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Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology (Review)

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[Intervention Review]

Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology

Demián Glujovsky[1], Andrea Marta Quinteiro Retamar[2], Cristian Roberto Alvarez Sedo[3], Agustín Ciapponi[4], Simone Cornelisse[5], Deborah Blake[6]

[1]Reproductive Medicine, CEGYR (Centro de Estudios en Genética y Reproducción), Buenos Aires, Argentina. [2]Eggs donation program – Genetics unit, CEGYR (Centro de Estudios en Ginecologia y Reproducción), Buenos Aires, Argentina. [3]Laboratory of Reproductive Biology and Genetics, CEGYR, Buenos Aires, Argentina. [4]Argentine Cochrane Centre, Instituto de Efectividad Clínica y Sanitaria (IECS-CONICET), Buenos Aires, Argentina. [5]Department of Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands. [6]Repromed Fertility Specialists, Auckland, New Zealand

Contact: Demián Glujovsky, demian.glujovsky@gmail.com.

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ABSTRACT

Background

Advances in embryo culture media have led to a shift in in vitro fertilisation (IVF) practice from cleavage-stage embryo transfer to blastocyst-stage embryo transfer. The rationale for blastocyst-stage transfer is to improve both uterine and embryonic synchronicity and enable self selection of viable embryos, thus resulting in better live birth rates.

Objectives

To determine whether blastocyst-stage (day 5 to 6) embryo transfer improves the live birth rate (LBR) per fresh transfer, and other associated outcomes, compared with cleavage-stage (day 2 to 3) embryo transfer.

Search methods

We searched the Cochrane Gynaecology and Fertility Group Specialised Register of controlled trials, CENTRAL, MEDLINE, Embase, PsycINFO, and CINAHL, from inception to October 2021. We also searched registers of ongoing trials and the reference lists of studies retrieved.

Selection criteria

We included randomised controlled trials (RCTs) which compared the effectiveness of IVF with blastocyst-stage embryo transfer versus IVF with cleavage-stage embryo transfer.

Data collection and analysis

We used standard methodological procedures recommended by Cochrane. Our primary outcomes were LBR per fresh transfer and cumulative clinical pregnancy rates (cCPR). Secondary outcomes were clinical pregnancy rate (CPR), multiple pregnancy, high-order multiple pregnancy, miscarriage (all following first embryo transfer), failure to transfer embryos, and whether supernumerary embryos were frozen for transfer at a later date (frozen-thawed embryo transfer). We assessed the overall quality of the evidence for the main comparisons using GRADE methods.

Main results

We included 32 RCTs (5821 couples or women).

The live birth rate following fresh transfer was higher in the blastocyst-stage transfer group (odds ratio (OR) 1.27, 95% confidence interval (CI) 1.06 to 1.51; I^2 = 53%; 15 studies, 2219 women; low-quality evidence). This suggests that if 31% of women achieve live birth after fresh cleavage-stage transfer, between 32% and 41% would do so after fresh blastocyst-stage transfer.

We are uncertain whether blastocyst-stage transfer improves the cCPR. A post hoc analysis showed that vitrification could increase the cCPR. This is an interesting finding that warrants further investigation when more studies using vitrification are published.

The CPR was also higher in the blastocyst-stage transfer group, following fresh transfer (OR 1.25, 95% CI 1.12 to 1.39; I^2 = 51%; 32 studies, 5821 women; moderate-quality evidence). This suggests that if 39% of women achieve a clinical pregnancy after fresh cleavage-stage transfer, between 42% and 47% will probably do so after fresh blastocyst-stage transfer.

We are uncertain whether blastocyst-stage transfer increases multiple pregnancy (OR 1.05, 95% CI 0.83 to 1.33; I^2 = 30%; 19 studies, 3019 women; low-quality evidence) or miscarriage rates (OR 1.12, 95% CI 0.90 to 1.38; I^2 = 24%; 22 studies, 4208 women; low-quality evidence). This suggests that if 9% of women have a multiple pregnancy after fresh cleavage-stage transfer, between 8% and 12% would do so after fresh blastocyst-stage transfer. However, a sensitivity analysis restricted only to studies with low or 'some concerns' for risk of bias, in the subgroup of equal number of embryos transferred, showed that blastocyst transfer probably increases the multiple pregnancy rate.

Embryo freezing rates (when there are frozen supernumerary embryos for transfer at a later date) were lower in the blastocyst-stage transfer group (OR 0.48, 95% CI 0.40 to 0.57; I^2 = 84%; 14 studies, 2292 women; low-quality evidence). This suggests that if 60% of women have embryos frozen after cleavage-stage transfer, between 37% and 46% would do so after blastocyst-stage transfer.

Failure to transfer any embryos was higher in the blastocyst transfer group (OR 2.50, 95% CI 1.76 to 3.55; I^2 = 36%; 17 studies, 2577 women; moderate-quality evidence). This suggests that if 1% of women have no embryos transferred in planned fresh cleavage-stage transfer, between 2% and 4% probably have no embryos transferred in planned fresh blastocyst-stage transfer.

The evidence was of low quality for most outcomes. The main limitations were serious imprecision and serious risk of bias, associated with failure to describe acceptable methods of randomisation.

Authors' conclusions

There is low-quality evidence for live birth and moderate-quality evidence for clinical pregnancy that fresh blastocyst-stage transfer is associated with higher rates of both than fresh cleavage-stage transfer. We are uncertain whether blastocyst-stage transfer improves the cCPR derived from fresh and frozen-thawed cycles following a single oocyte retrieval. Although there is a benefit favouring blastocyst-stage transfer in fresh cycles, more evidence is needed to know whether the stage of transfer impacts on cumulative live birth and pregnancy rates. Future RCTs should report rates of live birth, cumulative live birth, and miscarriage. They should also evaluate women with a poor prognosis to enable those undergoing assisted reproductive technology (ART) and service providers to make well-informed decisions on the best treatment option available.

PLAIN LANGUAGE SUMMARY

When trying to have a baby through assisted conception, is it better to transfer the embryo to the womb on day 3 or day 5?

Background

Many women and couples are unlikely to get pregnant and have a baby without medical treatment, due to infertility. Doctors have developed a variety of assisted reproductive technologies (ARTs), such as in vitro fertilisation (IVF), which involve the manipulation of eggs and sperm outside a woman's body, to try to increase her chances of getting pregnant.

Typically, in assisted conception, doctors collect eggs from a woman and fertilise them in a laboratory, leading to the formation of embryos. An embryo is the early stage of human development. Doctors commonly transfer one or several embryos into a woman’s womb (uterus) at one of two stages of embryo development: either the cleavage stage, which is 2 or 3 days after egg collection when an embryo typically consists of between 2 and 128 cells; or the blastocyst stage, which is 5 or 6 days after egg collection when an embryo consists of between 70 and 100 cells.

Until recently, doctors usually transferred embryos at the earlier, cleavage, stage. However, there has been a trend to transferring embryos at the later, blastocyst, stage. Researchers believe that only those embryos capable of surviving make it to the blastocyst stage; in other words, viable embryos will self-select. So, it is thought that transferring embryos at the later stage may improve a woman's chances of becoming pregnant and having a healthy baby.

Review question

We wanted to find out if transferring embryos into a woman's womb at cleavage stage (day 2 to 3) or blastocyst stage (day 5 to 6) is better, in terms of:

– number of babies born alive (live birth rate) following embryo transfers using only 'fresh' embryos; that is, embryos that have not been frozen and subsequently thawed;

– total number of pregnancies achieved following embryo transfers using both 'fresh' and frozen then thawed embryos, collected from a single egg collection procedure (cumulative clinical pregnancy rate);

– multiple pregnancy rate (when a woman is carrying more than one baby at a time);

- miscarriage rate (the loss of a pregnancy before the 20th week of development in the womb).

Study characteristics

We included 32 randomised controlled trials (studies in which participants are assigned randomly to 2 or more treatment groups), which included 5821 women or couples. The evidence is current to October 2021.

Key results

– Transferring 'fresh' embryos at the blastocyst stage (day 5 to 6) may lead to more live births than when 'fresh' embryos are transferred at the cleavage stage (day 2 to 3). This suggests that if 31% of women achieve live birth after 'fresh' cleavage-stage embryo transfer, between 32% and 41% would do so after 'fresh' blastocyst-stage transfer.

– Transferring 'fresh' embryos at the blastocyst stage probably leads to more clinical pregnancies – defined as evidence of fetal heart activity on an ultrasound scan – than when 'fresh' embryos are transferred at the cleavage stage. This suggests that if 39% of women achieve a clinical pregnancy after 'fresh' cleavage-stage transfer, between 42% and 47% will probably do so after 'fresh' blastocyst-stage transfer.

– We are uncertain whether blastocyst-stage transfer favors cumulative clinical pregnancy rates (i.e. pregnancies from both fresh and thawed cycles deriving from a single egg collection procedure).

– We are uncertain whether blastocyst-stage transfer increases multiple pregnancy rates compared to cleavage-stage transfer, when we consider all the studies that reported information on this.

– When we consider evidence only from higher-quality studies and studies that transferred the same number of embryos in both embryo stages, we found that multiple pregnancy rate is probably higher in the blastocyst-stage transfer group.

– We are uncertain whether blastocyst-stage transfer increases miscarriage rates compared to cleavage-stage transfer.

Future studies should report rates of live birth, cumulative live birth, and miscarriage, to enable women, couples and their doctors to make well-informed decisions on the best treatment option available.

Quality of the evidence

We have low to moderate confidence in the quality of the evidence for most outcomes. The main limitation was the failure of some studies to describe acceptable methods of assigning women or couples at random to treatment groups.

Summary of findings 1. Blastocyst-stage versus cleavage-stage embryo transfer for assisted reproductive technology

Blastocyst-stage versus cleavage-stage embryo transfer for assisted reproductive technology

Population: women and couples with subfertility
Settings: assisted reproductive technology
Intervention: blastocyst-stage embryo transfer
Comparison: cleavage-stage embryo transfer

Outcomes per couple	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Cleavage-stage embryo transfer	Blastocyst-stage embryo transfer				
Live birth rate per fresh transfer	**312 per 1000**	**365 per 1000** (324 to 406)	**OR 1.27** (1.06 to 1.51)	2219 (15 studies)	⊕⊕⊖⊖ Low[a,c]	When sensitivity analysis restricted to 9 studies with low or some concerns of overall risk of bias, it results in a similar effect (OR 1.26, 95% CI 1.04 to 1.54).
Cumulative pregnancy rate (slow freezing)	**565 per 1000**	**472 per 1000** (384 to 562)	**OR 0.69** (0.48 to 0.99)	512 (4 studies)	⊕⊕⊖⊖ Low[a,c]	
Cumulative pregnancy rate (vitrification)	**333 per 1000**	**550 per 1000** (369 to 719)	**OR 2.44** (1.17 to 5.12)	120 (1 study)	⊕⊕⊕⊖ Moderate[a]	
Clinical pregnancy rate	**362 per 1000**	**425 per 1000** (393 to 455)	**OR 1.25** (1.13 to 1.39)	5767 (32 studies)	⊕⊕⊖⊖ Moderate[a]	When sensitivity analysis restricted to 17 studies with low risk or some concerns of overall risk of bias, it results in a similar effect (OR 1.24, 95% CI 1.08 to 1.41).
Multiple pregnancy rate	**89 per 1000**	**99 per 1000** (81 to 119)	**OR 1.12** (0.90 to 1.38)	4208 (22 studies)	⊕⊕⊖⊖ Low[a,b,c]	When sensitivity analysis restricted to 15 studies with low risk or some concerns of overall risk of bias, it results in an increase of multiple pregnancy rate (OR 1.33, 95% CI 1.04 to 1.70).

Miscarriage rate	**67 per 1000**	**82 per 1000** (68 to 111)	**OR 1.24,** (0.98 to 1.57)	4106 (21 studies)	⊕⊕⊖⊖ Low[a,c]	
Embryo freezing rate	**594 per 1000**	**412 per 1000** (369 to 455)	**OR 0.48** (0.40 to 0.57)	2292 (14 studies)	⊕⊕⊖⊖ Low[a,b]	I^2 = 84%. Direction of effect largely consistent
Failure rate to transfer any embryos	**11 per 1000**	**26 per 1000** (19 to 37)	**OR 2.50** (1.76 to 3.55)	2577 (17 studies)	⊕⊕⊕⊖ Moderate[a]	I^2 = 36%

*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.

[a]Downgraded one level for serious risk of bias: most studies have some concerns of overall risk of bias, mainly due to the randomisation process, deviations from the intended intervention and selective reporting
[b]Downgraded one level for serious inconsistency
[c]Downgraded one level for serious imprecision: findings compatible with benefit or minimal effect

BACKGROUND

Description of the condition

Worldwide, 15% of reproductive-aged couples are affected by infertility (WHO 2022). The World Health Organization estimates that, globally, between 48 million couples and 186 million individuals live with infertility (WHO 2021). Assisted reproductive technologies (ARTs), such as in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI), and embryo freezing, are considered beneficial for many couples and women who are unlikely to conceive without treatment, and for whom less invasive forms of treatment have failed or are unlikely to be effective. The fledgling era of IVF, from 1980 to the mid-1990s, was characterised by relatively static successful pregnancy rates of around 20%. The past decade, however, has given rise to advances in ovarian stimulation, cell culture, embryo transfer, and new cryopreservation techniques as well as freeze-all cycles, that have culminated in significant overall improvements in successful pregnancies (Wirleitner 2016; Roque 2015). This is evident in the annual statistical reports from different areas of the globe. One such report, for example, has demonstrated a doubling of the pregnancy rate per embryo transfer cycle from 1994 to 2003, despite a decrease in the mean number of embryos transferred (Waters 2006).

