n = 1464, I^2 = 0%) and also in comparisons of blastocyst transfer (OR 0.25, 95% CI0.08 to 0.72, two studies, n = 148, I^2 = 67%). Heterogeneity in these analyses appeared to derive from a study at high risk of bias (Fernandez-Sanchez 2012). Treatment contamination (also known as 'cross-over') occurred in a high proportion of cases in this study and would be expected to attenuate any treatment difference. I^2 reduced to 0% when this study was excluded from the analyses, without materially affecting the conclusion.

In a sensitivity analysis restricted to studies which clearly reported methods of randomisation and allocation concealment and did not appear to be at high risk of bias, there were only three studies (ASSETT 2003; Lukassen 2005; ECOSSE 2006) with a total of 157 participants. Findings for live births for SET versus DET were no longer statistically significant (OR 0.68, 95% CI 0.35 to 1.34) but the findings for multiple pregnancy still significantly favoured SET (OR 0.13, 95% CI 0.02 to 0.74).

Secondary outcomes

3.3 Clinical pregnancy rate

3.3.1 SET (x 1) versus DET (x 1)

Six studies of cleavage-stage transfer and two of blastocyst transfer reported this outcome. See Analysis 3.3

When all studies were pooled, the clinical pregnancy rate per woman was significantly lower in those who had SET than those who had DET (OR 0.46, 95% CI 0.37 to 0.57, seven studies, n = 1521, I^2 = 0%).

These findings applied in comparisons of cleavage-stage transfer (OR 0.46, 95% CI 0.37 to 0.57, six studies, n = 1357, I^2 = 0%) and also in comparisons of blastocyst transfer (OR 0.37, 95% CI 0.18 to 0.76, two studies, n = 148, I^2 = 0%).

Miscarriage rate

Three studies of cleavage-stage transfer reported this outcome (Martikainen 2001; Thurin 2004; van Montfoort 2006). No significant

difference was found between the two groups (OR 0.85, 95% CI 0.54 to 1.34, three studies, n = 1113, I^2 = 61%), see Analysis 3.4

4. Other fresh cycle comparisons

Three studies tested other fresh cycle comparisons. Two were of cleavage-stage transfer (Vauthier-Brouzes 1994; Heijnen 2006). The day of transfer of the third study (Mostajeran 2006) was not reported. Specific interventions were as follows (with the number of cycles in brackets):

- DET (x 1) versus triple embryo transfer (TET) (x 1) (Heijnen 2006; Mostajeran 2006);
- DET (x 1) versus four embryo transfer (FET) (x 1) (Vauthier-Brouzes 1994);
- DET (x 2) versus TET (x 2) (Heijnen 2006);
- DET (x 3) versus TET (x 3) (Heijnen 2006).

Primary outcomes

4.1 Live birth rate or cumulative live birth rate across single or repeated IVF cycles

4.1.1 DET (x 1) versus TET (x 1)

No significant difference was found between the groups in the live birth rate (OR 0.40, 95% CI 0.09 to 1.85, one study, n = 45) (Heijnen 2006).

4.1.2 DET (x 1) versus FET (x 1)

No significant difference was found between the groups in the live birth rate (OR 0.35, 95% CI 0.11 to 1.05, one study, n = 56) (Vauthier-Brouzes 1994).

4.1.3 DET (x 2) versus TET (x 2)

No significant difference was found between the groups in the cumulative live birth rate after two cycles of SET versus two cycles of TET (OR 0.77, 95% CI 0.22 to 2.65, one study, n = 45) (Heijnen 2006).

4.1.4 DET (x 3) versus TET (x 3)

No significant difference was found between the groups in the cumulative live birth rate after three cycles of SET versus three cycles of TET (OR 0.77, 95% CI 0.24 to 2.52, one study, n = 45) (Heijnen 2006).

See Analysis 4.1.

4.2 Multiple pregnancy rate

4.2.1 DET (x 1) versus TET (x 1)

There was a significantly lower multiple pregnancy rate in the DET group than in the TET group (OR 0.36, 95% CI 0.13 to 0.99, two studies, n = 343) (Heijnen 2006; Mostajeran 2006).

4.2.2 DET (x 1) versus FET (x 1)

No significant difference was found between the groups in the multiple pregnancy rate (OR 0.44, 95% CI 0.10 to 1.97, one study, n = 56) (Vauthier-Brouzes 1994).

See analysis Analysis 4.3.

Secondary outcomes

4.3 Clinical pregnancy rate

4.3.1 DET (x 1) versus TET (x 1)

There was no significant difference between the groups in the clinical pregnancy rate (OR 0.67, 95% CI 0.42 to 1.08, one study, n = 343) (Heijnen 2006).

4.3.2 DET versus FET

No significant difference was found between the groups in the clinical pregnancy rate (OR 0.56, 95% CI 0.19 to 1.62, one study, n = 56) (Vauthier-Brouzes 1994).

4.4 Miscarriage rate

No studies reported this outcome.

5. Other fresh or frozen cycle comparisons

One study (Komori 2004) of cleavage-stage transfer compared DET versus TET among 169 participants. A total of 106 cycles of fresh or frozen embryos were apparently administered in each group, but study reporting was unclear and, moreover, outcomes were reported per cycle rather than per woman. Attempts to contact the authors were unsuccessful. Study findings were reported descriptively below.

Primary outcomes

5.1 Cumulative live birth rate

5.1.1 DET versus TET, apparently using fresh or frozen embryos for multiple cycles

No significant difference was found between the groups for this outcome using per cycle data (30 versus 26 live births resulting from 106 cycles in each group) (Komori 2004).

5.2 Multiple pregnancy rate

5.2.1 DET versus TET, apparently using fresh or frozen embryos for multiple cycles

There was a significantly lower incidence of multiple births per pregnancy in the DET group (6/40 pregnancies versus 14/29 pregnancies) (Komori 2004).

Secondary outcomes

5.3 Clinical pregnancy rate

5.3.1 DET versus TET, apparently using fresh or frozen embryos for multiple cycles

No significant difference was found between the groups for this outcome using per cycle data (40 versus 29 pregnancies resulting from 106 cycles in each group) (Komori 2004).

5.4 Miscarriage rate

This outcome was not reported.

Subgroup and sensitivity analyses

We did not perform our planned subgroup analyses to assess the efficacy of embryo replacement protocols in participant groups with differing prognostic characteristics because most studies did not identify such subgroups.

There were insufficient studies which clearly reported methods of randomisation and allocation concealment to conduct sensitivity analyses by study quality, other than for analysis 3.1. The overall findings did not materially change with the use of a random-effects model rather than a fixed-effect model or with use of risk ratios rather than odds ratios.

DISCUSSION

Summary of main results

Our findings indicate, as one would expect, that live birth and pregnancy rates following single embryo transfer (SET) are lower than those following double embryo transfer (DET) in a single fresh IVF cycle but that the risk of multiple pregnancy is much higher in the DET group. However, pooling of three studies of cleavage-stage transfer found no evidence of a significant difference in the cumulative live birth rate when a single cycle of DET was compared with repeated SET (either SET followed by transfer of a single frozen embryo in a natural or hormone-stimulated cycle (Thurin 2004; Thurin 2005), or two fresh cycles of SET (Lukassen 2005)). Confidence intervals for this finding were wide, and suggested that for a woman with a 42% chance of live birth following a single cycle of DET, the chance following repeated SET would be between 31% and 44%.

Thus, although DET achieves higher live birth rates per fresh cycle, the evidence suggests that the difference in effectiveness may be substantially offset when elective SET is followed by a further single fresh or frozen cycle, at least among women with a good prognosis.

Eleven studies compared one fresh cycle of SET versus one fresh cycle of DET. The live birth rate was 60% higher in the DET group but the risk of multiple pregnancy was eight times as high. One of this group of studies included a high proportion of women who chose not to comply with their randomised treatment, and inclusion of this study was associated with substantial heterogeneity for the outcome of multiple pregnancy. Otherwise there was little evidence of statistical heterogeneity in the review, suggesting that clinical differences between studies had little effect on overall findings.

Three studies of cleavage-stage transfer tested fresh cycle comparisons of DET versus transfer of three or four embryos. Live birth rates did not differ significantly, but there was a significantly lower multiple pregnancy rate in the DET group than in the three embryo transfer (TET) group.

Overall completeness and applicability of evidence

No studies compared repeated single versus repeated multiple embryo transfer within the same IVF cycle. This comparison was planned in one study (ECOSSE 2006) but the study was closed due to poor enrolment, with only 23 participants. This comparison would be a useful way to structure future trials in order to determine the safety and effectiveness of different embryo transfer policies, given that a number of embryos have been produced. Policy in this context means the strategy for using up the available embryos until success is achieved or the supply of embryos is exhausted. A comparison of repeated multiple versus repeated single embryo transfer would address the policy question by determining 'cumulative' success rates.

The vast majority of participants in the included studies had a good prognosis (aged under 36 years and with sufficient good quality embryos). Only two small studies (Thurin 2005; Heijnen 2006) focused on older women. As one of the studies (Gardner 2004) noted, there was a strong potential for self-selection bias, as only a small proportion of eligible women volunteered for the trial, probably due to the belief that single ET could result in lower pregnancy rates and that twin pregnancy is a desirable outcome: they commented that most volunteers were younger women. Future studies should include older women and those with previously failed IVF cycles or lack of good quality embryos

Per cycle, DET appears to be more expensive than SET (Tiitinen 2001; Gerris 2004; Thurin 2006; Chambers 2007; Fiddelers 2007). The higher cost is mainly due to the increased rate of multiple births and premature births in the DET group, and fewer pregnancies in the SET group. Long term costs related to multiple births and prematurity in the DET group have not yet been adequately assessed. However the additional costs of cryopreservation with SET + 1 FZET have not been evaluated. In order to implement a policy of multiple single embryo transfers per woman, providers require either an efficient cryopreservation service or the ability to provide multiple fresh IVF cycles. The former is likely to be a safer and less invasive option for the women concerned.

Only two studies (Gardner 2004; Fernandez-Sanchez 2012) specifically addressed blastocyst transfer.

Quality of the evidence

Many of the included studies were small, with half enrolling fewer than 60 participants. There was considerable clinical heterogeneity between the studies but little evidence of statistical heterogeneity for most analyses. The methodological quality of the studies was mixed. See Figure 2. Confidence intervals were wide for some analyses, and GRADEPro ratings for the primary outcomes ranged from high (for comparisons of DET versus SET in a single cycle) to low or very low (for comparisons of DET versus repeated SET). See Summary of findings table 3; Summary of findings table 4).

Potential biases in the review process

One of the review authors is primary investigator of one of the included studies (ECOSSE 2006).

Our comparison of one cycle of fresh SET versus one cycle of DET (Analysis 6.1) includes data from studies for which this was an interim analysis. This may be a potential source of bias, associated with placebo effects relating to participant anxiety. A post-hoc sensitivity analysis excluding these studies did not materially influence the live birth rate in this analysis.

We are unaware of any other potential biases in the review process.

Agreements and disagreements with other studies or reviews

Other studies and reviews are broadly in agreement with the current review.

A project commissioned by the UK National Institutes of Health Research Health Technology Assessment Programme (Roberts 2011) used statistical modelling, analysis of registry and cohort data, and exploration of consumer perspectives to explore options for increasing SET and reducing the incidence of multiple births. The analysis concluded that couples have approximately one-

third less chance of a live birth if they have one fresh cycle of SET rather than DET, but that use of repeat cycles using cryopreservation might compensate for the lost potential in each individual transfer while reducing the likelihood of multiple births. However, the authors recognised that a policy of repeat SET (with use of cryopreserved eggs) would involve challenges including appropriate patient selection, optimisation of freezing techniques, and the emotional, financial and physical burden associated with additional treatment cycles.