Description of the intervention

IVF involves the use of hormones to stimulate the ovaries to produce many eggs (oocytes), followed by egg collection (oocyte retrieval), addition or injection of sperm, fertilisation, embryo culture, and lastly, the return of a few selected embryos to the uterus (embryo transfer).

Conventionally, embryos have been transferred on either day 2 or day 3 when the embryos were two to eight cells, or 'cleavage stage', because the uterus was thought to provide the best environment for the survival of the embryo (Laverge 2001). The question of optimal timing for embryo transfer arises when examining the differences between IVF procedures and what happens naturally in vivo. Day 2 is an early time at which morphological grading of the embryos is possible, allowing selection of the 'best' embryos for transfer. Embryo morphology, along with other factors, is thought to be highly indicative of pregnancy outcome (De Placido 2002). Early replacement in the uterus may be advantageous for the embryos by limiting the time spent in the in vitro environment of the embryology laboratory.

Over the past decade, there has been a steady shift in practice to the transfer of embryos on day 5 or 6, when the embryos are 'blastocysts'. With the introduction of a variety of commercial preparations of sequential media in the late 1990s, the ART service sector witnessed an explosion of worldwide interest in blastocyst culture, with most clinics conducting research into its application in their own settings. As a result, a substantial volume of publications followed. These documented trials with conflicting results and reflected debates about the merits and drawbacks of extended culture (Sfontouris 2021; Sunde 2016; Sunde 2021).

One of the benefits of blastocyst-stage transfer could be the potential for an improved implantation rate, which could lead to a change in policy about the number of embryos to be transferred (Kamath 2020). The higher the implantation rate is, the lower the number of embryos are transferred.

The fact that many blastocyst-stage transfer trials were not prospectively randomised or were underpowered has contributed to the lack of a strong consensus about best practice for blastocyst culture. The need for an evidence-based approach using meta-analysis of small trials was, therefore, required to assist in deciphering the overall effect of blastocyst culture to help identify participant subsets and practices that might best benefit from this approach.

How the intervention might work

Blastocyst culture is not novel; indeed, the very first report of an IVF pregnancy was from a transferred blastocyst (Edwards 1995). Despite this, cleavage-stage transfer was adopted as standard global practice early in the history of IVF for two reasons: the low developmental rate of embryos cultured past this stage; and the observation that, unlike other primates, human embryos have an unusual propensity to survive when replaced prematurely into the uterus (Marston 1977). However, as knowledge of embryo metabolic requirements expanded, so did the range of more advanced culture media (Scholtes 1996), and co-culture techniques, culturing endometrial cells in co-culture with the embryo (Menezo 1990; Van Blerkom 1993; Yeung 1992). One important finding was that the in vitro environment in which a cleavage-stage embryo grows best is different from that for a blastocyst. This led to the evolution of stage-specific (or sequential) media (G1/G2) by Gardner in 1998 (Gardner 1998b): embryos are transferred on day 3 from a medium containing low concentrations of glucose and one or more amino acids to a medium containing higher concentrations of glucose and a wider range of amino acids (Gardner 1996). At this stage, the embryo undergoes cell compaction and genomic activation so that the embryo is no longer under the control of transcripts and ribonucleic acid (RNA) messages of maternal origin (Braude 1998). With the application of stage-specific media, there have been reports of blastocyst development rates as high as 60% to 65% (Schoolcraft 2001). Interestingly, with the development of time-lapse systems (TLS), stage-specific media is no longer considered essential (Armstrong 2019). Time-lapse systems take frequent digital images of embryos, allowing embryologists to assess their quality without physically removing them from the incubator.

There are two central arguments why blastocyst culture has possible advantages over traditional cleavage-stage transfer. Firstly, it is considered to be physiologically premature to expose early-stage embryos to the uterine environment, particularly one that has been subjected to superovulation and thus high levels of oestrogen (Valbuena 2001). In vivo, embryos travel through the fallopian tubes and do not reach the uterus before the morula (16-cell compacted) stage (Croxatto 1972), which equates to at least day 4 of in vitro culture. The uterus provides a different nutritional environment from the oviduct. Therefore, it is postulated that the uterine environment may cause stress on the embryo, if transferred at cleavage stage (Baart 2006; Munne 2002), and result in reduced implantation potential (Fanchin 2001; Gardner 1996).

The second argument for blastocyst-stage transfer is the reported higher implantation potential compared with cleavage-stage embryos. As a consequence of self selection, it is postulated that only the most viable embryos are expected to develop into

blastocysts. It is widely acknowledged that the morphological criteria used for selection of the best embryos on day 2 to 3 are limited. Many published studies that debate the correlation of morphological features with pregnancy rates can be found in the literature (Palmstierna 1998; Puissant 1987; Roseboom 1995; Scott 2000; Sjoblom 2006; Steer 1992). It is now understood that a disturbingly large proportion of morphologically-normal day 3 embryos are chromosomally abnormal or mosaic, thus contributing to the 80% to 90% rate of implantation failure post-transfer that is observed in cleavage-stage protocols (Magli 1998). While the transfer of day 5 blastocysts cannot ensure the absence of chromosomal abnormality (Magli 2000), Staessen 2004 demonstrated that, at least in women older than 36 years, the incidence can be reduced from 59% in day 3 embryos to 35% in day 5 blastocysts. The question that this review aims to answer is whether the higher implantation rates do translate into higher live birth rates.

Arguments against blastocyst culture are largely related to this process of self selection. Women undergoing blastocyst culture are expected to have a higher incidence of cycle cancellation due to failed embryo development (Marek 1999), and to have fewer embryos cryopreserved (frozen) (Tsirigotis 1998).

Overall utilisation rates have previously been described as the total number of embryos transferred plus the embryos thawed divided by the number of fertilised eggs. Whilst this approach presents information about the comparative number of pregnancy opportunities that each treatment approach can provide a woman or couple, it does not take into account the implantation potential for fresh and thawed embryos. Cumulative live birth rate is the only outcome that can assess this. An alternative efficacy formula was developed in the Schoolcraft 2001 study that does take cumulative pregnancy rate into account. Using the formula (mean number of embryos transferred multiplied by implantation rate) plus (mean number of embryos cryopreserved multiplied by implantation rate) minus (1 minus cancellation rate), this group of researchers was able to demonstrate a 19% greater efficiency in blastocyst culture compared to cleavage-stage transfers. Disappointingly, such a utilisation and efficiency analysis is not possible in the majority of RCTs due to the lack of reporting of frozen-thawed cycle outcomes within a reasonable time frame for trials. A frozen-thawed cycle is an embryo transfer that is performed at a later date with embryos that were frozen a few days after the oocyte retrieval. We would argue that a superior approach to both of the foregoing methods is to report the live birth rates for both fresh and frozen-thawed cycles following a single oocyte retrieval in women randomised to either cleavage-stage or blastocyst-stage transfers.

Why it is important to do this review

This is an update of a Cochrane Review first published in 2002, and previously updated in 2005, 2007, 2012, and 2016.

Advocates of blastocyst culture are confident that only the most viable embryos survive the extended culture to day 5 or 6. They argue that this results in a higher probability of implantation and requires fewer embryos to be transferred, thereby lowering the costly multiple birth rate (Gardner 1998b; Jones 1999). It is important to be aware that clinic policies may differ on the minimum criteria for blastocyst culture and the day on which this decision is made (for example, number of follicles, fertilised eggs, 8-cell embryos on day 3) (Milki 1999). It is also yet to be clarified if there are patient groups for whom blastocyst culture is disadvantageous. Most importantly, does blastocyst culture achieve the primary aim of providing the subfertile couple with a normal, healthy baby? Methods for identifying viable blastocysts are a popular research focus, involving a range of approaches which include identification of chromosomally-normal blastocysts by polar-body and blastomere, trophectoderm genetic analysis (using microarrays or next-generation sequencing known as pre-implantation genetic screening (Jones 2008)), and metabolomic analysis of culture media (Nel-Themaat 2011).

Critics of the blastocyst culture approach express concern at the increased incidence of women failing to have embryos available for transfer (Marek 1999), although the day of participant recruitment into the blastocyst programme is crucial to this argument.Other negative outcomes reported to be associated with blastocyst culture include a higher incidence of monozygotic twinning and altered sex ratio in favour of males (Menezo 1999; Spangmose 2020). Monozygotic twinning is frequently reported at above 1% in ART cycles (Sills 2000), whilst the background rate of monozygotic twins in spontaneous conceptions is in the order of 1 in 330. This twinning is associated with miscarriage, serious structural congenital anomalies, growth discrepancy and twin-to-twin transfusion syndrome. Extended culture of an embryo has been implicated as one of the interventions associated with an increase in monozygotic twinning (Behr 2000; Cohen 1990; De Felici 1982; Jain 2004), but a recent report suggests that improvements in cell culture techniques over time can result in a significant decrease in its incidence (Moayeri 2007). Similarly, as the underlying mechanisms that lead to an altered sex ratio are elucidated, whether it be media constituents or simply the morphological selection criteria (Luna 2007), the imbalance may also be rectified.

The aim of this review is to determine whether the number of days between oocyte retrieval and embryo transfer (that is, the embryo stage) has any effect on the success of ART treatment, and in particular, the live birth rate, the most important outcome for couples or women undergoing treatment as well as for service providers.

OBJECTIVES

To determine whether blastocyst-stage (day 5 to 6) embryo transfer improves the live birth rate (LBR) per fresh transfer, and other associated outcomes, compared with cleavage-stage (day 2 to 3) embryo transfer.

METHODS

Criteria for considering studies for this review

Types of studies

We included only individually-randomised parallel-group trials (RCTs). We excluded quasi-RCTs and cluster-randomised trials. We also excluded cross-over trials unless pre-cross-over data were available.

Types of participants

Inclusion criteria

We included couples or women affected by subfertility undergoing in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI)

for therapeutic reasons or for oocyte donation within all patient prognosis groups.

'Patient prognosis groups' (participant subsets or populations) is a term used to describe the categories that couples or women are assigned to based on several factors such as their age, type of subfertility, ovarian response to the superovulation drugs, and number of previous attempts. See the Subgroup analysis and investigation of heterogeneity section below for the categories.

Exclusion criteria

We excluded couples or women whose IVF or ICSI cycle, or both, involved in vitro matured oocytes or pre-implantation genetic screening. We also excluded couples or women whose frozen-thawed cycle results were shown, but where no data were available from the fresh cycle.

Types of interventions

Inclusion criteria

We included studies comparing blastocyst-stage (day 5 to 6) transfers to cleavage-stage (day 2 to 3) transfers in settings using single and sequential media culture methods for IVF and ICSI, where the embryos were grown for between 2 and 6 days in vitro prior to embryo transfer.

Exclusion criteria

We excluded studies using co-culture methods as an intervention.

We also excluded studies comparing blastocyst-stage transfers to cleavage-stage transfers in frozen-thawed cycles, but where no data were available from the fresh cycle.

Types of outcome measures

Primary outcomes

- Live birth rate per couple or woman (number of live births after week 20 of pregnancy per couple or woman) following fresh transfer
- Cumulative pregnancy rate (cCPR) per couple or woman (from both fresh and thawed cycles deriving from a single egg collection procedure)

Secondary outcomes

- Clinical pregnancy rate per couple or woman: number of couples or women achieving a clinical pregnancy following fresh transfer (defined by the demonstration of foetal heart activity on ultrasound scan)
- Multiple pregnancy rate per couple or woman following fresh transfer: number of multiple pregnancies per couple or woman
- High-order multiple pregnancy rate per couple or woman following fresh transfer: three or more foetal heartbeats per couple or woman
- Miscarriage rate for fresh transfer: number of occurrences per couple or woman and per pregnant woman
- Embryo freezing rates per couple or woman: number of couples or women that had supernumerary embryos for transfer at a later date per couple or woman
- Failure rate to transfer embryos (per couple or woman): percentage of couples or women that did not have an embryo transfer

Additional outcomes not appropriate for statistical pooling

We were unable to pool data per cycle or per embryo transfer or per oocyte pick up (OPU) (Vail 2003). If a study included multiple cycles, transfers, or OPUs per woman, then results reported per cycle/transfer/OPU cannot be validly pooled. If the study reported results per cycle/transfer/OPU and this did not coincide with the point of randomisation (i.e. if the denominator did not coincide with the point in the treatment where randomisation occurred), we calculated new results using randomised participants as the denominator and treated those excluded as having negative outcomes. However, due to the frequency that this form of data is reported in the literature, we have entered them into the 'Data and analyses tables' for the following outcomes.

- Live births per OPU and embryo transfer.
- Clinical pregnancy rate per OPU and embryo transfer.
- Implantation rate: the number of foetal sacs divided by the number of embryos transferred.

Search methods for identification of studies

We obtained all reports that described (or might have described) RCTs comparing cleavage-stage embryo transfer and blastocyst-stage transfer in the treatment of subfertility, using IVF or ICSI, using the search strategy developed by the Gynaecology and Fertility Group.

Electronic searches

We searched the following databases.

- The Cochrane Gynaecology and Fertility Group Specialised Register of Controlled Trials, searched 20 October 2021, ProCite platform (Appendix 1).
- CENTRAL via the Cochrane Register of Studies Online (CRSO), searched 20 October 2021, Web platform (Appendix 2).
- MEDLINE, searched from 1946 to 20 October 2021, Ovid platform (Appendix 3).
- Embase, searched from 1980 to 20 October 2021, Ovid platform (Appendix 4).
- PsycINFO, searched from 1806 to 20 October 2021, Ovid platform (Appendix 5).
- CINAHL (Cumulative Index to Nursing and Allied Health Literature), searched from 1961 to 4 April 2020, EBSCO platform (Appendix 6). Any later CINAHL search output is contained in the 2021 CENTRAL search output.

We combined the MEDLINE search with the Cochrane highly sensitive search strategy for identifying randomised trials, which appears in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019a, hereafter referred to as the *Cochrane Handbook*).

We combined the Embase search with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk/what-we-do/methodology/search-filters/). We did not impose any language restrictions in these searches.

Searching other resources

We searched the National Research Register, a register of ongoing and recently completed research projects funded by, or of interest to, the United Kingdom's National Health Service (NHS); entries from the Medical Research Council Clinical Trials Register; and details on reviews in progress that are collected by the NHS Centre for Reviews and Dissemination.

We also searched ClinicalTrials.gov and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (Appendix 7; Appendix 8).

We performed the search on titles, abstracts, and keywords of the listed articles. We also searched the citation lists of relevant publications, review articles, and included studies. We handsearched relevant conference abstracts and contacted experts in the field.

We conduct a search for new trials bi-annually and update the review as and when we find new trials to be incorporated.

Data collection and analysis

Selection of studies

Three review authors (DG, CAS, and AQR) performed the selection of trials for inclusion in the review after employing the search strategy described previously. Two review authors independently viewed each record. We resolved any disagreements about eligibility through discussion. We have presented details of the included studies in the Characteristics of included studies tables, which provide a context for assessing the reliability of results. We have described excluded studies in the Characteristics of excluded studies table.

Data extraction and management

Two of three review authors (DG, CAS, and AQR) extracted data from eligible studies using a data extraction form designed and pilot-tested by the authors. Two review authors independently viewed each record. We resolved any disagreements about data extraction through discussion. Data extracted included study characteristics and outcome data. Where studies had multiple publications, we used the main trial report as the primary reference and derived additional details from secondary papers. We corresponded with study investigators for further data, as required.

Assessment of risk of bias in included studies

Two of three review authors (DG, SC, and AC) independently assessed the included studies for risk of bias using the Cochrane risk of bias 2 (RoB 2) assessment tool (Higgins 2019b; Sterne 2019). We assessed: bias arising from the randomisation process; bias due to deviations from intended interventions; bias due to missing outcome data; bias in measurement of the outcome; and bias in selection of the reported result. The effect of interest was the effect of assignment to the intervention at baseline, regardless of whether the interventions were received as intended (the 'intention-to-treat effect').

A potential deviation from the intended protocols is an adherence issue, such as moving to the cleavage transfer arm if there were few available embryos on day 3. Another potential deviation is a difference that could be found in the number of transferred embryos in each of the arms. Although both of these deviations could occur in the real world, they could also occur as a result of participating in a trial.

The outcomes we selected to be assessed for risk of bias are the same as those reported in the summary of findings table: live birth, cumulative pregnancy, clinical pregnancy, multiple pregnancy, miscarriage, embryo freezing, and failure to transfer any embryo. We describe the measurement methods and time points in the Measures of treatment effect section.

Judgements were classified as 'low' or 'high' risk of bias, or as expressing 'some concerns', assigned as recommended in the *Cochrane Handbook*, Chapter 8 (Higgins 2019a). We resolved any disagreements about risk of bias judgements through discussion. We described all judgements fully and presented the consensus judgements in the main review document (e.g. as a table, or a figure, or within a forest plot of the results).

These domain-level judgements provided the basis for an overall risk of bias judgement for the specific trial result being assessed.

We used the RoB 2 Excel tool (available on the riskofbiasinfo.org website) to process the use of RoB 2 and to store data for presentation (Sterne 2019). We made risk of bias data available by providing detailed appendices.

We sought additional information on trial methodology or actual original trial data from the principal author of trials that appeared to meet eligibility criteria but were unclear in aspects of methodology, or where the data were in a form unsuitable for meta-analysis. We sent reminder correspondence when a reply was not received within three weeks.

Measures of treatment effect

For dichotomous data (for example, clinical pregnancy rate), we expressed results for each study as odds ratios (ORs) with 95% confidence intervals (CIs) and combined them for meta-analysis with RevMan Web 2020 (RevMan Web 2020).

Unit of analysis issues

The primary analysis was per woman randomised. We counted multiple live births (for example, twins or triplets) as one live birth event.

Dealing with missing data

We analysed the data on an intention-to-treat basis as far as possible (i.e. including all randomised participants in analysis, in the groups to which they were randomised). We attempted to obtain missing data from the original trialists. Where these were unobtainable, we imputed individual values to all the outcomes: they were assumed not to have occurred in participants without a reported outcome.

Assessment of heterogeneity

We considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. We assessed statistical heterogeneity by the measure of the I^2 statistic. An I^2 measurement greater than 50% was taken to indicate substantial heterogeneity. When we detected substantial

heterogeneity, we explored possible explanations in subgroup analyses. We took statistical heterogeneity into account when interpreting the results.

We examined heterogeneity between the results of different studies by inspecting the scatter of data points, the overlap in their confidence intervals, and more formally, by checking the results of the Chi^2 tests. A priori, we had planned to look at the possible contribution of differences in trial design to the heterogeneity identified. Where possible, we pooled the outcomes statistically.

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 to duplication of data. If there were ten or more studies in an analysis, we used 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

We performed statistical analyses in accordance with the *Cochrane Handbook* (Higgins 2019a). The primary analyses included all studies.

We pooled data for meta-analysis with RevMan Web 2020 (RevMan Web 2020), using the fixed-effect Mantel-Haenszel model method. We entered the data on the graphs so that, for beneficial outcomes (for example, pregnancy), data are displayed to the right of the line of no effect, and in detrimental outcomes (for example, miscarriage), to the left of the line of no effect.

Subgroup analysis and investigation of heterogeneity

We planned the following subgroup analyses for the outcomes live birth, cumulative pregnancy, clinical pregnancy, and multiple pregnancy.

- Studies that actively selected for good prognosis participants (for example, four or more zygotes, first two cycles, more than 10 follicles, young population, no male-factor individuals) versus participants with poor prognostic factors (for example, previous failed ART cycles or poor response to ovulation stimulation) versus studies with unselected participants.
- Studies that randomised at the start of the cycle (that is, prior to ovarian stimulation) were compared with those that randomised at the days immediately prior to and post-OPU (that is, day of final ultrasound scan and prior to human chorionic gonadotropin trigger up to and including the day of fertilisation check, when numbers of oocytes are anticipated).
- Studies where the policy for the number of embryos replaced was equal in both blastocyst-stage and cleavage-stage groups versus studies where fewer blastocyst-stage than cleavage-stage embryos were replaced.
- We also included a post hoc analysis to investigate substantial heterogeneity for one of the primary outcomes: studies where freezing technique was slow freezing versus studies where vitrification was used.
- We made a subgroup analysis for time-lapse s ystem s election (TLS selection) with algorithm which was not stated in the protocol, as TLS is a technology that was unavailable when the protocol was written. Subgroups would be:
  - Conventional cleavage stage versus conventional blastocyst stage;
  - TLS selection (with algorithm) cleavage stage versus conventional blastocyst stage;
  - TLS selection (with algorithm) cleavage stage versus TLS selection (with algorithm) blastocyst stage.

We planned to perform an overall assessment of risk of bias for subgroups.

Sensitivity analysis

Primary analysis pooled the data of all the studies. The selected outcomes assessed by a sensitivity analysis are live birth, cumulative pregnancy, clinical pregnancy, and embryo freezing.

Eligibility was restricted to studies with outcomes with 'low' or 'some concerns for' overall risk of bias.

Summary of findings and assessment of the certainty of the evidence

We prepared a summary of findings table using GRADEpro and Cochrane methods (GRADEpro GDT; Higgins 2019a). This table evaluated the overall quality of the body of evidence for the main review outcomes (live birth rate, cumulative pregnancy rate, clinical pregnancy rate, multiple pregnancy rate, miscarriage rate, embryo freezing rate and failure rate to transfer any embryos) for the main review comparison (blastocyst-stage transfer versus cleavage-stage transfer). We assessed the quality of the evidence using GRADE criteria: overall risk of bias (fed by RoB2 tool assessment), consistency of effect, imprecision, indirectness, and publication bias. Two review authors working independently made judgements about evidence quality (high, moderate, low, or very low), resolving any disagreements through discussion. We justified, documented, and incorporated our judgements into the reporting of results for each outcome.

We planned to extract study data, format our comparisons in data tables, and prepare a summary of findings table before writing the results and conclusions of our review.

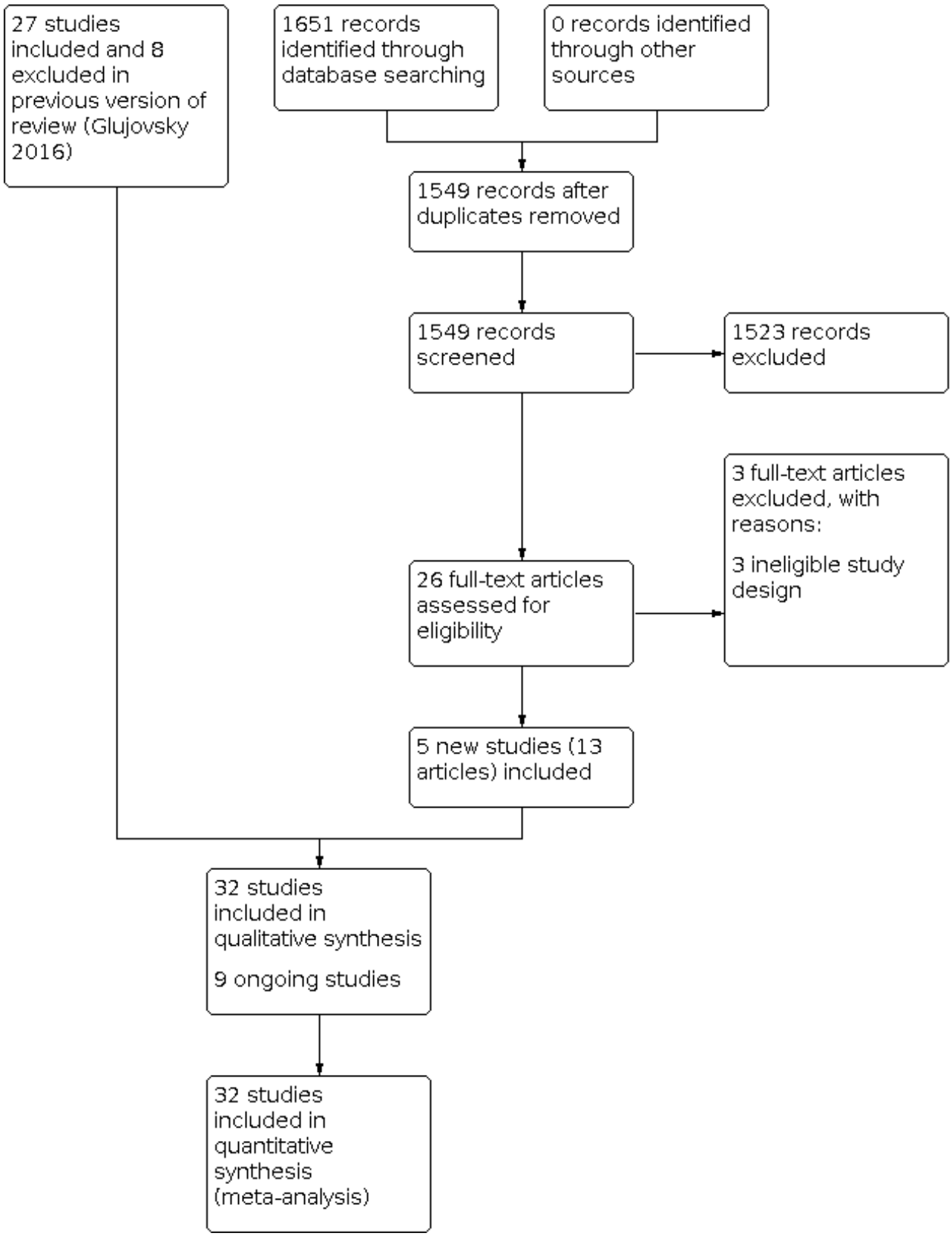
RESULTS

Description of studies

Results of the search

At the 2022 update we identified 1651 articles as potentially relevant for comparing blastocyst-stage versus cleavage-stage embryo transfer, and we retrieved 26 new articles in full text. In the 2022 update we included five new studies (Hatirnaz 2017; Kaser 2017; Levi-Setti 2018; Singh 2017; Yang 2018), and there were 3 newly excluded studies (Cornelisse 2018; Green 2016; Holden 2017). Including studies retrieved in the latest update, we now have 32 studies (47 articles) in the review. See Figure 1.

Figure 1. Study flow diagram: results of search from review inception to 2020 (update of the flow diagram published in 2016)



We also found 7 new ongoing studies. In total, there are 9 ongoing studies: one each from China (ChiCTR-ICR-15006184), Iran (NCT01107002), and the USA (Neuhausser 2020); two from Italy (ISRCTN48090543; NCT02639000), and the Netherlands (Cornelisse 2021); and three from Egypt (NCT04210414; PACTR201402000773124; PACTR201709002592834).

We attempted to contact study authors for information regarding methodology and outcome data. We received replies from 11 contact authors (Bungum 2003; Fernandez-Shaw 2015; Frattarelli 2003; Hreinsson 2004; Karaki 2002; Levitas 2004; Levron 2002; Livingstone 2002, Papanikolaou 2005; Papanikolaou 2006; Rienzi 2002). Cumulative live birth data were provided by Fernandez-Shaw 2015.