Recent systematic reviews (Gelbaya 2010; McLernon 2010) and a report from the American Society for Reproductive Medicine (ASRM 2012) have reached similar conclusions.

A large Dutch cohort study is currently in progress, which aims to assess the long term costs and health outcomes of IVF singleton and twin children and the long term cost-effectiveness of SET versus DET strategies. Outcomes will be reported at one year, five years and 18-year follow-up (van Heesch 2010).

AUTHORS' CONCLUSIONS

Implications for practice

This review indicates that in a single fresh IVF cycle, single embryo transfer is associated with a lower live birth rate than double embryo transfer. However, there is no evidence of a significant difference in the cumulative live birth rate when a single cycle of double embryo transfer is compared with repeated SET (either two cycles of fresh SET or one cycle of fresh SET followed by one cycle of frozen SET in a natural or hormone-stimulated cycle). Single embryo transfer is associated with much lower rates of multiple pregnancy than other embryo transfer policies. A policy of repeated SET may minimise the risk of multiple pregnancy in couples undergoing ART, without substantially reducing the likelihood of achieving a live birth. Most of the evidence currently available concerns younger women with a good prognosis.

Implications for research

More evidence is needed on policies for repeated embryo transfer, including the most safe and effective way to use available embryos within a single IVF cycle until success is achieved or the supply of embryos is exhausted. More research is needed to determine what characteristics of women and embryos are associated with multiple pregnancy and which, if present, should identify a need for single embryo transfer. As studies to date have been conducted largely among women with a good prognosis undergoing ART, future studies should include older women (above 36 years) and those with previously failed IVF cycles or lack of good quality embryos. Longer term cost-effectiveness analyses are also needed, which should take into account costs related to multiple births and also costs of cryopreservation in the various strategies. Finally, it is important to explore patient perspectives on multiple pregnancy and to increase consumer awareness that single embryo transfer is the best option for most women having IVF.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

ASSETT 2003

Methods	Multicentre randomised controlled trial

ASSETT 2003 *(Continued)*

Participants	Female age <35 yrs if no previous ART pregnancy, <40 if previous ART pregnancy. At least four good quality embryos or at least three if previous ART pregnancy successful 27 women randomised
Interventions	Cleavage-stage transfer: SET (n=1) versus DET (n=14) Eligibility into the trial was restricted to a single cycle of treatment. All subsequent cycles of treatment were performed under conditions of routine care.
Outcomes	Cumulative live birth, twin live birth, clinical ongoing pregnancy (fetal heartbeat), complications during pregnancy, delivery and neonatal period, perinatal mortality and morbidity, use of neonatal intensive care
Notes	Unpublished trial. This study was stopped because its implementation immediately and substantially altered patients' decision making, which more than tripled the rates of elective single embryo transfer during the study period, and reduced participation rates (M Davies, University of Adelaide, personal communication). Funded by National Health and Medical Research Council Grant no: 158006) (M Davies, University of Adelaide, personal communication)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Pre-randomised envelopes were used and stored in the laboratory, opened in numerical order
Blinding (performance bias and detection bias) All outcomes	Low risk	Patients were not informed of the number of embryos transferred nor the number of embryos suitable for freezing until immediately after their embryo transfer, doctors were also not informed of the randomisation until after their patient's embryo transfer, database manager and data analyser were also blinded until completion of data analysis by using codes to represent the two treatment groups. The code was held by an independent third party
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up
Selective reporting (reporting bias)	Low risk	All outcomes described in the protocol were reported
Other bias	Unclear risk	Day of randomisation on day of embryo transfer

ECOSSE 2006

Methods	Randomised controlled trial, computer-generated random sequence, n=23 women analysed
Participants	**Inclusion criteria** : all women receiving IVF or intra-cytoplasmic sperm injection (ICSI) treatment with an optimal chance of achieving pregnancy, i.e. women aged less than 37 years, first or second cycle of treatment, 4 or more good quality embryos at the time of embryo transfer

ECOSSE 2006 *(Continued)*

	Exclusion criteria : women undergoing pre-implantation genetic diagnosis, or assisted hatching, or a history of recurrent miscarriage
Interventions	Cleavage-stage transfer: SET fresh + multiple SET frozen (n=11) versus DET fresh + multiple DET frozen (n=12) Both groups: if a pregnancy does not result in the fresh cycle, women will be encouraged to return for replacement of frozen-thawed embryos in subsequent cycles over the next 12 months
Outcomes	Cumulative live birth, twin live birth, clinical pregnancy (at least one gestational sac with heartbeat), biochemical pregnancy (positive test), miscarriage, ectopic pregnancy preterm delivery, low birth weight, congenital abnormality
Notes	Unpublished trial. This study was stopped because of poor recruitment (planned for 700 women, enrolled only 23) Funded by The Wellcome Trust (UK) (grant ref: 067469) and The Bertarelli Foundation (Switzerland)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Telephone randomisation performed by the embryologist (call to the Aberdeen Fertility Centre)
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blinded, couples and clinician or nurse who performed the embryo transfer were blinded to the number of embryos transferred
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up
Selective reporting (reporting bias)	Low risk	All outcomes mentioned in the protocol were reported
Other bias	Unclear risk	Duration of infertility not reported

Fernandez-Sanchez 2012

Methods	Randomised open-label controlled trial, designed to show equivalence Patients were informed on day 3 of embryo culture of the assigned group by their physician. Randomised women were allowed to change group if they did not feel confident and expressed a desire to modify the day or number of transferred embryos. Both ITT and per protocol analysis reported
Participants	Inclusion criteria Women requesting fertility treatment, aged under 38 years, and first trial of in vitro fertilisation or intra-cytoplasmic sperm injection. At least four good quality embryos on day 3 of embryo development Exclusion criteria

Fernandez-Sanchez 2012 *(Continued)*

	Patients who underwent pre-implantation genetic diagnosis or oocyte donation treatments were excluded. Patients were also excluded if the sperm was not obtained from an ejaculate sample 199 women randomised
Interventions	Day 3 of embryo culture: Cleavage stage SET (n=50) Cleavage stage DET (n=49) Day 5 of embryo culture: Blastocyst stage SET (n=50) Blastocyst stage DET (n=50) The number of embryos transferred on subsequent thawed embryo cycles was determined independently of the randomised group the patient belonged to. Protocols for IVF, embryo culture, transfer and freezing reported in detail in study publication
Outcomes	Multiple birth, live birth, patient acceptance
Notes	In press December 2012 Study enrolment ceased before planned sample size (n=412) due to change in embryo cryopreservation programme at IVI Seville. Sponsored by the Instituto Valenciano de Infertilidad, Spain

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Use of web site Randomization.com to generate randomly permuted blocks of eight subjects per block
Allocation concealment (selection bias)	Low risk	The randomisation was kept in a locked drawer in the administration office where the clinical staff who enrolled participants had no access. The assigned group was requested by phone
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Open label
Incomplete outcome data (attrition bias) All outcomes	Low risk	ITT outcomes reported for all women randomised
Selective reporting (reporting bias)	Low risk	All expected outcomes reported
Other bias	High risk	Groups well-balanced at baseline High proportion of participants changed groups (mostly from SET to DET): Cleavage-stage SET = 30 (50 randomised) Cleavage-stage DET= 71 (49 randomised) Blastocyst-stage SET = 37 (50 randomised)

Fernandez-Sanchez 2012 *(Continued)*

	Blastocyst stage-DET =57 (50 randomised) Study data were analysed by intention to treat (as reported in this review) and also per protocol

Gardner 2004

Methods	Randomised controlled trial. 48 women randomised
Participants	Women aged up to 43 years, undergoing IVF and embryo transfer with their own oocytes. Day 3 FSH no more than 10 mUI/ml, E2 under 80 pg/ml, hysteroscopically normal endometrial cavity, at least 10 follicles over 12 mm in diameter on day of hCG administration
Interventions	Blastocyst stage transfer: Single versus double blastocyst transfer
Outcomes	Ongoing pregnancy (defined as gestational sac with cardiac activity noted on ultrasound exam at least 4.5 weeks after embryo transfer), multiple gestation
Notes	Supported in part by grants from Organon Inc. and Vitrolife AB

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Methods not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No dropouts mentioned, but results presented as percentages so it is unclear whether all women were included in analysis
Selective reporting (reporting bias)	Unclear risk	Live birth not reported
Other bias	Unclear risk	Baseline characteristics (indication for IVF, age, baseline ovarian reserve) similar. Duration of infertility not reported

Gerris 1999

Methods	Randomised controlled trial. States external concealment for concealment of allocation. Good quality embryos transferred, morphology of good quality embryos defined. Protocols for ovarian stimulation, oocyte retrieval, insemination and embryo transfer clearly described. Natural progesterone used for luteal phase support. Semen was prepared using mini-percoll gradient prior to insemination. Medi-Cult medium used for embryo culture. Wallace embryo transfer catheter was used for transfer. Embryo transfer was performed on day 3, 64-67 hours after insemination, results expressed using 95% confidence intervals analysis

Gerris 1999 *(Continued)*

	53 women randomised
Participants	First IVF/ICSI cycle. Female age <34 years. Average duration of infertility 3.5 years
Interventions	One embryo transfer versus two embryo transfer
Outcomes	Clinical pregnancy rate, live birth rate, multiple pregnancy rate per woman or couple and implantation rates
Notes	Method of randomisation not mentioned. Blinding not stated. Power calculation not reported. Intention-to-treat analysis not performed. Withdrawals and dropouts not mentioned clearly. Indication for treatment not mentioned. Previous treatment not mentioned Sponsored by the Foundation Marguerite-Marie Delacroix, dedicated to the prevention of cerebral palsy, Belgium

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	States external concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women included in analysis
Selective reporting (reporting bias)	Low risk	Reports live birth and multiple pregnancy rates
Other bias	Unclear risk	Duration of infertility reported. Indication for treatment not mentioned. Previous treatment not mentioned

Heijnen 2006

Methods	Two-centre randomised controlled trial. Randomisation performed before embryo quality was known 45 women randomised
Participants	Patients on the waiting list for IVF/ICSI. Women >38 years and had an indication for IVF/ICSI either for the first time or after a previous IVF/ICSI childbirth
Interventions	Cleavage stage transfer (day 3 or 4): two embryo transfer in the first 3 cycles versus 3 embryo transfer in the first three treatment cycles
Outcomes	Cumulative live birth rate, live birth rate, multiple pregnancy rate
Notes	Chi^2 test and Mann-Whitney U test used for analysis. Randomisation was performed before information on embryo quality was available. Power calculation not mentioned

Heijnen 2006 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Remote: "Randomization was carried out using sealed envelopes opened by the study coordinator on the phone"
Allocation concealment (selection bias)	Low risk	Remote: "Randomization was carried out using sealed envelopes opened by the study coordinator on the phone
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding not mentioned
Incomplete outcome data (attrition bias) All outcomes	Low risk	All 45 women analysed by intention to treat
Selective reporting (reporting bias)	Low risk	Reports cumulative live birth rate, live birth rate, multiple pregnancy rate.
Other bias	Low risk	Duration of infertility reported