Included studies

Study design and setting

We included 32 parallel-design RCTs in this review (5821 participants). The size of trials ranged from 20 in Fisch 2007 to 460 couples or women in Kolibianakis 2004, including both comparison groups.

The majority of trials were carried out in less than six months, except for the two largest studies. All studies were reported to have been performed at single private clinics or university-based clinics. Twelve countries were represented in the included studies, with Belgium being the most prolific, providing six studies. The countries represented were: Australia (Livingstone 2002); Belgium (Devreker 2000; Emiliani 2003; Kolibianakis 2004; Papanikolaou 2005; Papanikolaou 2006; Van der Auwera 2002); Brazil (Motta 1998); China (Yang 2018); Denmark (Bungum 2003); Egypt (Elgindy 2011; Gaafar 2015); France (Brugnon 2010); Greece (Pantos 2004); India (Kaur 2014; Singh 2017); Iran (Aziminekoo 2015); Israel (Coskun 2000; Levitas 2004; Levron 2002); Italy (Levi-Setti 2018; Rienzi 2002; Schillaci 2002); Jordan (Karaki 2002); Spain (Fernandez-Shaw 2015; Ten 2011); Sweden (Hreinsson 2004); Turkey (Hatirnaz 2017); and the USA (Fisch 2007; Frattarelli 2003; Gardner 1998a; Kaser 2017).

Participants

Participant selection criteria comprised three main groups: unselected participants (Emiliani 2003; Fernandez-Shaw 2015; Gaafar 2015; Hatirnaz 2017; Karaki 2002; Kolibianakis 2004; Motta 1998; Pantos 2004; Schillaci 2002; Van der Auwera 2002); good prognostic factors where participants were positively selected; that is, those who would be expected to do well with blastocyst culture (Brugnon 2010; Bungum 2003; Coskun 2000; Elgindy 2011; Fisch 2007; Frattarelli 2003; Gardner 1998a; Hreinsson 2004; Kaser 2017; Kaur 2014; Levi-Setti 2018; Levron 2002; Livingstone 2002; Papanikolaou 2005; Papanikolaou 2006; Rienzi 2002; Singh 2017; Ten 2011; Yang 2018); and poor prognostic factors, where couples or women were selected who had experienced multiple failures with conventional treatment or had a poor response to ovulation induction (Aziminekoo 2015; Devreker 2000; Levitas 2004). Most studies recruited women under 40 years of age, except for Fernandez-Shaw 2015, Gardner 1998a, and Gaafar 2015, which had no age limit. The mean age across all the studies varied from 29 years to 34 years.

We found no studies of participants using donor eggs.

Interventions

Twenty trials used sequential media, of which 13 used Vitrolife G1/G2, while the remaining media were combinations of brands or made in-house. Five did not state the media used (Table 1).

Freezing of embryos in both experimental groups was reported in 14 of the 32 included trials (Brugnon 2010; Bungum 2003; Fernandez-Shaw 2015; Gardner 1998a; Hreinsson 2004; Karaki 2002; Kolibianakis 2004; Levron 2002; Motta 1998; Pantos 2004; Papanikolaou 2006; Rienzi 2002; Ten 2011; Van der Auwera 2002). Coskun 2000 reported no provision for day 5 freezing. Levitas 2004 stated that most of the remaining embryos were not suitable for freezing. Other interventions, such as assisted hatching, were either not provided or not reported on for the majority of trials. Gardner 1998a was the only trial that practised assisted hatching, but only for the day 3 embryo transfer group.

All the studies compared blastocyst-stage versus cleavage-stage embryo transfers. For the cleavage-stage transfer groups, most transfers were on day 3, except for five trials that did the embryo transfers on day 2 (Devreker 2000; Emiliani 2003; Gaafar 2015; Motta 1998; Van der Auwera 2002), and one study that had a policy of transferring on day 2 or 3 (Levitas 2004).

The trials that provided details on the ovarian stimulation regimen mostly reported using a similar gonadotropin-releasing hormone pituitary down-regulation protocol prior to human menopausal gonadotropin (HMG) and follicle stimulating hormone (FSH) administration. However, in some trials (Kolibianakis 2004; Levi-Setti 2018; Papanikolaou 2005; Papanikolaou 2006; Singh 2017), gonadotropin-releasing hormone antagonists were used in varying degrees.

Two studies (Kaser 2017; Yang 2018) evaluated the effects of adding time-lapse system (TLS) to cleavage-stage transfer.

Outcomes

- 15/32 studies reported live birth rate per fresh embryo transfer
- 5/32 studies reported cumulative pregnancy rate
- 32/32 studies reported clinical pregnancy rate
- 22/32 studies reported multiple pregnancy rate
- 13/32 studies reported high-order multiple pregnancy rate
- 21/32 studies reported miscarriage rate
- 14/32 studies reported embryo freezing rate (when there are supernumerary embryos for transfer at a later date)
- 17/32 studies reported failure rate to transfer embryos

Excluded studies

We excluded 10 studies from the review for the following reasons. Six were not truly randomised studies (Cornelisse 2018; Green 2016; Holden 2017; Levron 2001; Utsonomiya 2004; Zech 2007). Three studies used co-culture (Bungum 2002; Guerin 1991; Menezo 1992). In the remaining study, fresh embryo transfers on day 5 or 6 were not the main intervention (Loup 2009).

One study (Clua Obrado 2020) randomised Spanish recipients between 18 and 50 years old in their first or second synchronous cycle to D3 or D5 embryo transfer. The authors will not provide the missing information until its publication, therefore it awaits classification.

Risk of bias in included studies

We attempted to obtain additional information regarding all aspects of randomisation, blinding, power analysis, and intention-to-treat from all trial authors.

We accessed the RoB 2 tool on 20 August 2020. Risk of bias assessments for each outcome, including all domain judgements and support for the judgement, are located within each included study, and at the side of all forest plots. To access further detailed risk of bias assessment data, please use the following link.

The risk of bias judgements for outcomes across all studies were predominantly of 'some concerns' and 'high'. The most common reason for 'some concerns' of overall risk of bias was the lack of publication of the protocol in a trial registry, while overall high risk of bias was mainly due to lack of reporting of allocation concealment.

Detailed risk of bias assessment data (with consensus responses to the signalling questions) are available upon reasonable request to the authors.

Effects of interventions

See: **Summary of findings 1** Blastocyst-stage versus cleavage-stage embryo transfer for assisted reproductive technology

Blastocyst-stage versus cleavage-stage transfer

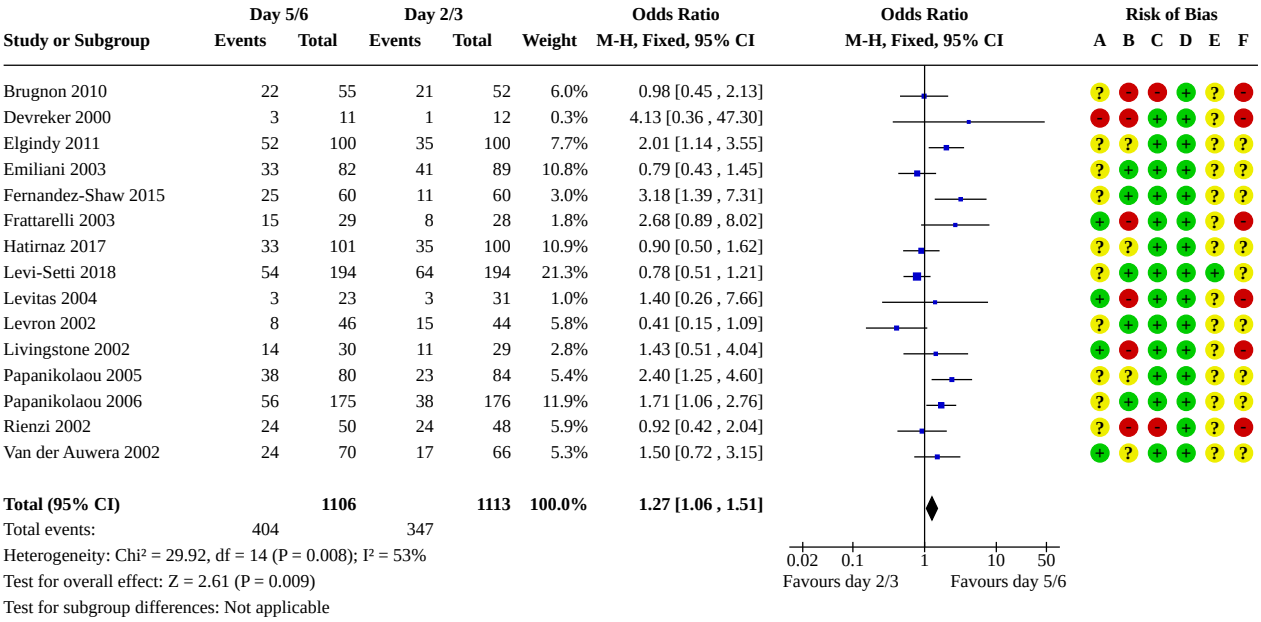
For an overview of our main analyses, please see Summary of findings 1.

Primary outcomes

1. Live birth rate per couple or woman

The live birth rate per fresh embryo transfer was higher in the fresh blastocyst transfer group (odds ratio (OR) 1.27, 95% confidence interval (CI) 1.06 to 1.51; I^2 = 53%; 15 studies, 2219 women; low-quality evidence; Analysis 1.1; Figure 2). This suggests that if 31% of women achieve live birth after fresh cleavage-stage transfer, between 32% and 41% would do so after fresh blastocyst-stage transfer.

Figure 2. Forest plot of comparison: 1 Blastocyst- versus cleavage-stage transfer: live birth rate, outcome: 1.1 live birth per couple



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Subgroup and sensitivity analyses

We did not find evidence that the treatment effect differed between fresh cleavage-stage and blastocyst-stage transfer based either on number of embryos transferred (test for subgroup differences: P = 0.48, I^2 = 0%; Analysis 1.2) or on the prognosis (test for subgroup differences: P = 0.73, I^2 = 0%; Analysis 1.3).

Sensitivity analysis including only those studies with low or some concerns of overall risk of bias found a higher live birth rate in the blastocyst group (OR 1.26, 95% CI 1.04 to 1.54; I^2 = 69%; 9 studies, 1821 women; moderate-quality evidence).

2. Cumulative clinical pregnancy rate per couple or woman (following fresh and frozen-thawed transfer)

We are uncertain whether blastocyst-stage transfer improves cumulative clinical pregnancy rate (OR 0.89, 95% CI 0.64 to 1.22; I^2 = 71%; 5 studies, 632 women; very low-quality evidence; Analysis 2.1). There was substantial heterogeneity for this outcome, with differing directions of effect. The heterogeneity was largely attributable to two studies (Fernandez-Shaw 2015; Rienzi 2002). We investigated statistical heterogeneity by conducting a post hoc subgroup analysis according to the method of freezing. The test for subgroup differences showed a significant difference between the subgroups (Chi^2 = 9.12, degrees of freedom (df) = 1 (P = 0.003), I^2 = 89.0%). The only study using vitrification showed evidence of higher cumulative pregnancy rate in blastocyst transfers (OR 2.44, 95% CI 1.17 to 5.12; moderate-quality evidence; Fernandez-Shaw 2015), whilst the four studies with slow freezing showed that the confidence interval is too wide to know if blastocyst transfer decreases the cumulative pregnancy rate (OR 0.69, 95% CI 0.48 to 0.99; low-quality evidence). This is an interesting finding which should be investigated further when more studies using vitrification are published.

Subgroup and sensitivity analyses

We did not find evidence that the treatment effect differed between fresh cleavage-stage and blastocyst-stage transfer based on number of embryos transferred (test for subgroup differences: P = 0.89, I^2 = 0%) or day of randomisation (test for subgroup differences: P = 0.42, I^2 = 0%), and no conclusive evidence of a difference based on prognosis (test for subgroup differences: P = 0.05, I^2 = 73.6%; Analysis 2.2; Analysis 2.3; Analysis 2.4).