Komori 2004

Methods	Single-centre RCT
Participants	Women attending IVF clinic: 169 analysed (212 cycles)
Interventions	Cleavage-stage transfer (day two): two versus three embryo transfer, number of cycles unclear
Outcomes	Clinical pregnancy (gestational sac), ongoing pregnancy, live birth, multiple pregnancy
Notes	Per cycle data only

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method not described; "patients were randomly divided into two groups"
Allocation concealment (selection bias)	Unclear risk	Method not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding not mentioned
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Dropouts and withdrawals not reported, per cycle data only
Selective reporting (reporting bias)	Unclear risk	Reports expected outcomes, but only as per cycle data

Komori 2004 *(Continued)*

Other bias	Unclear risk	No information reported about baseline characteristics

Lukassen 2005

Methods	Randomised controlled trial 107 women randomised
Participants	First IVF/ICSI cycle. Female age <35 years, FSH < 10IU/L. At least one good quality embryo should be available
Interventions	Cleavage-stage transfer (day 3): SET (2 cycles) versus DET transfer In the second cycle protocol violations occurred in 4 patients (received two embryos)
Outcomes	Clinical pregnancy rate, live birth rate, multiple pregnancy rates and miscarriage rates per woman/couple. Cumulative pregnancy rates, Cumulative live birth rates, Cumulative multiple pregnancy rates and miscarriage rates for one plus one fresh embryo transfer
Notes	Good quality embryos transferred, but morphologic characteristics not defined clearly. Embryo transfer took place on day 3 after insemination. Patients and physicians not blinded to treatment. Power calculation reported. Details of those lost to follow-up given. Duration of infertility and indication for treatment provided. Protocols for IVF/ICSI described. Methods of statistical analysis mentioned Chi^2 test and student's t- test were used for analysis

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation
Allocation concealment (selection bias)	Unclear risk	"Allocation to the randomized group by an opaque, sealed envelope took place just before embryo transfer by the laboratory personnel to maintain concealment to the last moment". Does not specify that envelopes were consecutively numbered.
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Patients and physicians not blinded to treatment
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women analysed
Selective reporting (reporting bias)	Low risk	Reports cumulative live birth rate, live birth rate, multiple pregnancy rate
Other bias	Low risk	Duration of infertility reported

Martikainen 2001

Methods	Multicentre randomised controlled trial 144 women randomised
Participants	Fresh IVF/ICSI treatment who had/not had more than one previous failed treatment. Frozen embryo transfers were analysed separately. At least 4 good quality embryos should be available for inclusion in the trial.
Interventions	Cleavage-stage transfer: one embryo transfer (n=74) versus two embryo transfer (n=70). Good quality embryos transferred. Morphology of good quality embryos described clearly. Protocols for IVF/ ICSI clearly defined. Effectiveness of one versus two embryo transfer in frozen replacement cycles analysed separately. All centres involved used various age limits for inclusion of women. Embryos cultured in Medi-Cult medium. IVF-500 medium or Sydney IVF medium (Cook IVF) catheters were used for embryo transfer. Embryo transfer performed 46 - 50 hours after oocyte recovery. Natural progesterone used for luteal phase support. Chi^2 test and two-tailed t-tests used for statistical analysis
Outcomes	Reports clinical pregnancy rate, live birth rate, multiple pregnancy rates per woman/couple. Implantation and miscarriage rates
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated random number table, balanced in sets of 10
Allocation concealment (selection bias)	Unclear risk	Not clear: allocation done by laboratory personnel
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated
Incomplete outcome data (attrition bias) All outcomes	Low risk	All women included in analysis
Selective reporting (reporting bias)	Low risk	Reports cumulative live birth rate, live birth rate, multiple pregnancy rate
Other bias	Unclear risk	Duration of infertility not mentioned

Mostajeran 2006

Methods	Single-centre RCT
Participants	ART candidates referred to university clinic, 298 analysed
Interventions	One cycle of double embryo transfer (155 analysed) versus triple embryo transfer (143 analysed). Day of transfer not reported
Outcomes	Clinical pregnancy (fetal heart on ultrasound); multiple pregnancy

Mostajeran 2006 *(Continued)*

Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method not stated: "the subjects were randomly divided into two groups"
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding not mentioned
Incomplete outcome data (attrition bias) All outcomes	High risk	Women who did not follow the prescribed drug regimen or who had OHSS were excluded (numbers not reported). Three women with ectopic pregnancy also excluded - not stated which group they were in
Selective reporting (reporting bias)	Unclear risk	Live birth not reported
Other bias	Unclear risk	Duration of infertility not mentioned

Thurin 2004

Methods	Multicentre randomised controlled trial 661 women randomised
Participants	First or second IVF cycle who had at least 2 embryos of good quality available for transfer or freezing. Female age <36 years. Duration and cause for infertility mentioned
Interventions	Transfer on day two (93%), day three (5%) (cleavage stage), or day 5 (2%-3%) (blastocyst stage) a. One embryo transfer (n=330) versus two embryo transfer (n=331) b. One fresh plus one thawed embryo transfer cycle versus two embryo transfer (fresh)
Outcomes	Clinical pregnancy rate, live birth rate, multiple pregnancy rates and miscarriage rates per woman/couple. Cumulative pregnancy rates, Cumulative live birth rates, Cumulative multiple pregnancy rates and miscarriage rates for one embryo transfer plus one thawed embryo transfer cycle
Notes	Power calculation performed. Good quality embryos transferred, morphologic characteristics defined clearly. Embryo transfer took place on day 2, 3 or 5 days after oocyte retrieval. Women lost to follow-up mentioned. Fisher's non-parametric permutation test and Fisher's exact test used for statistical analysis and 95% confidence intervals calculated. Eight women in each group (2.4%) had blastocyst transfer at day 5 Supported by a grant from Serono Nordic

Risk of bias

Bias	Authors' judgement	Support for judgement

Thurin 2004 *(Continued)*

Random sequence generation (selection bias)	Low risk	Computer-generated randomisation at a ratio of 1:1
Allocation concealment (selection bias)	Unclear risk	Method not reported
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blind study
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women analysed
Selective reporting (reporting bias)	Low risk	Cumulative live birth rate, live birth rate, multiple pregnancy rate
Other bias	Unclear risk	No mean duration of infertility given. Eight women in each group (2.4%) had blastocyst transfer at day 5

Thurin 2005

Methods	Multicentre randomised controlled trial. Computer-generated randomisation at a ratio of 1:1 27 women randomised
Participants	Female age ≥36 years. First or second IVF/ICSI cycle. At least two good quality embryos available
Interventions	Transfer at cleavage stage (23/27; 85%) or blastocyst stage (4/27; 15%) DET fresh versus SET fresh + SET frozen
Outcomes	Reports live birth rate per woman, multiple live birth per woman
Notes	Unpublished trial, pilot study, part of a thesis Supported by a grant from Serono Nordic

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation at a ratio of 1:1
Allocation concealment (selection bias)	Unclear risk	Method not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blind study
Incomplete outcome data (attrition bias) All outcomes	Low risk	Women lost to follow-up mentioned ITT performed

Thurin 2005 *(Continued)*

Selective reporting (reporting bias)	Low risk	Reports cumulative live birth rate, live birth rate, multiple pregnancy rate
Other bias	Unclear risk	No mean duration of infertility given

van Montfoort 2006

Methods	Randomised controlled trial 308 women randomised
Participants	First IVF cycle. Participants had to have at least 2 oocytes (2PN embryos)
Interventions	Cleavage-stage transfer (day two or three): one embryo versus two embryo transfer
Outcomes	Reports clinical pregnancy rate, multiple pregnancy rate per woman/couple
Notes	Randomisation performed immediately prior to embryo transfer, but method of randomisation not stated. Patient population was stratified with respect to female age (<38 and >38 years), fertilisation technique (IVF/ICSI). Power calculation performed. Number lost to follow-up mentioned. Duration and cause for infertility mentioned. Analysis of variance (ANOVA) with Tukey's multiple test procedure and Chi^2 test were used for statistical analysis

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method not described
Allocation concealment (selection bias)	Unclear risk	"by using a nontransparent box containing the sealed opaque envelopes, the randomization procedure was blinded". Does not state that envelopes were consecutively numbered
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blinded study
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women included in analysis
Selective reporting (reporting bias)	Low risk	Reports pregnancy rate, multiple pregnancy rate, miscarriage rate
Other bias	Unclear risk	Duration of infertility not provided

Vauthier-Brouzes 1994

Methods	Randomised controlled trial 56 women included in analysis

Vauthier-Brouzes 1994 *(Continued)*

Participants	Fresh IVF/ICSI cycle. Frozen embryo transfers analysed separately. Age ≤35 years. Cleavage rate ≥70% for IVF. Good quality embryos transferred. Morphological characteristics of good quality embryos defined. Study and control groups were comparable in terms of age, number of hMG ampoules required for ovarian stimulation, mean number of oocytes obtained and the number of embryos obtained. Indications for IVF was also comparable in both groups. Protocols for IVF/ICSI defined. HCG and natural progesterone used for luteal phase support. IVF using donor sperm was also included and the number of patients who used donor sperm for IVF was also comparable in the two groups. Patients who had a single, successful previous IVF attempt were also included
Interventions	Cleavage stage transfer: two (n=28) versus four (n=28) embryo transfer
Outcomes	Clinical pregnancy rate, live birth rate and multiple pregnancy rate per woman/couple
Notes	Method of randomisation not mentioned. Blinding not stated. Allocation concealment not clear. Power calculation not reported. Intention-to-treat analysis not performed. Details of withdrawals, dropouts not given. Duration of infertility and indication for treatment not provided. Methods of statistical analysis not clearly mentioned. Embryo culture medium and catheter used for embryo transfer not described. Day of embryo transfer also unclear

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Unclear
Allocation concealment (selection bias)	Unclear risk	Unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not stated
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Details of withdrawals, dropouts not given.
Selective reporting (reporting bias)	Low risk	Reports live birth rate and multiple pregnancy rate per woman/couple
Other bias	Unclear risk	Day of embryo transfer also unclear

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

Study	Reason for exclusion
Bowman 2004	Non-randomised study of double blastocyst transfer versus single blastocyst plus frozen transfers. NB same publication also includes Livingstone 2001 (an RCT awaiting assessment).
Elgindy 2011	Compares cleavage versus blastocyst transfer. On average more embryos were transferred in the cleavage-stage group but this was not prespecified policy.
Forman 2012	Compares quantitative chromosome-screened SET versus morphology-based DET.

Study	Reason for exclusion
Frattarelli 2003	Compares cleavage versus blastocyst transfer. On average more embryos were transferred in the cleavage-stage group but this was not prespecified policy.
Gardner 1998	Compares cleavage versus blastocyst transfer. On average more embryos were transferred in the cleavage-stage group but this was not prespecified policy.
Guerif 2011	Not randomised controlled trial.
Heijnen 2007	The ovarian stimulation regimes used for the two randomised groups (SET versus DET) were significantly different.
Levitas 2004	Compares cleavage versus blastocyst transfer. On average more embryos were transferred in the cleavage-stage group but this was not prespecified policy.
Livingstone 2001	No comparison of interest - compares double cleavage-stage embryo versus single blastocyst-stage embryo. Mentioned in same paper as Bowman 2004.
Motta 1998 A & B	RCT comparing 3-5 cleavage-stage versus 1-3 blastocyst-stage embryos.
Moustafa 2008	Quasi-randomised trial - days of week used.
Pantos 2004	Compares cleavage versus blastocyst transfer. On average more embryos were transferred in the cleavage-stage group but this was not prespecified policy.
Staessen 1993	Not randomised controlled trial.
van Montfoort 2005	Not randomised controlled trial.

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

Obrado 2012

Methods	Randomised parallel-group study
Participants	Oocyte donor women aged 18-50 years in first or second donor cycle, with a minimum of 5 available embryos, women accepting transfer of frozen-thawed embryos
Interventions	DET versus SET
Outcomes	Cumulative live birth, cumulative pregnancy, multiple pregnancy
Notes	NCT01228474. This study was terminated due to the high number of multiple pregnancies in the double embryo transfer group, we have decided to stop the enrolment of patients at 07/31/2012. Have emailed author for data. Details of intervention unclear (e.g. whether cleavage or blastocyst-stage embryos used)

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

Abuzeid 2012

Trial name or title	Comparison of pregnancy rates following transfer of one embryo versus two in patients undergoing fertility treatment

Abuzeid 2012 *(Continued)*

Methods	RCT
Participants	Included: 1. Patients going through a cycle of IVF/ET 2. Have signed a consent form 3. Age 18 - 35 years old 4. Follicle-stimulating hormone (FSH) level on cycle day 2 or 3 < 10 Excluded: 1. Day 2 or 3 FSH level > 10 2. Previous history of poor response to stimulation drugs 3. Previous history of more than one failed IVF cycle
Interventions	Compares transfer of one blastocyst versus two
Outcomes	Delivery, pregnancy
Starting date	2008-2012
Contact information	Dr Mostafa Abuzeid
Notes	reprod1@hurleymc.com

Scott 2013

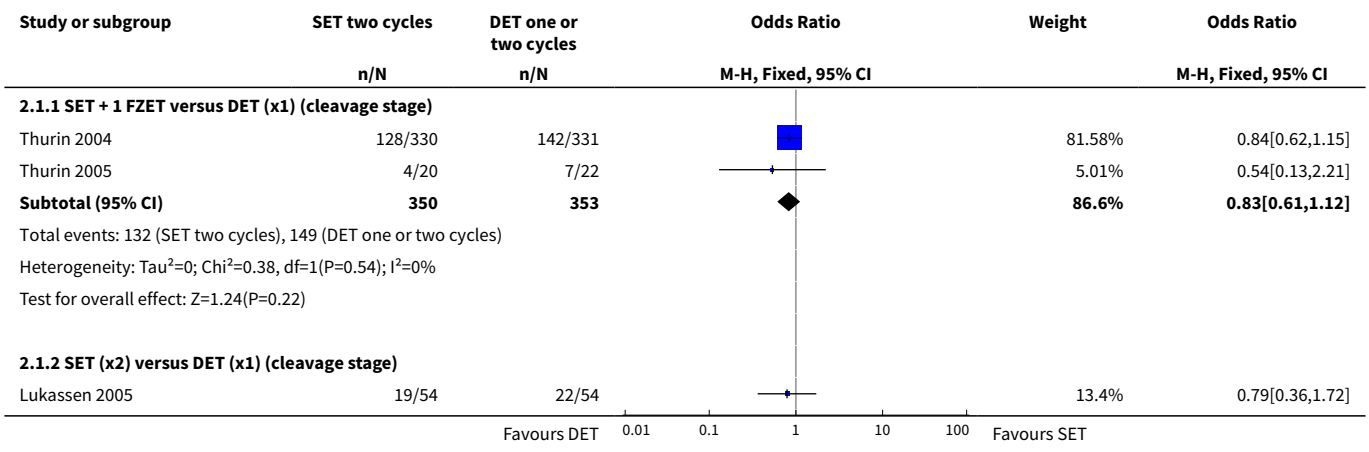
Trial name or title	Single Embryo Transfer of a Euploid Embryo Versus Double Embryo Transfer
Methods	Open-label RCT
Participants	Women undergoing IVF
Interventions	Patients who are a candidate for fresh transfer will be randomized into either the single embryo transfer of a chromosomally normal embryo group or the double, untested embryo group. Additional embryos will be cryopreserved. Patients in the double embryo transfer group will undergo a two embryo transfer. Additional embryos will be cryopreserved. If patients are not a candidate for a fresh transfer they will still be randomized into either the single or double embryo transfer group. and will have all embryos biopsied for CCS prior to being frozen. Patients will then immediately undergo a synthetic frozen embryo transfer cycle in accordance with their randomization. Any patient who does not become pregnant during their fresh transfer cycle will immediately undergo a synthetic frozen embryo transfer cycle in accordance with their original randomization.
Outcomes	Live birth, multiple pregnancy
Starting date	2011-15
Contact information	http://www.clinicaltrials.gov/ct2/show/NCT01408433?term=embryo+transfer&rank=1
Notes	Compares transfer of one tested embryo versus two untested embryos

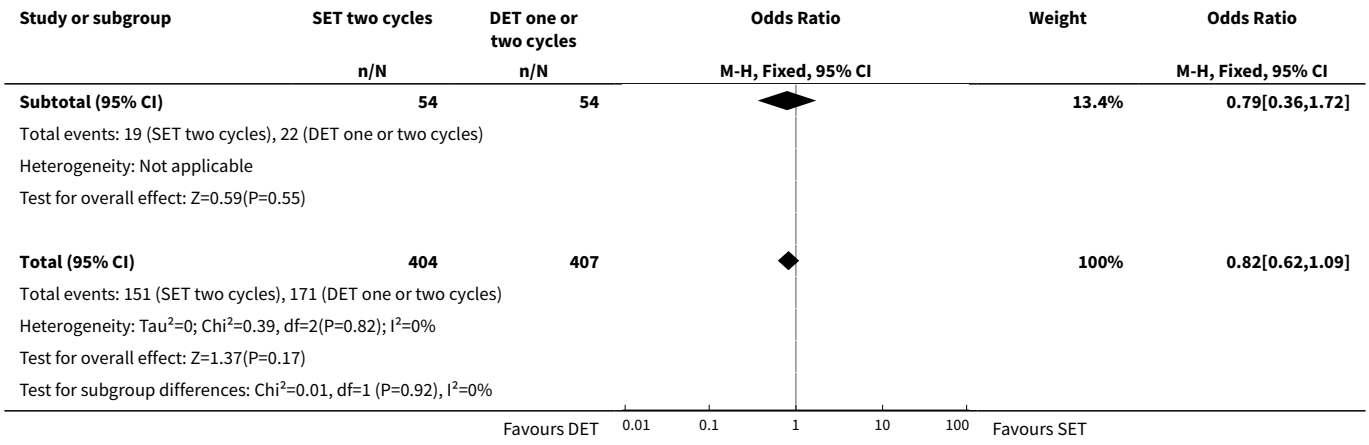
DATA AND ANALYSES

Comparison 2. Repeated single versus mixed policies

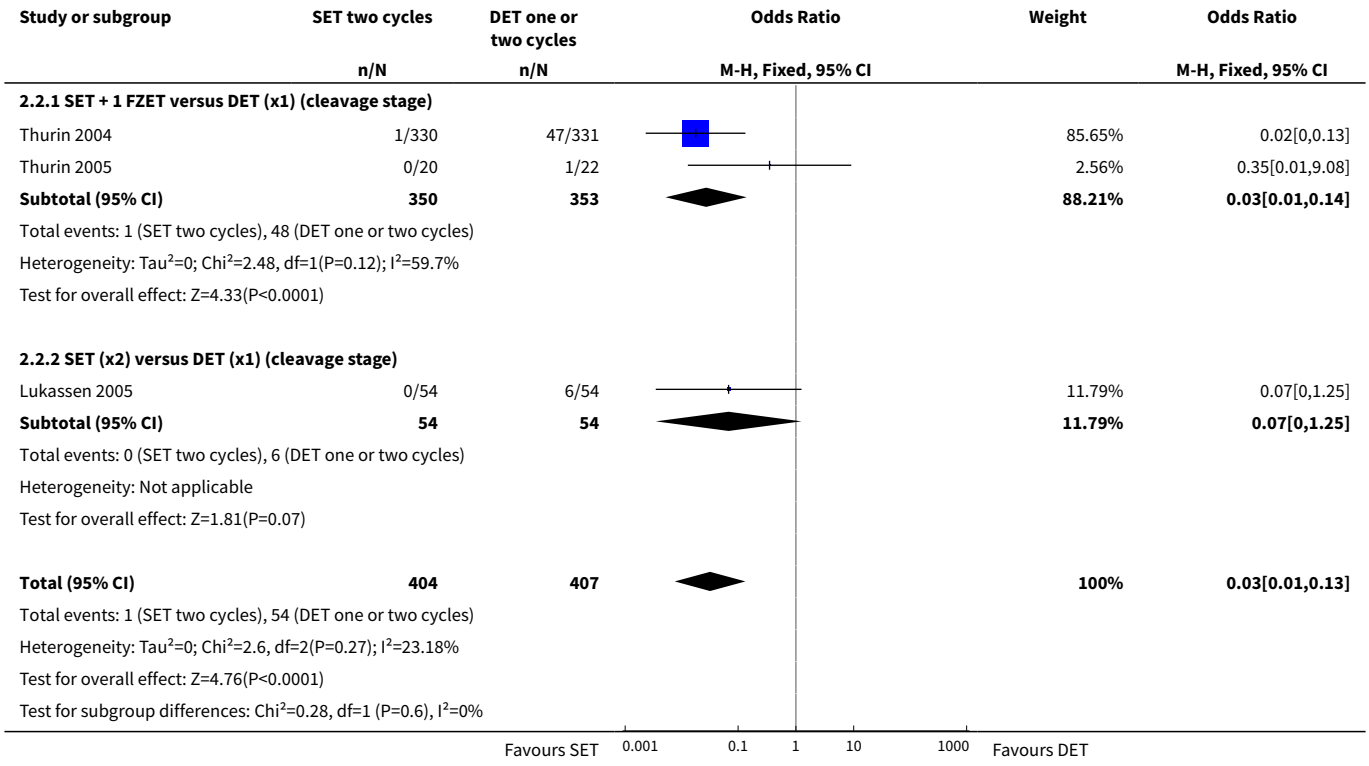
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Cumulative live birth	3	811	Odds Ratio (M-H, Fixed, 95% CI)	0.82 [0.62, 1.09]
1.1 SET + 1 FZET versus DET (x1) (cleavage stage)	2	703	Odds Ratio (M-H, Fixed, 95% CI)	0.83 [0.61, 1.12]
1.2 SET (x2) versus DET (x1) (cleavage stage)	1	108	Odds Ratio (M-H, Fixed, 95% CI)	0.79 [0.36, 1.72]
2 Multiple pregnancy	3	811	Odds Ratio (M-H, Fixed, 95% CI)	0.03 [0.01, 0.13]
2.1 SET + 1 FZET versus DET (x1) (cleavage stage)	2	703	Odds Ratio (M-H, Fixed, 95% CI)	0.03 [0.01, 0.14]
2.2 SET (x2) versus DET (x1) (cleavage stage)	1	108	Odds Ratio (M-H, Fixed, 95% CI)	0.07 [0.00, 1.25]
3 Clinical pregnancy rate	2	768	Odds Ratio (M-H, Fixed, 95% CI)	0.81 [0.61, 1.08]
3.1 SET + 1 FZET versus DET (x1) (cleavage stage)	1	661	Odds Ratio (M-H, Fixed, 95% CI)	0.83 [0.61, 1.12]
3.2 SET (x2) versus DET (x1) (cleavage stage)	1	107	Odds Ratio (M-H, Fixed, 95% CI)	0.71 [0.33, 1.53]
4 Miscarriage	1		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 SET (x2) versus DET (x1) (cleavage stage)	1	107	Odds Ratio (M-H, Fixed, 95% CI)	0.60 [0.18, 1.97]

Analysis 2.1. Comparison 2 Repeated single versus mixed policies, Outcome 1 Cumulative live birth.