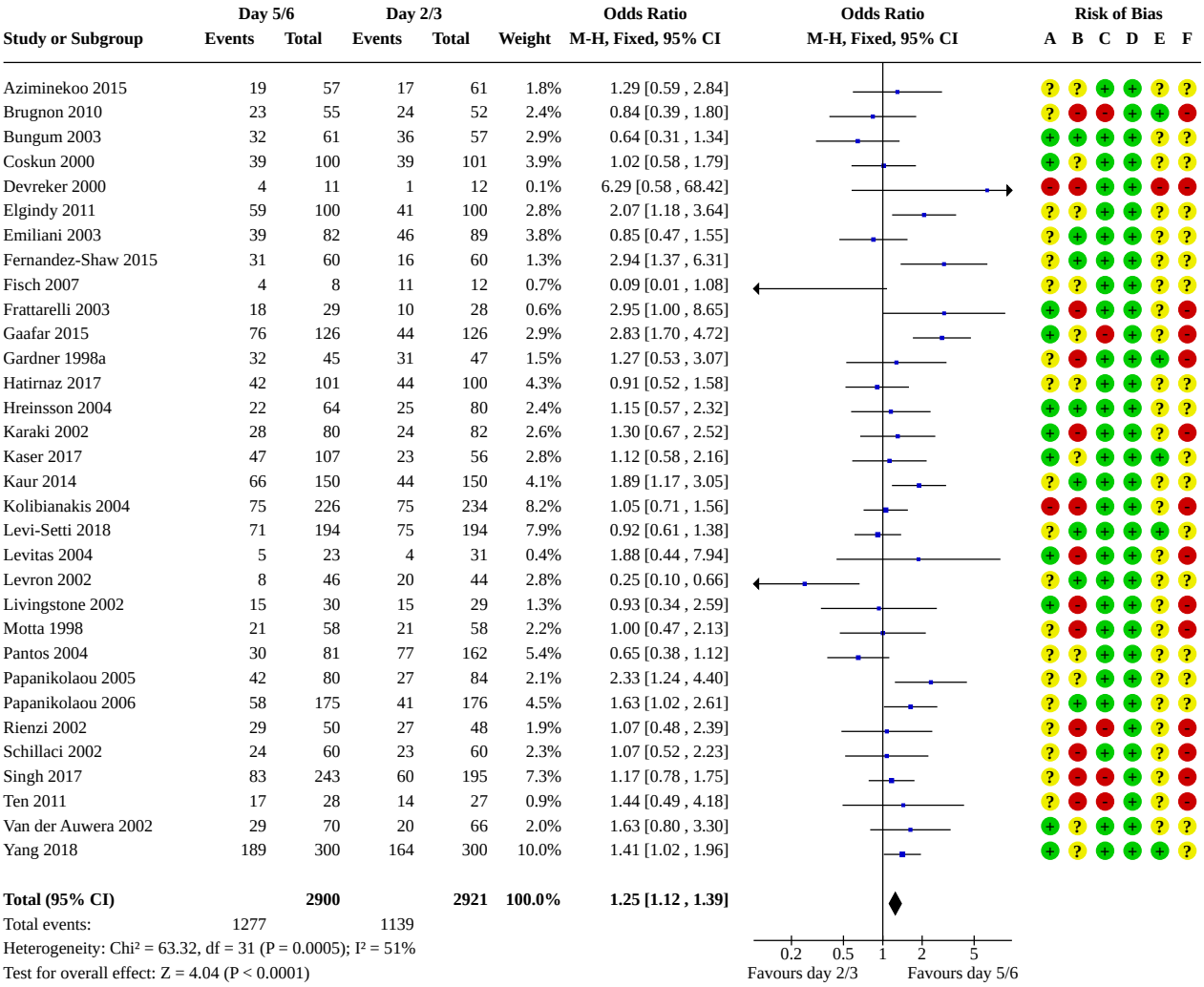
We are also uncertain whether blastocyst-stage transfer improves the cumulative pregnancy rate when we run a sensitivity analysis including only those studies with low or some concerns of overall risk of bias (OR 1.10, 95% CI 0.74 to 1.61; I^2 = 73%; 3 studies, 427 women; very low-quality evidence).

Secondary outcomes

3. Clinical pregnancy rate per couple or woman

The clinical pregnancy rate was higher in the fresh blastocyst-transfer group (OR 1.25, 95% CI 1.12 to 1.39; I^2 = 51%; 32 studies, 5821 women; moderate-quality evidence; Figure 3). This suggests that if 39% of women achieve a clinical pregnancy after fresh cleavage-stage transfer, between 42% and 47% would do so after fresh blastocyst-stage transfer.

Figure 3. Forest plot of comparison: 2 Blastocyst- versus cleavage-stage transfer: clinical pregnancy rate, outcome: 2.1 clinical pregnancy rate per couple



Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Subgroup and sensitivity analyses

We did not find evidence that the treatment effect differed between fresh cleavage-stage and blastocyst-stage transfer for clinical pregnancy rate. However, the separate analysis of subgroups shows that clinical pregnancy rate may be higher in the fresh blastocyst-stage transfer group when the number of transferred embryos is equal (OR 1.31, 95% CI 1.16 to 1.48; I^2 = 48%; 20 studies; 4434 women) and for single embryo transfers (OR 1.31, 95% CI 1.04 to 1.65; I^2 = 43; 5 studies; 1241 women). We also found that good prognosis participants (OR 1.25, 95% CI 1.09 to 1.43; I^2 = 51%; 9 studies, 3645 women) and unselected participants (OR 1.22, 95% CI 1.01 to 1.46; I^2 = 64%; 10 studies, 1981 women) may have a higher clinical pregnancy rate if fresh embryo transfer was done at blastocyst stage. When analysing a fresh blastocyst transfer without TLS versus TLS cleavage-stage transfer, the blastocyst transfer group also may have a higher pregnancy rate (OR 1.41, 95% CI 1.04 to 1.90; I^2 =0%; 2 studies, 709 women). However, when analysing TLS blastocyst-stage transfer versus TLS cleavage-stage transfer, no differences were found (OR 0.91, 95% CI 0.43 to 1.96; 1 study, 110 women).

Sensitivity analysis including only those studies with low risk of bias for allocation concealment did not substantially influence our findings (OR 1.52, 95% CI 1.19 to 1.94; I^2 = 68%; 8 studies, 1097 women), though heterogeneity was high. However, we found

that transferring at fresh blastocyst stage had a higher clinical pregnancy rate when randomisation was done on day 2 or 3 after oocyte pick up (OPU) (OR 1.59, 95% CI 1.13 to 2.23; I^2 = 63%; 4 studies, 537 women), but no differences were found when randomisation was performed earlier.

4. Multiple pregnancy rate per couple or woman

We are uncertain of the effect of the embryo stage on the multiple pregnancy rate in fresh cycles (OR 1.12, 95% CI 0.90 to 1.38; I^2 = 24%; 22 studies, 4208 women; low-quality evidence). This suggests that if 9% of women have a multiple pregnancy after fresh cleavage-stage transfer, between 8% and 12% would do so after fresh blastocyst-stage transfer.

Subgroup and sensitivity analyses

We did not find evidence that the treatment effect differed between fresh cleavage-stage and blastocyst-stage transfer based on number of embryos transferred, prognosis, or day of randomisation (Analysis 4.2; Analysis 4.3; Analysis 4.4).

Sensitivity analysis including only studies with low or some concerns of overall risk of bias showed that transferring at blastocyst stage probably increases the multiple pregnancy rate (OR 1.33, 95% CI 1.04 to 1.70; moderate-quality evidence).

We are uncertain of the effect of the embryo stage on the high-order multiple pregnancy rate in fresh cycles (OR 0.45, 95% CI 0.18 to 1.15; I^2 = 0%; 13 studies, 2335 women).

5. Miscarriage rate for fresh transfer

We are uncertain of the effect of the embryo stage on the miscarriage rate per couple or woman in fresh cycles (OR 1.24, 95% CI 0.98 to 1.57; I^2 = 0%; 21 studies, 4106 women; low-quality evidence).

6. Embryo freezing rate per couple or woman

Rates of embryo freezing when there are supernumerary embryos for transfer at a later date per couple or woman were lower in the blastocyst transfer group (OR 0.48, 95% CI 0.40 to 0.57; I^2 = 84%; 14 studies, 2292 women; low-quality evidence). There was very high heterogeneity, but the direction of effect was consistent in most studies.

7. Failure rate to transfer embryos

Rates of failure to transfer any embryos were higher in the blastocyst transfer group (OR 2.50, 95% CI 1.76 to 3.55; I^2 = 36%; 17 studies, 2577 women; moderate-quality evidence).

8. Other data

Blastocyst formation rates

As reported in Table 2, blastocyst formation rates, which show the proportion of 2PN embryos that get to blastocyst stage (day 5 to 6 transfer only) ranged from 22.4% in Aziminekoo 2015 to 60.3% in Schillaci 2002.

Implantation data

For blastocyst-stage transfer, the implantation rate varied from 4.2% to 55.8%. For cleavage-stage transfer, the implantation rate varied from 3% to 43.9% (see Table 2).

Assessment of publication bias

We generated funnel plots for the outcomes of live birth and clinical pregnancy. They did not suggest publication bias (see Figure 4; Figure 5).

Figure 4. Funnel plot of comparison: 1 Blastocyst- versus cleavage-stage transfer: live birth rate, outcome: 1.1 live birth per couple

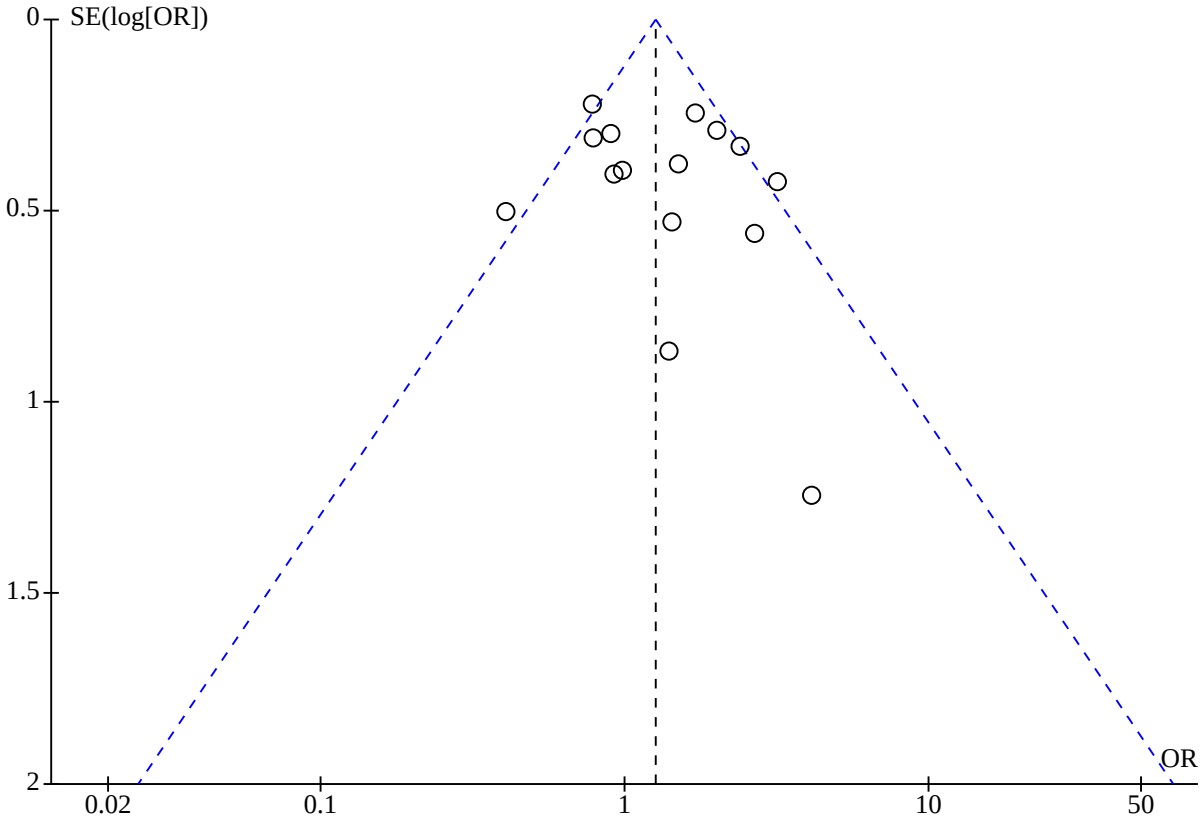
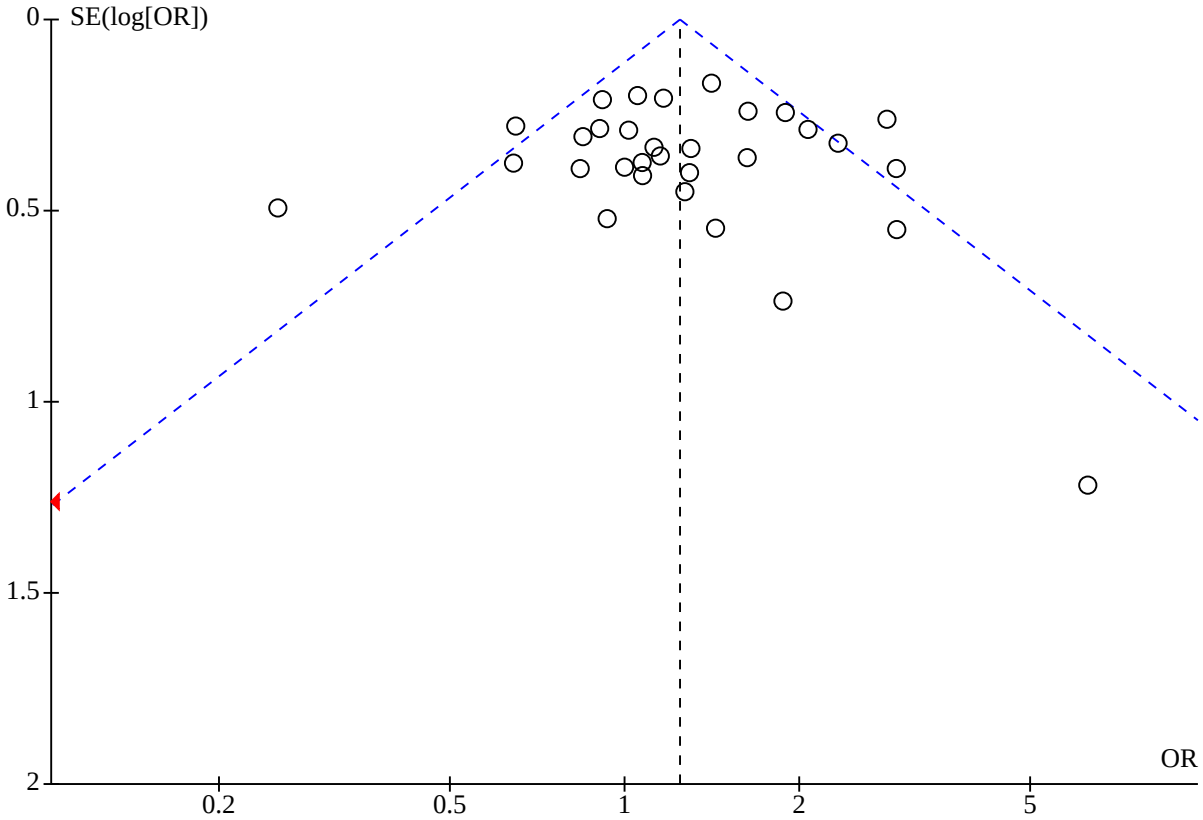


Figure 5. Funnel plot of comparison: 2 Blastocyst- versus cleavage-stage transfer: clinical pregnancy rate, outcome: 2.1 clinical pregnancy rate per couple



DISCUSSION

Summary of main results

In the 15 RCTs that reported live birth rates, there was low-quality evidence of a benefit in live birth rate per couple or woman in the fresh blastocyst transfer group and there was no evidence of a difference in the miscarriage rate. Clinical pregnancy rates were also higher in the fresh blastocyst transfer group, based on moderate-quality evidence. When the comparator was TLS cleavage-stage transfer, the benefit favoured blastocyst transfer as well.