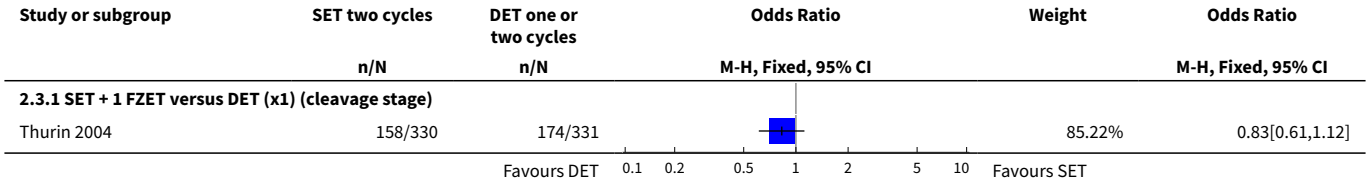


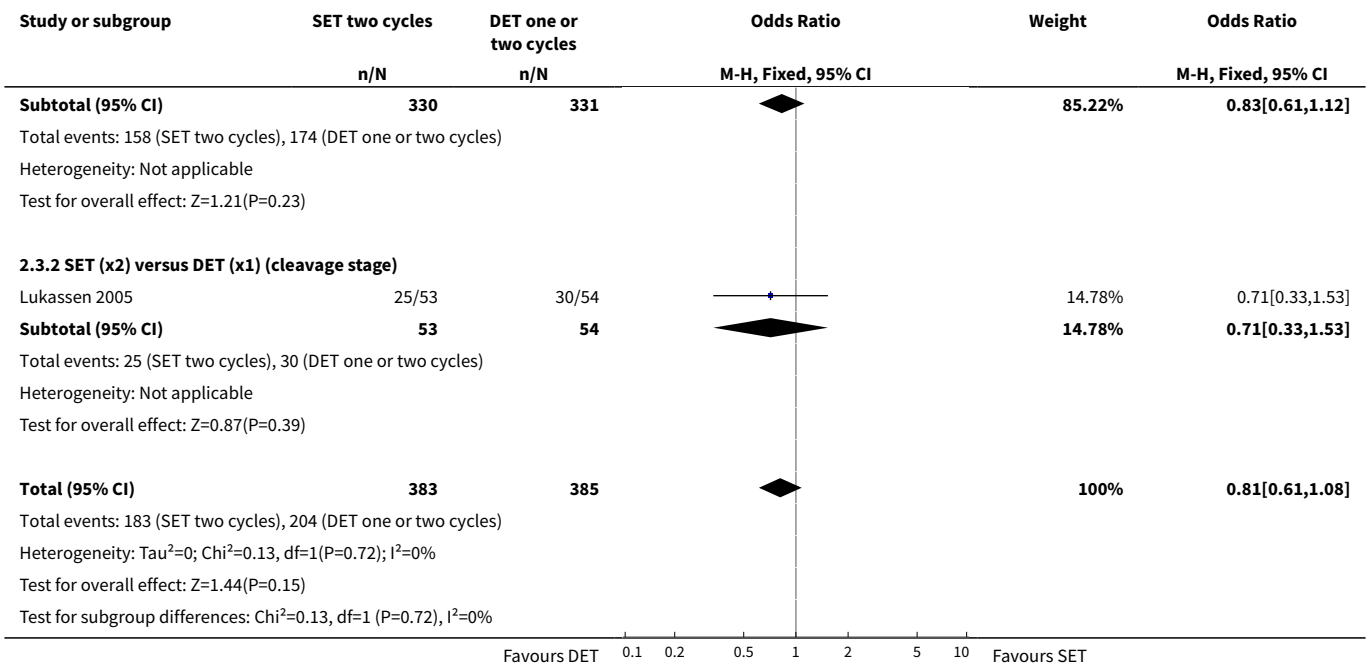


Analysis 2.2. Comparison 2 Repeated single versus mixed policies, Outcome 2 Multiple pregnancy.

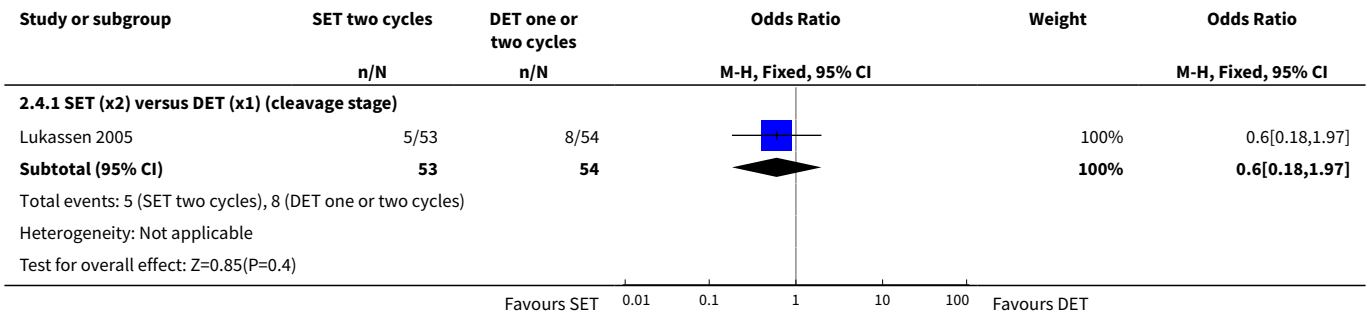


Analysis 2.3. Comparison 2 Repeated single versus mixed policies, Outcome 3 Clinical pregnancy rate.





Analysis 2.4. Comparison 2 Repeated single versus mixed policies, Outcome 4 Miscarriage.



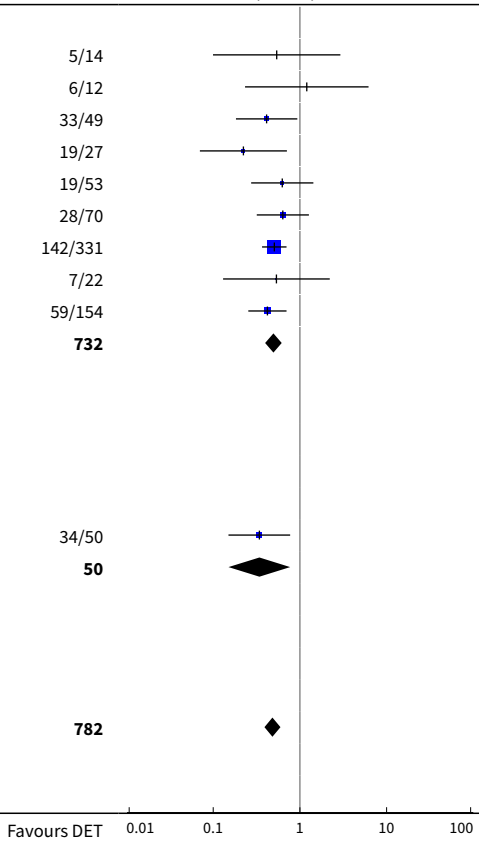
Comparison 3. Single versus multiple (in a single cycle)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth	9	1564	Odds Ratio (M-H, Fixed, 95% CI)	0.48 [0.39, 0.60]
1.1 SET (x1) versus DET (x1) (cleavage stage)	9	1464	Odds Ratio (M-H, Fixed, 95% CI)	0.49 [0.40, 0.62]
1.2 SET (x1) versus DET (x1) (blastocyst stage)	1	100	Odds Ratio (M-H, Fixed, 95% CI)	0.34 [0.15, 0.77]
2 Multiple pregnancy	10	1612	Odds Ratio (M-H, Fixed, 95% CI)	0.12 [0.07, 0.20]

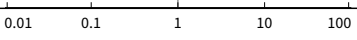
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 SET (x1) versus DET (x1) (cleavage stage)	9	1464	Odds Ratio (M-H, Fixed, 95% CI)	0.10 [0.05, 0.18]
2.2 SET (x1) versus DET (x1) (blastocyst stage)	2	148	Odds Ratio (M-H, Fixed, 95% CI)	0.25 [0.08, 0.72]
3 Clinical pregnancy rate	7	1521	Odds Ratio (M-H, Fixed, 95% CI)	0.46 [0.37, 0.57]
3.1 SET (x1) versus DET (x1) (cleavage stage)	6	1373	Odds Ratio (M-H, Fixed, 95% CI)	0.47 [0.37, 0.59]
3.2 SET (x1) versus DET (x1) (blastocyst stage)	2	148	Odds Ratio (M-H, Fixed, 95% CI)	0.37 [0.18, 0.76]
4 Miscarriage	3		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 SET (x1) versus DET (x1) (cleavage stage)	3	1113	Odds Ratio (M-H, Fixed, 95% CI)	0.85 [0.54, 1.34]

Analysis 3.1. Comparison 3 Single versus multiple (in a single cycle), Outcome 1 Live birth.

Study or subgroup	SET n/N	DET n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.1.1 SET (x1) versus DET (x1) (cleavage stage)					
ASSETT 2003	3/13	5/14		1.51%	0.54[0.1,2.93]
ECOSSE 2006	6/11	6/12		1.06%	1.2[0.23,6.19]
Fernandez-Sanchez 2012	23/50	33/49		7.33%	0.41[0.18,0.93]
Gerris 1999	9/26	19/27		4.97%	0.22[0.07,0.71]
Lukassen 2005	14/54	19/53		5.79%	0.63[0.27,1.43]
Martikainen 2001	22/74	28/70		8.24%	0.63[0.32,1.27]
Thurin 2004	91/330	142/331		41.84%	0.51[0.37,0.7]
Thurin 2005	4/20	7/22		2.17%	0.54[0.13,2.21]
van Montfoort 2006	32/154	59/154		19.05%	0.42[0.25,0.7]
Subtotal (95% CI)	**732**	**732**		**91.96%**	**0.49[0.4,0.62]**
Total events: 204 (SET), 318 (DET)					
Heterogeneity: Tau^2=0; Chi^2=4.37, df=8(P=0.82); I^2=0%					
Test for overall effect: Z=6.25(P<0.0001)					
3.1.2 SET (x1) versus DET (x1) (blastocyst stage)					
Fernandez-Sanchez 2012	21/50	34/50		8.04%	0.34[0.15,0.77]
Subtotal (95% CI)	**50**	**50**		**8.04%**	**0.34[0.15,0.77]**
Total events: 21 (SET), 34 (DET)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.58(P=0.01)					
Total (95% CI)	**782**	**782**		**100%**	**0.48[0.39,0.6]**
Total events: 225 (SET), 352 (DET)					
Heterogeneity: Tau^2=0; Chi^2=5.11, df=9(P=0.82); I^2=0%					

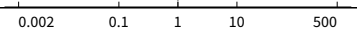


Study or subgroup	SET n/N	DET n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Test for overall effect: Z=6.71(P<0.0001)					
Test for subgroup differences: Chi²=0.74, df=1 (P=0.39), I²=0%					



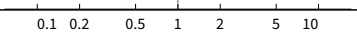
Analysis 3.2. Comparison 3 Single versus multiple (in a single cycle), Outcome 2 Multiple pregnancy.

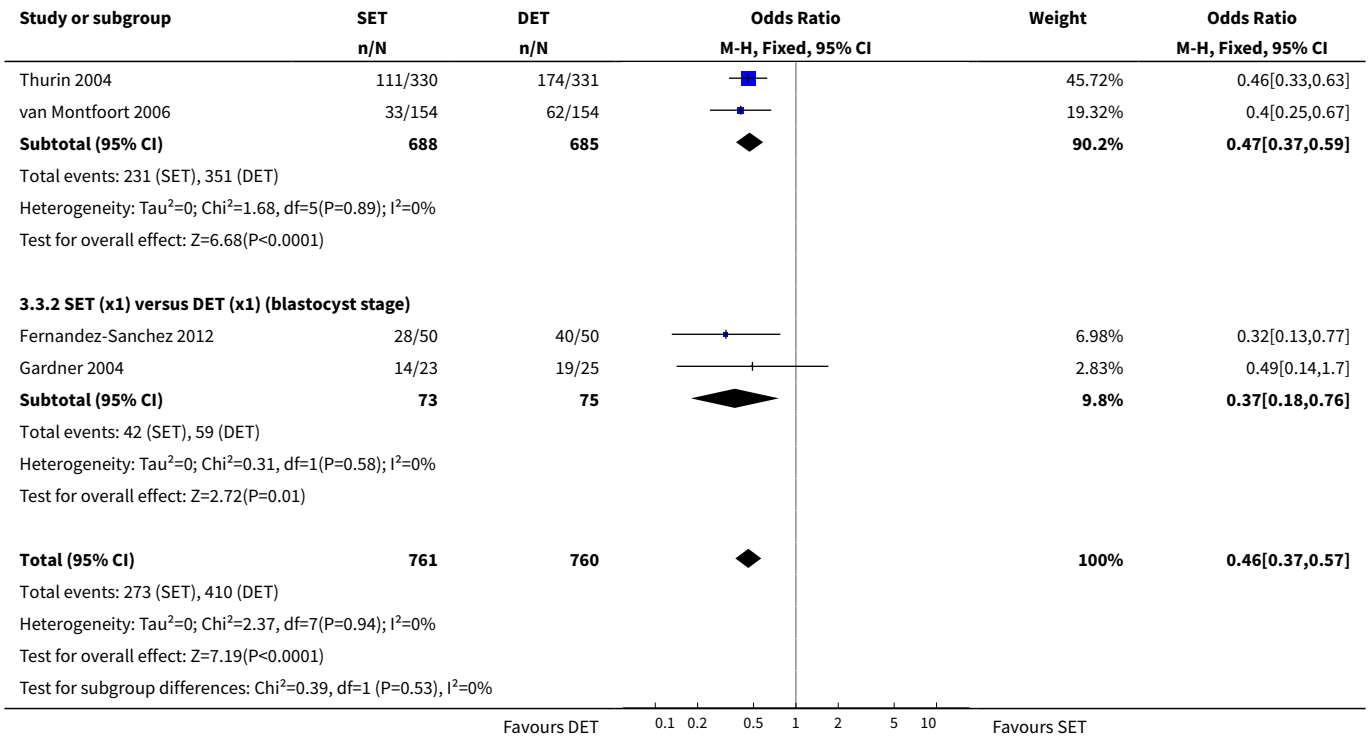
Study or subgroup	SET n/N	DET n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.2.1 SET (x1) versus DET (x1) (cleavage stage)					
ASSETT 2003	0/13	1/14		1.21%	0.33[0.01,8.93]
ECOSSE 2006	0/11	1/12		1.2%	0.33[0.01,9.07]
Fernandez-Sanchez 2012	7/50	13/49		9.79%	0.45[0.16,1.25]
Gerris 1999	1/26	6/27		4.91%	0.14[0.02,1.26]
Lukassen 2005	0/54	7/53		6.5%	0.06[0,1.02]
Martikainen 2001	1/74	11/70		9.66%	0.07[0.01,0.59]
Thurin 2004	1/330	47/331		40.55%	0.02[0,0.13]
Thurin 2005	0/20	1/22		1.21%	0.35[0.01,9.08]
van Montfoort 2006	0/154	13/154		11.66%	0.03[0,0.58]
Subtotal (95% CI)	**732**	**732**		**86.68%**	**0.1[0.05,0.18]**
Total events: 10 (SET), 100 (DET)					
Heterogeneity: Tau²=0; Chi²=13.77, df=8(P=0.09); I²=41.91%					
Test for overall effect: Z=7.33(P<0.0001)					
3.2.2 SET (x1) versus DET (x1) (blastocyst stage)					
Fernandez-Sanchez 2012	4/50	7/50		5.58%	0.53[0.15,1.95]
Gardner 2004	0/23	9/25		7.74%	0.04[0,0.68]
Subtotal (95% CI)	**73**	**75**		**13.32%**	**0.25[0.08,0.72]**
Total events: 4 (SET), 16 (DET)					
Heterogeneity: Tau²=0; Chi²=3.01, df=1(P=0.08); I²=66.73%					
Test for overall effect: Z=2.56(P=0.01)					
Total (95% CI)	**805**	**807**		**100%**	**0.12[0.07,0.2]**
Total events: 14 (SET), 116 (DET)					
Heterogeneity: Tau²=0; Chi²=18.21, df=10(P=0.05); I²=45.08%					
Test for overall effect: Z=7.86(P<0.0001)					
Test for subgroup differences: Chi²=2.07, df=1 (P=0.15), I²=51.77%					



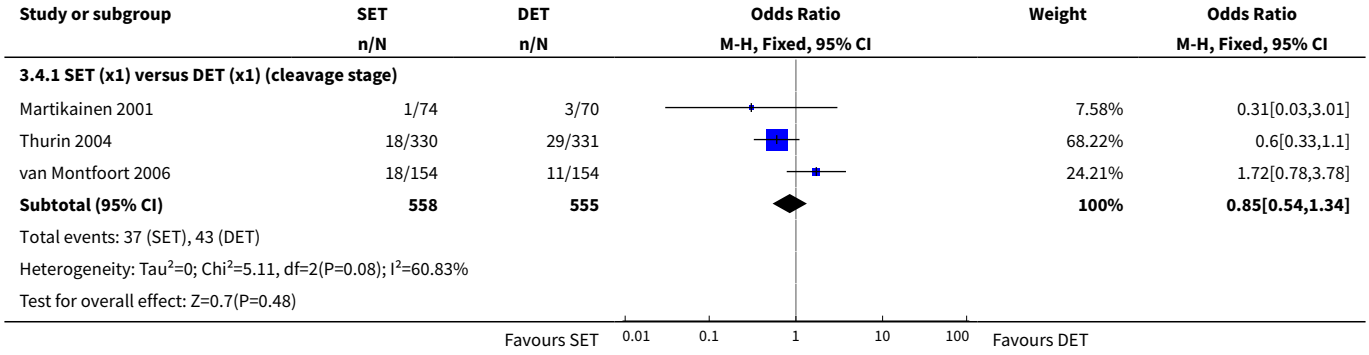
Analysis 3.3. Comparison 3 Single versus multiple (in a single cycle), Outcome 3 Clinical pregnancy rate.

Study or subgroup	SET n/N	DET n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
3.3.1 SET (x1) versus DET (x1) (cleavage stage)					
Fernandez-Sanchez 2012	29/50	36/50		6%	0.54[0.23,1.24]
Gerris 1999	14/26	21/27		3.77%	0.33[0.1,1.1]
Lukassen 2005	20/54	25/53		6.3%	0.66[0.3,1.43]
Martikainen 2001	24/74	33/70		9.09%	0.54[0.27,1.06]





Analysis 3.4. Comparison 3 Single versus multiple (in a single cycle), Outcome 4 Miscarriage.



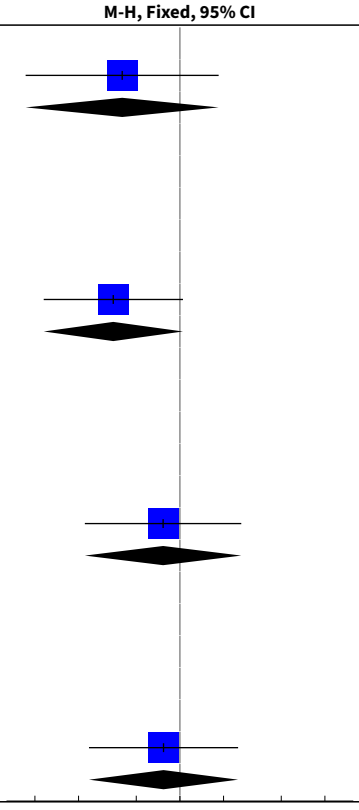
Comparison 4. Other fresh cycle comparisons

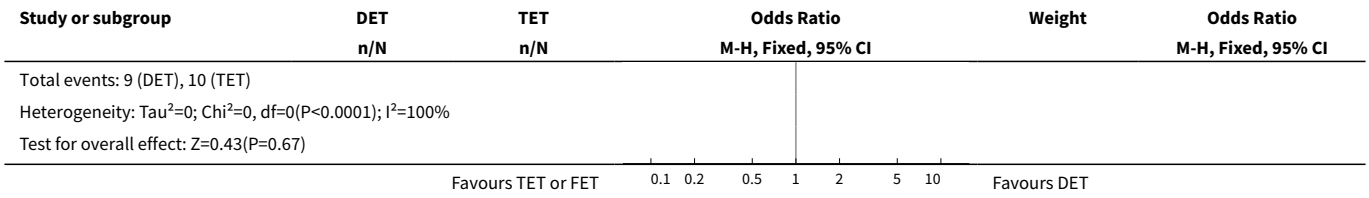
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live or cumulative live birth	2		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 DET (x1) versus TET (x1)	1	45	Odds Ratio (M-H, Fixed, 95% CI)	0.4 [0.09, 1.85]
1.2 DET (x1) versus FET (x1)	1	56	Odds Ratio (M-H, Fixed, 95% CI)	0.35 [0.11, 1.05]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.3 DET (x2) versus TET (x2)	1	45	Odds Ratio (M-H, Fixed, 95% CI)	0.77 [0.22, 2.65]
1.4 DET (x3) versus TET (x3)	1	45	Odds Ratio (M-H, Fixed, 95% CI)	0.77 [0.24, 2.52]
2 Multiple pregnancy	3		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 DET versus TET (cleavage stage)	2	343	Odds Ratio (M-H, Fixed, 95% CI)	0.36 [0.13, 0.99]
2.2 DET versus FET (cleavage stage)	1	56	Odds Ratio (M-H, Fixed, 95% CI)	0.44 [0.10, 1.97]
3 Clinical pregnancy	3		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 DET (x1) versus TET (x1) (cleavage stage)	2	343	Odds Ratio (M-H, Fixed, 95% CI)	0.67 [0.42, 1.08]
3.2 DET (x1) versus FET (x1) (cleavage stage)	1	56	Odds Ratio (M-H, Fixed, 95% CI)	0.56 [0.19, 1.62]