We did not find any evidence of a difference when pooling the five RCTs that reported cumulative pregnancy rates (derived from fresh and thawed cycles from a single oocyte retrieval cycle): three were published in 2002/2003, one in 2010, and one in 2015. However, a post hoc analysis showed an interesting finding in which a blastocyst-stage transfer may improve the cumulative pregnancy rate when the method of freezing was vitrification, but not when slow freezing was used (low-quality evidence). This finding should be regarded cautiously as it resulted from a post hoc subgroup analysis.

Embryo freezing rates and failure rates to transfer embryos favoured early cleavage-stage transfers. We did not find any evidence of a difference between fresh blastocyst-stage and cleavage-stage transfers for rates of miscarriage, multiple pregnancies, or high-order multiple pregnancies.

Overall completeness and applicability of evidence

The data in this review of 32 RCTs are incomplete. Of 32 RCTs, only five reported cumulative pregnancy rates. Three of the five new studies (for the 2022 update) failed to report live birth data and all five failed to report cumulative pregnancy rates. This lack of data from the subsequent frozen-thawed cycles and, therefore, the lack of data on cumulative pregnancy rates, means that couples and women are not adequately informed about the outcomes.

For a well-informed decision between a cleavage-stage or blastocyst-stage embryo transfer policy, professionals, couples and women consider multiple variables, such as the chance of pregnancy, the time to pregnancy, the safety of the treatment, its burden, and the costs involved. The fact that the freezing rate is higher in cleavage-stage transfer highlights the importance of getting the cumulative pregnancy rates data, in order to draw more complete conclusions (Cornelisse 2018).

The applicability of the evidence to everyday practice is somewhat limited by the many variables present in an in vitro fertilisation (IVF) cycle, which directly result from different clinic policies. Although the 32 studies all compared cleavage-stage with blastocyst-stage embryo transfer, they differed with respect to media used, freezing protocols, policies about the numbers of embryos transferred, the use of time-lapse systems for embryo selection, and embryo quality scores. However, the most serious limitation was the lack of data from the frozen-thawed cycles, as alluded to in the previous paragraph. For the most part, the variation in the different protocols

can be a strength, as it means that in spite of the variation in cycles, the effect is still seen. With so few studies reporting the most useful outcome (cumulative pregnancy rate), it is difficult to report the applicability of the evidence.

Blastocyst culture is expected to result in higher implantation rates (number of fetal sacs observed divided by the number of embryos transferred). However, pooling of implantation data could not be included in the meta-analysis as this would not generate valid estimates or confidence intervals due to the unit of analysis used (Vail 2003). Implantation rate is also no longer considered a useful outcome for a number of methodological reasons (Griesinger 2016).

There was a significantly higher failure rate to transfer any embryos in the blastocyst-stage group, leading to cycle cancellation. The increased rates of failure to transfer with blastocyst stage is largely because of embryos with arrested development prior to the day of embryo transfer. Indeed, many of the studies that transferred fewer blastocysts than cleavage-stage embryos did so from a lack of options rather than by policy. Only the reporting of fresh and frozen-thawed cycle embryos can overcome this challenge. Although it could be better for some women to learn that their embryos failed to develop by day 5 than go through with a transfer at cleavage stage with embryos that had a low potential for success, there has been little research into the emotional status of couples or women given such choices (Borg 2000). Avoiding unnecessary embryo transfer needs to be balanced against the need for an additional oocyte retrieval. The possibility that extended culture may cause harm to viable embryos, through suboptimal culture conditions, must be considered, particularly when there are large variations between trials in blastocyst development rates.

There are no good-quality studies showing the data of women with poor prognosis, such as few available embryos, or women at advanced reproductive age. Both scenarios are extremely common, yet the evidence to make an educated decision in these scenarios is still missing. We found one ongoing study Neuhausser 2020) that intends to analyse these subgroups. Thus, the next update of this review may be able to assess this information.

The varying embryo transfer policies between the two experimental groups was also a concern: a significant number of the studies had a policy to transfer fewer embryos in the blastocyst-stage group than in the cleavage-stage group (Table 3). There are two primary reasons for this difference. First, there is a reduced survival rate of day 5 to 6 blastocysts. Second, many clinics worried about the high incidence of multiple pregnancies with blastocysts will have a policy to transfer no more than two blastocyst-stage embryos. Some clinics state that by employing blastocyst culture, they have been able to reduce the multiple pregnancy rate whilst maintaining the pregnancy rate. In this review, many of the studies were still transferring two to three embryos. Single-embryo transfers for selected patient groups are now considered standard practice in many clinics throughout the world (Hamberger 2005).

The importance of selecting the single most viable embryo for transfer has intensified the search to improve assessment of the quality of embryos. Performing blastocyst culture may offer one of those mechanisms (Gardner 2004; Milki 2004). In this meta-analysis, significantly fewer embryos were transferred in the blastocyst-stage group than in the cleavage-stage group. When we performed a subgroup analysis for trials where equal numbers of embryos were transferred (including single-embryo transfers), the clinical pregnancy rate remained unchanged. It could be argued that this is the most valid comparison, because trials with a greater number of cleavage-stage embryos being transferred are probably advantaged inappropriately.

Regardless of the embryo transfer policy, for many women, there is simply a lack of choice, as only one, if any, embryo reaches the blastocyst stage. Only three studies in this review had a policy for single-blastocyst transfer, although only one reported the live birth rate. None of the new studies added to this updated review had a single-embryo transfer policy.

Studies have shown that women with a high oocyte yield and good-quality 8-cell embryos on day 3 are more likely to have blastocysts by day 5 to 6 than poor responders and those with no 8-cell embryos by day 3. Therefore, we considered whether outcomes might be influenced by the time of randomisation. In subgroup analysis for live birth and pregnancy outcomes, we compared studies that randomised couples or women prior to the start of the treatment cycle (at a time when neither the number of oocytes retrieved nor fertilised nor the number of 8-cell embryos could be anticipated) versus studies that randomised women at a later stage. We found no evidence of a statistically significant difference between the subgroups.

Miscarriage rates were reported in just over half of the included trials. Theoretically, the rate of miscarriage might be expected to be lowest with the transfer of highly selected embryos into a better synchronous uterine environment, such as in blastocyst culture. However, the results to date reveal little change from earlier reviews that showed no evidence of a difference in miscarriage rates for couples or women randomised (odds ratio (OR) 1.15, 95% confidence interval (CI) 0.88 to 1.50; 21 studies). Only seven of the included trials reported on the presence or absence of monozygotic twinning, so this analysis remains underpowered to comment meaningfully on monozygotic twin rates. A total of three sets of monozygotic twins were reported, two with cleavage-stage embryo transfers and only one set of monozygotic twins from blastocyst transfer. Monozygotic twin rates in assisted reproductive technologies (ARTs) are thought to be underestimated, with up to one-third being missed without genetic testing (Vitthala 2009).

Overall, this review found that women in the blastocyst group were less likely to have any embryos frozen, but there was no clear evidence of a difference in cumulative pregnancy rate (fresh and frozen-thawed cycle transfers). The number of embryos frozen is an important consideration when assessing the effectiveness of a treatment, as it may offer women an additional opportunity to achieve a pregnancy. When considering an alteration in treatment procedure from cleavage-stage to blastocyst-stage transfer, the benefits of possible higher implantation rates are weighed up against the disadvantages of not only higher failure rates to transfer, but also lower cryopreservation rates.

Five trials reported data on pregnancies following transfer of frozen-thawed embryos in both groups (Brugnon 2010; Emiliani 2003; Fernandez-Shaw 2015; Rienzi 2002; Van der Auwera 2002). Van der Auwera 2002 reported a cumulative live birth rate of 47% in the blastocyst-stage transfer group and 52% in the cleavage-stage transfer group. That is, the added benefit of a higher cryopreservation rate in the cleavage-stage group cancelled out the higher implantation rates of the fresh day 5 to 6 transfers. Similarly,

Rienzi 2002 and Brugnon 2010 reported no evidence of a difference in cryo-augmented pregnancy rates when at least one thawed cycle was carried out in the cleavage-stage group. Emiliani 2003, on the other hand, reported significantly higher cumulative pregnancy rates in the cleavage-stage group, presumably correlating to the much lower cryosurvival rate they reported in their blastocyst group (cleavage stage: 46%; blastocyst stage: 27%). However, Fernandez-Shaw 2015 reported significantly higher cumulative pregnancy rates in the blastocyst-stage group (day 3: 43.3%; day 5: 56.8%). This study was the only study to use vitrification, and also included women with a good prognosis. The pooled data for cumulative clinical pregnancy has 71% heterogeneity and this appears to be explained by the differences in freezing methods. This is supported by other reports of improved blastocyst outcomes with vitrification (Edgar 2012; Gardner 2003; Iwayama 2011; Richter 2016).

Although the evidence is limited, there is a growing trend for freeze-only cycles to further reduce the risk of ovarian hyperstimulation (OHSS) and improve pregnancy outcomes if the progesterone levels are elevated (Roque 2015; Zaat 2021). This trend will also have an impact on the decision about cleavage-stage or blastocyst-stage transfers and should be considered in the next update.

Quality of the evidence

The overall quality of the evidence in this review was low for most outcomes and moderate for clinical pregnancy rate and failure to transfer any embryos. The main limitations in evidence quality were serious imprecision and serious risk of bias. Blinding is not possible with this intervention. Only half of the included studies adequately described their method of sequence generation, and less than one-third reported their allocation concealment method. For some outcomes, we also downgraded the quality of evidence for imprecision. The large imprecision impacts most of the outcomes: both in cases in which we cannot say if the intervention increases or decreases the outcome rates, and also in some cases in which we cannot say if the intervention increases those rates a little or a lot.

Live birth rates were reported in 15 trials, providing low-quality evidence (serious risk of bias and imprecision). Data for cumulative pregnancy rates after fresh and frozen-thawed embryo transfer were only reported in five trials, in which heterogeneity was found. The most likely explanation for the heterogeneity was the difference in freezing protocols, with one study using vitrification and reporting benefit for blastocyst-stage transfer for the cumulative pregnancy rate (Fernandez-Shaw 2015), and the other four studies using slow freezing and reporting benefit for cleavage-stage transfer.

Potential biases in the review process

As far as possible, we adhered to the protocol methodology in order to limit any potential biases. However, we added one new outcome – cumulative pregnancy rates (derived from fresh and thawed cycles) – in the 2016 update. This outcome reflects the policy of fresh embryo transfer and freezing remaining embryos for transfer later and more correctly reflects modern IVF practice than a single cycle transfer. Although we contacted study authors to try to obtain data for cumulative pregnancy rates, most did not collect this information.

There is an important distinction between the outcomes 'live birth rate following fresh transfer' and ‘cumulative pregnancy rate’. In the last decade, many studies only reported the outcome live birth rate following the first transfer, and trialists are generally used to reporting this outcome. Due to the extended embryo culture in blastocyst-stage transfers, the selected embryos are potentially of higher quality, but the number available for transfer is reduced, which could potentially affect the cumulative pregnancy outcomes. Still, low-quality evidence showed that cumulative pregnancy rate, when using vitrification, may benefit blastocyst transfer as well.

The issue of publication bias is important in systematic reviews as it may result in incorrect conclusions being reached. For example, it might be expected that the pressure for clinics to obtain high implantation rates with blastocyst culture could lead to a bias in publication towards those that do achieve this. However, the funnel plots for live birth and clinical pregnancy did not suggest publication bias.

Another point to consider is the widely varying policies for assessing minimal quality of embryos for transfer that may have existed amongst trials. Some trials accepted transfer of developmentally-delayed embryos in blastocyst stage, whilst other trials were more selective and denied transfer of embryos that had not reached the late morula or early blastocyst stage. A strict selection policy for blastocyst transfer could have a potential bias in favor of the cleavage stage arm for two outcomes, embryo freezing rate per couple and failure rate to transfer embryos per couple. The freezing rate can be higher and the failure rate to transfer embryos lower in the cleavage stage group.

Blastocyst formation rates may also influence the pregnancy rate per embryo transfer for each trial. They ranged from 22.4% in the Aziminekoo 2015 trial to 60% in the Schillaci 2002 trial. Of the included trials that provided information on the media used, all used sequential media for the culture of blastocysts. However, while the majority reported using various versions of Vitrolife G1/G2, others used a combination of different brands, or made the media in-house. This highlights the possibility that different brands and formulations are likely to influence the blastulation rates and subsequent outcomes. Improvements in culture media and embryo laboratory procedures should prompt new studies.

We note that there are six ongoing studies that started more than five years ago, and which have not yet published findings. These may raise a concern about publication bias.

Finally, although we took steps to conduct an exhaustive search for eligible trials, it is possible that our search strategy failed to find some studies, resulting in bias.