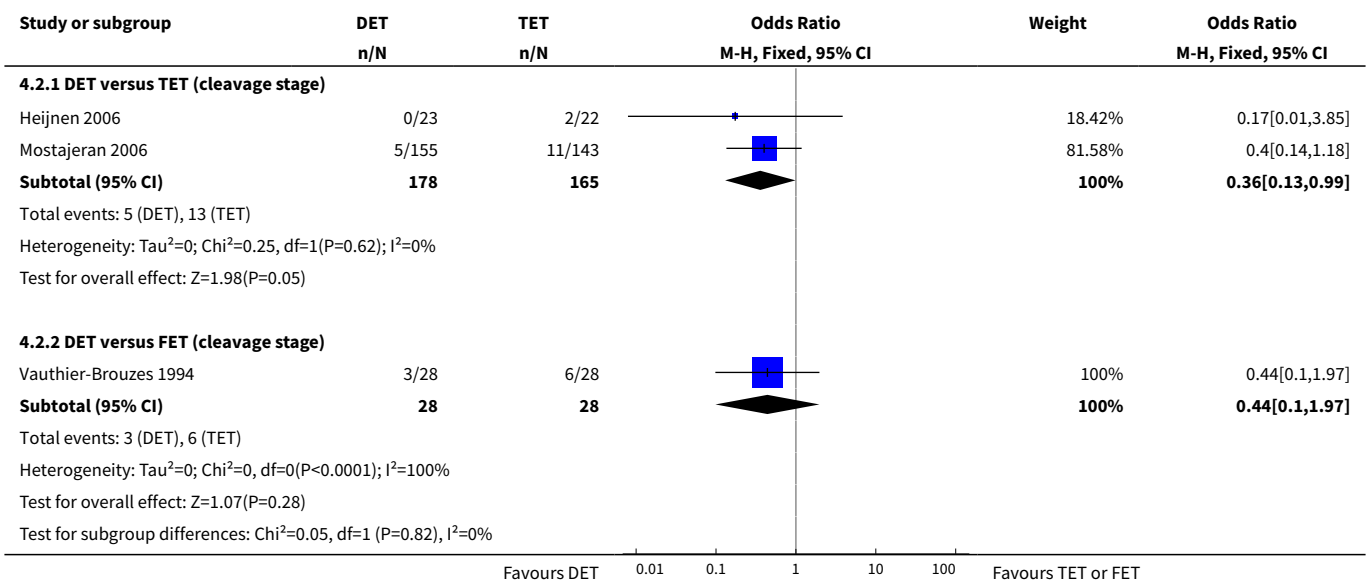
Analysis 4.1. Comparison 4 Other fresh cycle comparisons, Outcome 1 Live or cumulative live birth.

Study or subgroup	DET n/N	TET n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
4.1.1 DET (x1) versus TET (x1)					
Heijnen 2006	3/23	6/22		100%	0.4[0.09,1.85]
Subtotal (95% CI)	**23**	**22**		**100%**	**0.4[0.09,1.85]**
Total events: 3 (DET), 6 (TET)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.17(P=0.24)					
4.1.2 DET (x1) versus FET (x1)					
Vauthier-Brouzes 1994	8/28	15/28		100%	0.35[0.11,1.05]
Subtotal (95% CI)	**28**	**28**		**100%**	**0.35[0.11,1.05]**
Total events: 8 (DET), 15 (TET)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.88(P=0.06)					
4.1.3 DET (x2) versus TET (x2)					
Heijnen 2006	7/23	8/22		100%	0.77[0.22,2.65]
Subtotal (95% CI)	**23**	**22**		**100%**	**0.77[0.22,2.65]**
Total events: 7 (DET), 8 (TET)					
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%					
Test for overall effect: Z=0.42(P=0.67)					
4.1.4 DET (x3) versus TET (x3)					
Heijnen 2006	9/23	10/22		100%	0.77[0.24,2.52]
Subtotal (95% CI)	**23**	**22**		**100%**	**0.77[0.24,2.52]**

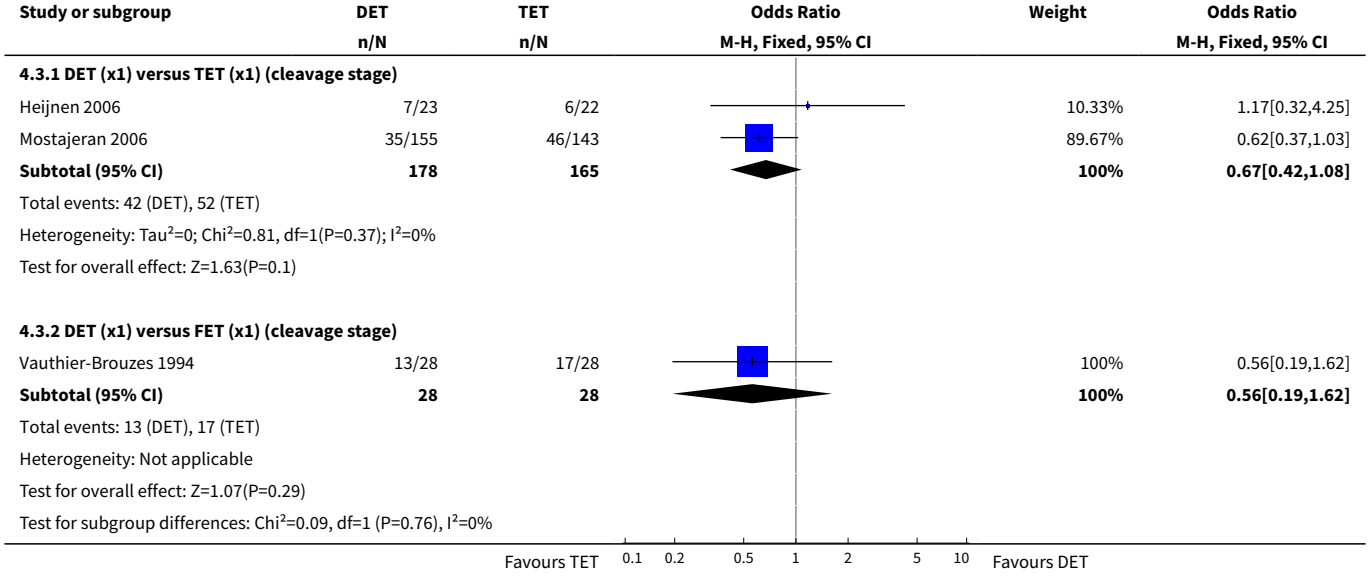




Analysis 4.2. Comparison 4 Other fresh cycle comparisons, Outcome 2 Multiple pregnancy.



Analysis 4.3. Comparison 4 Other fresh cycle comparisons, Outcome 3 Clinical pregnancy.



ADDITIONAL TABLES

Table 1. Prognostic factors in included studies

Study author and year	Age Eligibility criteria (mean participant age, where stated)	Duration of infertility	Previous failed cycle	Frozen cycles	Prim/Sec infertility	FSH	Quality of embryo
Fernandez-Sanchez 2012	Under 38 years (mean age 33)	Mean 2.6 to 3.2 years	First IVF/ICSI cycle.	Frozen cycles included	Not stated	Not stated	good
Gerris 1999	less than 34 years	Average duration of infertility 3.5 years.	First IVF/ICSI cycle.	Not included	unclear	not mentioned	good
Heijnen 2007	38-45 years (mean age 41)	Average duration of infertility in DET group was 3.7(+/- 2.5) and in TET group was 3.2(+/- 2.4)	First cycle and previous successful cycle	Not included	yes	not mentioned	good
Komori 2004	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Good
Lukassen 2005	<35 years (mean age 30-31)	not stated	First IVF/ICSI cycle or after previous successful cycle .	Not included	yes	FSH < 10IU/L.	good
Martikainen 2001	various, no age criteria, ranged between 22-40 years (mean age 31)	not stated	women who had / not had more than one previous failed treatment.	Frozen cycles included	yes, but not mentioned	not mentioned	good
Mostajeran 2006	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Good
Thurin 2004	<36 years (mean age 31)	0-12 years	First or second IVF cycle	Frozen cycles included	yes	not mentioned	good, blastocysts included
Thurin 2005 Unpublished trial, pilot study, part of a thesis	≥36 years	0-12 years	First or second IVF/ICSI cycle	Frozen cycles included	yes	not mentioned	At least two good quality embryos available
van Montfoort 2006	Various ages, no criteria (mean age 33)	SET- 3.3+/-1.8, DET- 3.3+/- 2.1	First IVF cycle	Not included	yes	not mentioned	good

Table 1. Prognostic factors in included studies *(Continued)*

Vauthier-Brouzes 1994	≤35 years	Not mentioned	First or previous successful cycle	Frozen cycles included	yes	not mentioned	good
ASSETT 2003 unpublished trial	Female age <35 if no previous ART pregnancy, <40 if previous ART pregnancy.	Not mentioned	First or previous successful cycle	Frozen cycles included	yes	not mentioned	At least four good quality embryos or at least three if previous ART pregnancy successful
ECOSSE 2006 unpublished trial	≤37 years	Not mentioned	first or second cycle of treatment	frozen cycles included	yes	not mentioned	4 or more good quality embryos available at the time of embryo transfer

APPENDICES

Appendix 1. MDSG search string

The following Medical Subject Headings (MeSH terms) and all combinations of these words were used: embryo transfer, multiple pregnancy, IVF, in vitro fertil$, ICSI, intra cytoplasmic sperm injection, infertility, subfertility, single/one embryo, two/double embryo, three/four/multiple embryos, effectiveness, ART, Assisted reprod$ techn$, randomised controlled trial, clinical trial.

Appendix 2. MEDLINE search

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Nov 2012>

1 Embryo Transfer/ (11908)

2 (Embryo$ adj5 Transfer$).tw. (12767)

3 (blastocyst$ adj5 transfer$).tw. (1490)

4 exp embryo, mammalian/ or exp blastocyst/ (75947)

5 or/1-4 (89708)

6 (two adj2 embryo$).tw. (2775)

7 (double adj2 embryo$).tw. (431)

8 DET.tw. (591)

9 (three adj2 embryo$).tw. (1449)

10 (triple adj2 embryo$).tw. (32)

11 TET$.tw. (321295)

12 (two adj2 blastocyst$).tw. (168)

13 (double adj2 blastocyst$).tw. (13)

14 (three adj2 blastocyst$).tw. (77)

15 (triple adj2 blastocyst$).tw. (3)

16 DBT.tw. (1071)

17 TBT.tw. (1157)

18 (one adj2 embryo$).tw. (1781)

19 (single adj2 embryo$).tw. (1179)

20 SET.tw. (286512)

21 (one adj2 blastocyst$).tw. (141)

22 (single adj2 blastocyst$).tw. (170)

23 SBT.tw. (1139)

24 (four adj2 embryo$).tw. (727)

25 (four adj2 blastocyst$).tw. (55)

26 FET.tw. (1134)

27 FZET.tw. (0)

28 (multiple$ adj2 embryo$).tw. (448)

29 (multiple$ adj2 blastocyst$).tw. (9)

30 (quadruple adj2 embryo$).tw. (4)

31 (quadruple adj2 blastocyst$).tw. (1)

32 or/6-31 (614694)

33 5 and 32 (6467)

34 randomized controlled trial.pt. (342319)

35 controlled clinical trial.pt. (85680)

36 randomized.ab. (257751)

37 placebo.tw. (145527)

38 clinical trials as topic.sh. (163663)

39 randomly.ab. (187932)

40 trial.ti. (110917)

41 (crossover or cross-over or cross over).tw. (55492)

42 or/34-41 (838746)

43 exp animals/ not humans.sh. (3809972)

44 42 not 43 (773670)

45 33 and 44 (251)

Appendix 3. EMBASE search

Database: Embase <1980 to 2012 Week 45>

Search Strategy:

---

1 Embryo Transfer/ (18351)

2 (Embryo$ adj5 Transfer$).tw. (16394)

3 (blastocyst$ adj5 transfer$).tw. (2079)

4 exp embryo, mammalian/ or exp blastocyst/ (49471)

5 or/1-4 (69463)

6 (two adj2 embryo$).tw. (3029)

7 (double adj2 embryo$).tw. (539)

8 DET.tw. (881)

9 (three adj2 embryo$).tw. (1624)

10 (triple adj2 embryo$).tw. (35)

11 TET$.tw. (351760)

12 (two adj2 blastocyst$).tw. (204)

13 (double adj2 blastocyst$).tw. (27)

14 (three adj2 blastocyst$).tw. (84)

15 (triple adj2 blastocyst$).tw. (4)

16 DBT.tw. (1414)

17 TBT.tw. (1560)

18 (one adj2 embryo$).tw. (2077)

19 (single adj2 embryo$).tw. (1717)

20 SET.tw. (332164)

21 (one adj2 blastocyst$).tw. (176)

22 (single adj2 blastocyst$).tw. (298)

23 SBT.tw. (1713)

24 (four adj2 embryo$).tw. (780)

25 (four adj2 blastocyst$).tw. (70)

26 FET.tw. (1399)

27 FZET.tw. (0)

28 (multiple$ adj2 embryo$).tw. (584)

29 (multiple$ adj2 blastocyst$).tw. (12)

30 (quadruple adj2 embryo$).tw. (6)

31 (quadruple adj2 blastocyst$).tw. (2)

32 or/6-31 (693129)

33 5 and 32 (6682)

34 Clinical trial/ (873896)

35 Randomized controlled trials/ (22276)

36 Random Allocation/ (59995)

37 Single-Blind Method/ (16629)

38 Double-Blind Method/ (111820)

39 Cross-Over Studies/ (35508)

40 Placebos/ (207982)

41 Randomi?ed controlled trial$.tw. (80804)

42 RCT.tw. (10393)

43 Random allocation.tw. (1194)

44 Randomly allocated.tw. (17927)

45 Allocated randomly.tw. (1847)

46 (allocated adj2 random).tw. (713)

47 Single blind$.tw. (12761)

48 Double blind$.tw. (132065)

49 ((treble or triple) adj blind$).tw. (288)

50 Placebo$.tw. (181564)

51 Prospective Studies/ (218579)

52 or/34-51 (1286058)

53 Case study/ (17669)

54 Case report.tw. (234557)

55 Abstract report/ or letter/ (850195)

56 or/53-55 (1097609)

57 52 not 56 (1249761)

58 animal/ (1801042)

59 human/ (13859436)

60 58 not 59 (1346252)

61 57 not 60 (1224281)

62 33 and 61 (498)

63 (2011$ or 2012$).em. (2193008)

64 62 and 63 (88)

Appendix 4. CENTRAL search

1 Embryo Transfer/ (736)

2 (Embryo$ adj5 Transfer$).tw. (1117)

3 (blastocyst$ adj5 transfer$).tw. (92)

4 exp embryo, mammalian/ or exp blastocyst/ (506)

5 or/1-4 (1702)

6 (two adj2 embryo$).tw. (96)

7 (double adj2 embryo$).tw. (21)

8 DET.tw. (46)

9 (three adj2 embryo$).tw. (62)

10 (triple adj2 embryo$).tw. (2)

11 TET$.tw. (4765)

12 (two adj2 blastocyst$).tw. (7)

13 (double adj2 blastocyst$).tw. (1)

14 (three adj2 blastocyst$).tw. (3)

15 (triple adj2 blastocyst$).tw. (0)

16 DBT.tw. (61)

17 TBT.tw. (17)

18 (one adj2 embryo$).tw. (44)

19 (single adj2 embryo$).tw. (52)

20 SET.tw. (6351)

21 (one adj2 blastocyst$).tw. (4)

22 (single adj2 blastocyst$).tw. (17)

23 SBT.tw. (81)

24 (four adj2 embryo$).tw. (23)

25 (four adj2 blastocyst$).tw. (1)

26 FET.tw. (72)

27 FZET.tw. (0)

28 (multiple$ adj2 embryo$).tw. (13)

29 (multiple$ adj2 blastocyst$).tw. (1)

30 (quadruple adj2 embryo$).tw. (0)

31 (quadruple adj2 blastocyst$).tw. (0)

32 or/6-31 (11591)

33 5 and 32 (255)

Appendix 5. CINAHL search

1 Embryo Transfer/ (96)
2 (Embryo$ adj5 Transfer$).tw. (90)

3 (blastocyst$ adj5 transfer$).tw. (9)
4 or/1-3 (154)
5 (single embryo$ or one embryo$).tw. (14)
6 (two embryo$ or double embryo$).tw. (7)
7 (three embryo$ or four embryo$).tw. (5)
8 (multiple embryo$ or (number adj5 embryo$)).tw. (28)
9 or/5-8 (44)
10 4 and 9 (30)
11 from 10 keep 1-30 (30)

Appendix 6. PsycINFO search

Database: PsycINFO <1806 to July 17 2013>

1 exp Infertility/ or exp Reproductive Technology/ (2388)

2 (Embryo$ adj5 Transfer$).tw. (121)

3 (blastocyst$ adj5 transfer$).tw. (3)

4 or/1-3 (2434)

5 (two adj2 embryo$).tw. (27)

6 (double adj2 embryo$).tw. (6)

7 DET.tw. (88)

8 (three adj2 embryo$).tw. (10)

9 (triple adj2 embryo$).tw. (0)

10 (two adj2 blastocyst$).tw. (0)

11 (three adj2 blastocyst$).tw. (0)

12 (one adj2 embryo$).tw. (22)

13 (single adj2 embryo$).tw. (13)

14 (four adj2 embryo$).tw. (7)

15 or/5-14 (165)

Appendix 7. CINAHL search strategy

#	Query	Results
S28	S12 AND S26	86
S27	S12 AND S26	124
S26	S13 OR S14 or S15 or S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25	Display
S25	TX allocat* random*	Display
S24	(MH "Quantitative Studies")	Display

(Continued)

S23	(MH "Placebos")	Display
S22	TX placebo*	Display
S21	TX random* allocat*	Display
S20	(MH "Random Assignment")	Display
S19	TX randomi* control* trial*	Display
S18	TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))	Display
S17	TX ((trebl* n1 blind*) or (trebl* n1 mask*))	Display
S16	TX ((trebl* n1 blind*) or (trebl* n1 mask*))	Display
S15	TX clinic* n1 trial*	Display
S14	PT Clinical trial	Display
S13	(MH "Clinical Trials+")	Display
S12	S3 AND S11	306
S11	S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10	1,189,815
S10	TX three	165,919
S9	TX triple	3,133
S8	TX two	258,377
S7	TX double	670,325
S6	TX multiple	139,923
S5	TX one	264,468
S4	TX single	73,642
S3	S1 OR S2	530
S2	"blastocyst transfer"	23
S1	(MM "Embryo Transfer") OR "embryo transfer"	526

WHAT'S NEW

Date	Event	Description
1 June 2014	Amended	Analyses of single embryo transfer versus double embryo transfer changed so that single embryo transfer is now regarded as

Date	Event	Description
		the intervention and double embryo transfer as the control, in order to make the nature of the comparison more clinically appropriate. Text and summary of findings table edited accordingly. Errors in display of some of tables of analysis corrected in order to show OR consistently. Assessed as up to date and Search dates corrected.

HISTORY

Protocol first published: Issue 1, 2002
Review first published: Issue 4, 2004

Date	Event	Description
25 July 2013	New citation required but conclusions have not changed	No change to conclusions
25 July 2013	New search has been performed	The search was updated to July 2013 Comparisons of different numbers of blastocysts were added (previously only cleavage-stage embryo comparisons were included) Seven extra completed studies were included (ASSETT 2003, Gardner 2004, Komori 2004, Thurin 2005, ECOSSE 2006, Mostajeran 2006, Manuel-Fernandez 2012) The structure of the table of comparisons was reformatted
29 August 2011	New search has been performed	Review updated Aug 2011. Objective- wording has been changed Three unplished trials (ASSETT 2003; Thurin 2005; ECOSSE 2006) have been added to comparison 1 Comparison 2 has been changed to DET vs SET (2 or more cycles), the sub comparisons now include DET vs 2 fresh SET, DET vs SET plus 1FZET, DET plus FZET vs SET +FZET and has additional data from 2 unpublished trials. The original Comparison 3 from previous review has therefore been deleted and included in comparison 2. Comparison 5 from previous review has also been deleted and added to comparison 4 of the updated review. This updated review will have 3 comparisons.
8 May 2008	Amended	Converted to new review format.
8 May 2008	New search has been performed	A new literature search was performed on 30/03/2008 by two reviewers independently (ZP, OO). Five new trials were identified using the Cochrane search strategy for identifying new trials.Search redesigned and run March 2008 . Three new trials were added to the review. One trial (Thurin 2004) included blastocyst transfers. Blastocyst transfers were excluded from the data analysed.

Date	Event	Description
		Two trials (Thurin2004 and van Montfoort 2006) compared one embryo transfer versus two embryo transfer. One trial (Thurin 2004) also compared one embryo transfer followed by a frozen-thawed single embryo transfer versus two embryo transfer. Livebirth rates from Van Montfoort 2006 study was derived from another publication from the same study and appears as van Montfoort* 2006 in the review and references. A single trial (Heijnen 2006) compared two embryo transfer versus three embryo transfer. The trial also determined the cumulative effect of multiple transfers of two and three embryos. A trial included in the original review (Lukassen 2002) that compared single embryo transfer versus double embryo was updated and published in 2005. This review has also been updated with this trial. Two trials (Komori 2004; Mostajeran 2006) that compared three embryo transfer versus two embryo transfer were identified with the new literature search but were excluded as the method of randomisation was unclear in both trials. The review has been converted into the new Rev man 5 format. The order of appearance of the comparisons have been changed. Two additional tables (1, 2) has been added.
12 June 2007	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

Zabeena Pandian: checked literature searches, data extraction, study selection, quality assessment, entered and checked data, data analysis, completed and checked risk of bias tables, wrote the first draft of the review.

Jane Marjoribanks: 2013 update of literature search, redrafted text and comparison tables, added new studies and summary of findings table, entered and checked data, completed and checked risk of bias tables.

Ozturk Ozkan: development of protocol, literature search in 2009.

Gamal Serour: revised the final draft of the 2009 review.

Siladitya Bhattacharya: study selection, quality assessment, responsible for final draft of the review.

DECLARATIONS OF INTEREST

None

SOURCES OF SUPPORT

Internal sources

- Department of Obstetrics & Gynaecology, University of Aberdeen, UK.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The structure of the comparisons was reformatted to prioritise comparisons of repeat single embryo transfer. Live birth and cumulative live birth rates were amalgamated as a single primary outcome.

Studies of blastocyst transfer were added (previously only cleavage-stage transfers included).

INDEX TERMS

Medical Subject Headings (MeSH)

*Fertilization in Vitro; *Pregnancy Rate; Blastocyst; Cleavage Stage, Ovum [transplantation]; Embryo Transfer [*adverse effects] [*methods]; Pregnancy, Multiple; Randomized Controlled Trials as Topic; Sperm Injections, Intracytoplasmic

MeSH check words

Female; Humans; Pregnancy