Agreements and disagreements with other studies or reviews

This Cochrane Review is in agreement with the Papanikolaou 2008 systematic review, which reported that cycles where equal numbers of embryos were transferred had higher live birth rates in blastocyst-stage transfers than in cleavage-stage tranfers. Our review also includes the cumulative pregnancy rate. Another systematic review was published by Wang 2014, which only included RCTs published after 2004. Wang 2014 shows similar results with respect to live birth rates, but reported lower miscarriage rates in the blastocyst group, which was not observed

in our review. Two published reviews that cited the previous version of this review are in agreement that better studies are required (Maheshwari 2016; Martins 2016), and argue that analysing obstetric and neonatal outcomes results is critical in drawing clearer conclusions. Another systematic review by Martins 2017 emphasises that there is still too much uncertainty to conclude that transferring at one stage is better than the other.

Alviggi 2018 published a systematic review of retrospective studies, analysing perinatal outcomes. They showed that blastocyst transfers may slightly increase the preterm and very preterm birth rates but may decrease the small-for-gestational-age rates in fresh cycles. However, these associations were not seen in frozen transfers.

Busnelli 2019 published a systematic review including mostly retrospective studies, analysing the risk factors for monozygotic twins. They showed that blastocyst transfer may be one of the factors to increase the risk of monozygotic twins.

Spangmose 2020 published a large retrospective cohort study analysing obstetric and perinatal outcomes, after fresh blastocyst or cleavage transfer. They showed that fresh blastocyst transfer increases the risk of placenta previa, preterm birth, and monozygotic twins. Furthermore, an altered male-female ratio with more males following blastocyst transfer was seen.

The same study group published a large retrospective cohort study (Ginström 2019), analysing obstetric and perinatal outcomes, after frozen-thawed transfer of vitrified blastocysts or slow-frozen cleavage-stage embryos. They showed a higher risk of preterm birth after blastocyst-stage transfer. For maternal outcomes, no significant differences were found, although the precision was limited.

AUTHORS' CONCLUSIONS

Implications for practice

There is low-quality evidence that blastocyst-stage transfer, in comparison to cleavage-stage transfer, is associated with higher rates of live birth, and moderate-quality evidence showing that clinical pregnancy rate is also higher in blastocyst-stage transfer. We are uncertain whether blastocyst-stage transfer improves the cumulative pregnancy rate (derived from fresh and thawed cycles). Given that the embryo freezing rate may be higher in cleavage-stage transfer, more evidence about cumulative pregnancy rate and a cost-effectiveness analysis are needed to draw firmer conclusions. Finally, there is low-quality evidence showing that the addition of a time-lapse system (TLS) to a cleavage-stage transfer is still less effective than a blastocyst-stage transfer without TLS in terms of pregnancy rate.

Implications for research

Although this review provides evidence that there is a significant difference in live birth rates from fresh cycles in favour of blastocyst-stage transfer compared to cleavage-stage transfer cycles, the findings for cumulative clinical pregnancy rates (derived from fresh and thawed cycles) still raise questions. Further new studies of blastocyst cycles must report live birth, cumulative live birth rates (especially when vitrification is used), and miscarriage rates to enable consumers and service providers to make well-informed decisions on the best treatment option. Also, more studies evaluating poor prognosis women will help to obtain more information about this specific subgroup. Finally, more studies with single-embryo transfer are also needed in order to identify the best option with the fewest adverse event rates.

Based on the results of this review, we recommend the following to ensure valuable data are produced.

- Adherence to Consolidated Standards of Reporting Trials (CONSORT) recommendations for randomised controlled trials (RCTs), especially methods of concealment (Begg 1996).
- Research into the best participant selection and inclusion criteria; consider prognostic factors such as age and outcomes from previous cycles.
- Same media composition and brand for both groups up to the cleavage stage.
- Explicit prespecified embryo transfer policies for both groups; preferably single-embryo transfer.
- Long-term follow-up reports of cumulative live birth rates (including embryo thaws) presented as a survival analysis. A minimum of a one-year time period after randomisation for follow-up of cumulative live birth rates within studies (as part of study protocol), and a possible follow-up of results of transfers outside this time window in a later report (if applicable, i.e. if not all transfers have been performed within one year).
- Research into improved blastocyst cryopreservation techniques, e.g. vitrification.
- Reporting of miscarriage, live birth, and cumulative pregnancy rates.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Aziminekoo 2015

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Iran
Participants	• Number of participants randomised: 118 • Age: BS 34.9 and CS 35.1 • Number of previous treatments: BS 3.7 and CS 3.1 • Infertility duration: BS 10.3 and CS 8.5 years • Prognosis: poor prognostic factors • Inclusion criteria: infertile women with at least two previous failures of implantation • Exclusion criteria: females aged more than 40 years old with severe untreated uterus abnormalities, and couples with severe male factor infertility, such as severe oligospermia

Interventions	• BS group: N = 57
	• CS group (day 3): N = 61
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ Injected oocytes were transferred to culture dish with cleavage medium (Sydney IVF cleavage medium, Cook Medical)
	  ○ In blastocyst group, on day 3, embryos were transferred to blastocyst medium (Sydney IVF blastocyst medium) for 48 h extended culture. Then, the day 5 embryos (blastocysts) were transferred to uterus.
	  ○ All embryo transfers were performed using the Sydney IVF catheter (K-JETS-7019-SIVF; Cook Medical)
	  ○ To support luteal phase, all participants received progesterone suppository.

Outcomes	Clinical pregnancy rate Multiple pregnancy rate

Aziminekoo 2015 *(Continued)*

	Miscarriage rate
	Cancellation
Notes	

Brugnon 2010

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: France
Participants	• Number of participants randomised: 107 • Age: BS and CS: not stated • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: if more than five oocytes were retrieved and three top-quality embryos (4 blastomeres, < 20% fragmentation without multinuclear blastomeres) were observed at day two, the couples were included in the study. • Exclusion criteria: Not described

Interventions	• BS group: N = 52
	• CS group (day 3): N = 56
	• Description of the cycles:
	  ○ Sequential media was used in both groups.
	  ○ The freezing method was standard slow freezing method at day 3(FreezeKit, Vitrolife) and at day 5/6 (G- FreezeKit Blast, Vitrolife)

Outcomes	Cumulative pregnancy rate Clinical pregnancy rate per embryo transfer
Notes	

Bungum 2003

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Denmark
Participants	• Number of participants randomised: 118 • Age: BS 31.2 and CS 31.3 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis patients • Inclusion criteria: D3 3 or more 8-cell embryos < 20% fragmentation, eligible participants under 40 years of age, BMI < 30, FSH < 12 • Exclusion criteria: not stated

Bungum 2003 *(Continued)*

Interventions	• BS group: N = 61
	• CS group (day 3): N = 57
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ HEPES-buffered medium, was used to rinse the oocytes(Vitrolife, Gothenburg,Sweden)
	  ○ Embryos cultured for either 3 or 5 days in the sequential media system used in the standard IVF/ICSI programme (G1/G2 Vitrolife, Gothenburg, Sweden).
	  ○ All embryo transfers were performed with a Cook Soft 5000catheter (Cook, Australia)
	  ○ To support luteal phase, all participants received progesterone suppository

Outcomes	Clinical pregnancy rate Multiple rate Miscarriage Embryo freezing rate Implantation rate
Notes	3 or more 8-celled D3 Lower blast rate in ICSI than IVF Letter sent and reply received

Coskun 2000

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Israel
Participants	• Number of participants randomised: 201 • Age: BS 30.4 and CS 30.7 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: all IVF/ICSI cycles from consenting patients with four or more fertilized oocytes. • Exclusion criteria: no exclusion criteria

Interventions	• BS group: N = 100
	• CS group (day 3): N = 101
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ IVF medium(Medi-Cult) was used to rinse the oocytes.
	  ○ Embryos cultured for day 3 were cultured in IVF medium(Medi-cult). Embryos for day 5 were cultured in the sequential media system (G1/G2 Vitrolife, Gothenburg, Sweden).
	  ○ To support luteal phase, all participants received progesterone suppository(100mg/daily i.m)

Outcomes	Clinical pregnancy rate Multiple pregnancy rate High-order multiple pregnancy rate

Coskun 2000 *(Continued)*

	Miscarriage rate
Notes	Prognosis: good 4 or more zygotes Young women Good ET policy Mixture of media brands Low blast rate High implantation rate considering No dropouts unusual

Devreker 2000

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Belgium
Participants	• Number of participants randomised: 23 • Age: not stated • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: poor prognostic • Inclusion criteria: age < 40 years old and > four previous cycles • Exclusion criteria: not stated
Interventions	• BS group: N = 11 • CS group (day 2): N = 12 • Description of the cycles: not stated
Outcomes	Live birth rate Clinical pregnancy rate Miscarriage rate
Notes	Prognosis: poor Abstract only Letter sent regarding randomisation

Elgindy 2011

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Egypt
Participants	• Number of participants randomised: 200 • Age: BS 28.47 and CS 27.7 • Number of previous treatments: not stated • Infertility duration: BS 6.22 and CS 6.84 years • Prognosis: not stated

Elgindy 2011 *(Continued)*

	• Inclusion criteria: participants under 35 years of age, with regular cycles, serum day-3 FSH concentration < 9.5 IU/L and antral follicle count > 6. At least four good-quality embryos on day 3 • Exclusion criteria: not described

Interventions	• BS group: N = 100
	• CS group (day 2): N = 100
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH and HCG)
	  ○ Embryos were cultured in sequential media (Sage; Cooper Surgical, USA)

Outcomes	Live birth rate per fresh embryo transfer Clinical pregnancy rate Miscarriage rate Failure rate to transfer embryos
Notes	

Emiliani 2003

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Belgium
Participants	• Number of participants randomised: 171 • Age: BS 32 and CS 31 • Number of previous treatments: BS 2.0 and CS 1.7 • Infertility duration: not stated • Prognosis: mixed unselected • Inclusion criteria: participants under 39 years of age, 3 or fewer previous cycles, 4 or more 2PN on day 1 • Exclusion criteria: not described

Interventions	• BS group: N = 82
	• CS group (day 2): N = 89
	• Description of the cycles:
	  ○ Ovarian stimulation was performed using GnRH analogue (buserelin acetate: Suprefact spray; Hoechst, Germany), hMG (Humegon; Organon, The Netherlands) and hCG (Pregnyl; Organon).
	  ○ For culture between day 3 and day 5 after insemination, similar dishes were prepared, containing blastocyst medium (in-house sequential media).

Outcomes	Live birth rate per fresh embryo transfer Cumulative pregnancy rate Clinical pregnancy rate Multiple pregnancy rate Failure rate to transfer embryos
Notes	Prognosis: mixed unselected Outcome: discontinued blast culture Gives cumulative fresh thawed rates

Emiliani 2003 *(Continued)*

	Not all different women - per cycle data only

Fernandez-Shaw 2015

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Spain
Participants	• Number of participants randomised: 120 • Age: BS 35.2 and CS: 36.3 • Number of previous treatments: BS 0 and CS 0 • Infertility duration: BS 23.9 months and CS 27.7 months • Prognosis: mixed unselected • Inclusion criteria: first IVF or ICSI cycle; presence of normal uterine cavity; ejaculated sperm origin; absence of any contraindications to pregnancy • Exclusion criteria: oocyte donation cycles; vitrified oocytes cycles; non-ejaculated sperm; PGD

Interventions	• BS group: N = 60
	• CS group (day 3): N = 60
	• Description of the cycles:
	  ○ Three ovarian stimulation protocols were used for the 120 participants in the study depending on their age and diagnosis: long GnRH agonist protocol with intranasal nafarelin; short GnRH agonist protocol with nafarelin; GnRH antagonist protocol where the ganirelix was started on day 6 of the stimulation. All with recombinant daily FSH. Some participants were given added hMG when needed.
	  ○ Oocytes and embryos were cultured in sequential media of Vitrolife Sweden (G5 series, Kungsbacka, Sweden) using IVF, G1 and G2 medium as recommended by the manufacturer.
	  ○ All embryo transfers were performed using a Wallace catheter with EmbryoGlue media.
	  ○ To support luteal phase, all participants received progesterone suppository.
	  ○ Frozen embryos were vitrified in the day 3 and day 5 groups respectively. The vitrification followed the Irvine Scientific procedure.

Outcomes	Cumulative live birth rate* Live birth rate* Cumulative pregnancy rate Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate The percentage of participants with embryos that were vitrified Three participants from the day 3 group and 2 from the day 5 group (with a mean number of 3 vitrified embryos) had not done a frozen embryo transfer cycle at the time of closing our interim analysis.
Notes	*Additional unpublished data received from contact author May 2016 in personal communication (email) to Demián Glujovsky. This is source of data on live births (analysis 1.1)

Fisch 2007

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: USA
Participants	• Number of participants randomised: 20 • Age: BS 31.2 and CS: 31.3 • Number of previous treatments: BS 0 and CS 0 • Infertility duration: BS 23.9 months and CS 27.7 months • Prognosis: good prognosis • Inclusion criteria: women under 41 years of age, with ≤ 2 prior fresh cycles with at least one embryo on day 3 with graduated embryo score ≥ 70 and soluble human leukocyte antigen-G (sHLA-G): 0.148–0.210 • Exclusion criteria: not described
Interventions	• BS group: N = 12 • CS group (day 3): N = 8 • Description of the cycles: not stated
Outcomes	Clinical pregnancy rate
Notes	

Frattarelli 2003

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Hawaii
Participants	• Number of participants randomised: 57 • Age: BS 30.2 ± 3.2 and CS 31.0 ± 2.8 • Number of previous treatments: BS 0 and CS 0 • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: women under 35 years of age, no previous cycles, a day 3 FSH < 12 mIU/ml, ten or more follicles of 14mm or more on the day of hCG administration, and six or more high-grade embryos on day 3 • Exclusion criteria: not described

Interventions	• BS group: N = 26
	• CS group (day 3): N = 23
	• Description of the cycles:
	  ○ In both groups they used the same media: sequential culture media.
	  ○ More information about the cycle is not available.

Outcomes	Live birth rate per fresh embryo transfer Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate

Frattarelli 2003 *(Continued)*

	Failure rate to transfer embryos
Notes	Prognosis: good 6 or more high-grade embryos D3, 1st cycle, young, high numbers of oocytes No dropouts due to lack of blasts High blast implantation rate

Gaafar 2015

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Egypt
Participants	• Number of participants randomised: 326 • Age: not stated • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: unselected • Inclusion criteria: couples suffering from male infertility, with indication for ICSI • Exclusion criteria: not described
Interventions	• BS group: N = 126 • CS group (day 2): N = 126 • Description of the cycles: ○ Long agonist protocol was used followed by ICSI procedure. More information about the cycle is not available.
Outcomes	Clinical pregnancy rate Miscarriage rate
Notes	

Gardner 1998a

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: USA
Participants	• Number of participants randomised: 92 • Age: BS 33.6 and CS 34.5 • Number of previous treatments: BS 0.61 and CS 0.21 • Infertility duration: not stated • Prognosis: good prognosis patients • Inclusion criteria: the female age must be under 45 years of age, FSH < 15 mIU/ml, presence of normal uterine cavity, adequate semen for IVF or ICSI. In addition, at least 10 follicles 12 or more mm in diameter were required on day of hCG trigger • Exclusion criteria: any contraindications for pregnancy
Interventions	• BS group: N = 45

Gardner 1998a *(Continued)*

	• CS group (day 3): N = 47
	• Description of the cycles:
	  ○ Ovarian hyperstimulation was initiated with leuprolide acetate.
	  ○ Embryos were cultured until day 3 in Ham's F10+ (Flow Laboratories,McLean, VA,USA) fetal cord serum (FCS), until D5 in Vitrolife G1 /G2
	  ○ All embryo transfers were performed using a Wallace catheter (Edwards-Wallance catheter; Marlow Technologies)
	  ○ To support luteal phase, all participants received steroids and tetracycline for 4 days post-OPU and progesterone suppository, 50 mg IM in oil.

Outcomes	Clinical pregnancy rate Embryo freezing rate Implantation rate
Notes	Prognosis: good < 1 previous cycle and > 10 follicles Different media used for each arm of study Excluded participants not mentioned 'Number of ET' policy change partway through Unblinded interim analysis: initially participants received three blastocysts.

Hatirnaz 2017

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Turkey
Participants	• Number of participants randomised: 201 • Age: BS 30.4 and CS 29.4 • Number of previous treatments: not stated • Infertility duration: BS 8.1 and CS 7.8 years • Prognosis: unselected participants • Inclusion criteria: the retrieval of at least four fertilized oocytes was set up as a criterion for eligibility to keep the risk for treatment discontinuation and cycle cancellation at a minimum. • Exclusion criteria: high risk for ovarian hyperstimulation.

Interventions	• BS group: N = 95
	• CS group (day 3): N = 95
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ Embryos for day 3 were cultured in the standard culture medium, blastocysts for day 5 were moved into G1.2 and G2.2 media (Scandinavian IVF Sciences, Gothenburg, Sweden) on day 1 and day 3, respectively.
	  ○ The luteal phase was supported by 50 mg intramuscular progesterone in oil once daily (Progestan®, Koçak Farma, İstanbul, Turkey) and estradiol, two 100 µg transdermal patches (Estraderm TTS®, Novartis Pharma AG, Basel, Sweden) with daily replacements.

Outcomes	Live birth rate Clinical pregancy rate Miscarriage rate Multiple rate

Hatirnaz 2017 *(Continued)*

	Implantation rate
Notes	

Hreinsson 2004

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Sweden
Participants	• Number of participants randomised: 144 • Age: BS 32.1 and CS 33.1 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good; excluded poor responders • Inclusion criteria: women undergoing IVF or ICSI treatment cycles, who had at least six follicles as observed at the final ultrasound scan before hCG administration were allocated to the study, after they had given informed consent. • Exclusion criteria: not described

Interventions	• BS group: N = 64
	• CS group (day 3): N = 80
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH) or short protocol with Cetrorelix
	  ○ Injected oocytes were collected into IVF-medium (Vitrolife, Gothenburg, Sweden).
	  ○ The embryos were cultured from day 1 to day 3 in IVF-medium and from D3 to D5-6 in CCM-medium'(Vitrolife). In the first 50 cases, sequential media was used(G1/G2 Vitrolife).

Outcomes	Clinical pregnancy rate
Notes	Prognosis: good; excluded poor responders Mixture of media types and ET policy change over course of study Outcome no advantage of blast culture Letter sent and reply received

Karaki 2002

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Jordan
Participants	• Number of participants randomised: 162 • Age: BS 30.0 and CS 29.0 • Number of previous treatments: BS 0.9 and CS 1.1 • Infertility duration: BS 6.8 and CS 6.7 years • Prognosis: unselected participants • Inclusion criteria: all patients needed at least five two-pronuclei embryos. • Exclusion criteria: not described

Karaki 2002 *(Continued)*

Interventions	• BS group: N = 80
	• CS group (day 3): N = 82
	• Description of the cycles:
	  ○ Ovarian stimulation with GnRH agonist administrated in either long (down-regulation) or short(flare) protocol in addition to HP-FSH or recFSH.
	  ○ Embryos in the day 3 group were cultured in IVF medium (Medi-Cult, Jyllinge, Denmark). Embryos for the blastocyst group were transferred to G1.2 and G2.2 media(Scandinavian IVF Sciences, Gothenburg, Sweden).
	  ○ All embryo transfers were performed using the Edward-Wallace catheter.
	  ○ To support luteal phase, all participants received progesterone suppository.

Outcomes	Clinical pregnancy rate Multiple rate Miscarriage rate Failure rate to transfer embryos Implantation rate
Notes	Prognosis: moderate, young women, moderately high oocyte numbers. Large difference in embryo ET# between groups Sent letter

Kaser 2017

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: USA
Participants	• Number of participants randomised: 163 • Age: BS 34.4 and CS 34.6 years • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: all participants aged 18 to 40 years with a planned fresh single embryo transfer • Exclusion criteria: more than three prior retrievals without an intervening clinical pregnancy; use of donor oocytes, a gestational carrier, PGD, or in-vitro maturation; and presence of uninterrupted hydrosalpinx or intrauterine adhesions. Participants were excluded if all embryos were frozen due to ovarian hyperstimulation, or if they had fewer than four zygotes.

Interventions	• BS group: N = 107
	• CS group (day 3): N = 56
	• Description of the cycles:
	  ○ Three groups. D3 + time-laps system/ D5 + time laps system/ D5 conventional.
	  ○ After fertilisation check, zygotes were placed in a 12 well Eeva dish(Global total with HSA, LifeGlobal, CT, USA) within a time-lapse system.

Outcomes	Clinical pregnancy rate Cumulative pregnancy rate was not evaluated as the randomization was broken after the first transfer, and all embryos were cryopreserved at the blastocyst stage.

Kaser 2017 *(Continued)*

Notes	This study was funded by Progyny, Inc, which participated in the initial study design and approved the final embryo selection algorithms. All data handling, statistical analyses, and interpretation was performed independent of Progyny. The sponsor solely provided comments on the manuscript, and did not have editorial control in the manuscript preparation or submission. The trial was terminated prematurely in February 2016 by the sponsor due to a change in funding priorities. Enrolment opened in August 2014 and closed in February 2016. Clinicaltrials.gov NCT02218255

Kaur 2014

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: India
Participants	• Number of participants randomised: 300 • Age: BS 32.0 and CS: 34.4 • Number of previous treatments: not stated • Infertility duration: BS 7.7 and CS 8.9 years • Prognosis: good prognosis group • Inclusion criteria: participants aged 25-40 years with 2-20 years of infertility; having minimum five oocytes at oocyte pick up and endometrial thickness of 7 mm and more indicating good ovarian response; having normal uterine cavity and basal FSH < 10 mIU/mL; availability of minimum three good quality embryos • Exclusion criteria: not described

Interventions	• BS group: N = 150
	• CS group (day 3): N = 150
	• Description of the cycles:
	  ◦ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ◦ Retrieved oocytes were incubated in IVF-30 media. The fertilised oocytes were transferred into cleavage medium. In the blastocyst group extended culture till day 5 in G2 plus media.
	  ◦ All transfers were performed using Edward–Wallace catheter.
	  ◦ Luteal support was given in the form of micronised vaginal progesterone in dose of 200 mg twice a day. Injection HCG 2000 IU was given IM on days 5th, 8th, and 11th after retrieval

Outcomes	Clinical pregnancy Multiple pregnancy Miscarriage rate Implantation rate
Notes	Attempt to contact authors for more details of randomisation methods was not successful

Kolibianakis 2004

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups

Kolibianakis 2004 *(Continued)*

	• Country: Belgium
Participants	• Number of participants randomised: 460 • Age: BS 31.5 and CS 31.1 years • Number of previous treatments: BS 0.8 and CS 0.7 • Infertility duration: not stated • Prognosis: unselected participants • Inclusion criteria: women under 43 years of age and an indication for IVF • Exclusion criteria: preimplantation genetic screening and azoospermia.

Interventions	• BS group: N = 226
	• CS group (day 3): N = 234
	• Description of the cycles:
	  ○ Two ovarian stimulation protocols were used in the present study. Initially, the long protocol (down-regulation with GnRH agonist and stimulation with rFSH). A combination of GnRH antagonist and recombinant gonadotropins was introduced in turn, and gradually replaced the long agonist protocol.
	  ○ Embryos were cultured in sequential media G1/G2 (Vitrolife, Goethenburg,Sweden).
	  ○ To support luteal phase, all participants received progesterone suppository.

Outcomes	Clinical pregnancy rate Miscarriage rate Embryo freezing rate Failure to transfer embryos
Notes	Prognosis: mixed unselected

Levi-Setti 2018

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Italy
Participants	• Number of participants randomised: 388 • Age: BS 33.5 and CS 33.4 years • Number of previous treatments: BS 2.05 and CS 2.06 • Infertility duration: BS 3.96 and CS 4.42 years • Prognosis: good prognosis • Inclusion criteria: couples with a diagnosis of primary or secondary infertility with a clinical indication for IVF/intracytoplasmic sperm injection (ICSI), and under 39 years of age were enrolled, if they had more than three fertilised oocytes (zygotes) the day after insemination/injection. • Exclusion criteria: couples with three or more failed previous IVF/ICSI cycles. Couples involved in other clinical or embryological trials or at high risk for ovarian hyperstimulation syndrome (OHSS) were also excluded.
Interventions	• BS group: N = 188 • CS group (day 3): N = 188

Levi-Setti 2018 *(Continued)*

	• Description of the cycles:
	  ○ All enrolled patients underwent a stimulation treatment for IVF/ICSI.
	  ○ For patients in the cleavage stage group, two embryos were transferred, while for the ones in the blastocyst group, two blastocysts were transferred, when available.
	  ○ Embryo transfer was performed under ultrasound guidance using a soft catheter by a professional with at least 6 months of training

Outcomes	Live birth rate Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate Implantation rate
Notes	

Levitas 2004

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Israel
Participants	• Number of participants randomised: 54 • Age: BS 29.1 years and CS:31.2 years • Number of previous treatments: BS 4.9 and CS 4.3 • Infertility duration: BS 7.1 and CS 7.0 years • Prognosis: Poor prognosis, multiple IVF failures • Inclusion criteria: females aged younger than 37 years who were being treated mainly for tubal or male infertility, who had evidence of a normal uterine cavity, and who had no contraindication to pregnancy. • Exclusion criteria: women with poor response on previous IVF cycles (peak estradiol level below 500 pg/mL or retrieval of fewer than three oocytes). Also patients with embryo transfer from donor oocytes or frozen-thawed embryos

Interventions	• BS group: N = 23
	• CS group (day 2 or 3): N = 31
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ In blastocyst group, embryos cultured according to the sequential media system: the first 72 hours of culture in G1.2 medium (IVF Science, Scandinavia) followed by G2.2 medium (Scandinavia IVF Science, Gothenburg, Sweden) to day 5–7.
	  ○ To support luteal phase, all participants received five injections of hCG1,250 U every other day starting 48 hours after oocyte retrieval or daily IM administration.

Outcomes	Live birth rate Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate Failure rate to transfer embryos

Levitas 2004 *(Continued)*

	Implantation rate
Notes	Note: young women with large numbers of failed cycles Uneven number in each group Similar figures to the 2001 abstract Letter sent and reply received

Levron 2002

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Israel
Participants	• Number of participants randomised: 90 • Age: BS 30.9 years and CS 31.5 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: maternal age less than 38 years, fewer than five previous IVF attempts, and presence of more than five zygotes on day 1 • Exclusion criteria: not described
Interventions	• BS group: N = 46 • CS group (day 3): N = 44 • Description of the cycles: ○ Gamete and embryo handling procedures were done by using a commercial sequential IVF medium (Cook, Eight-Miles Plains, Queensland, Australia).
Outcomes	Live birth rate Clinical pregnancy rate Multiple pregnancy rate Embryo freezing rate Failure to transfer emvryos Implantation rate
Notes	Prognosis: moderate Young women Moderately high numbers of oocytes Clarification letter sent Same ET policy

Livingstone 2002

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Australia

Livingstone 2002 *(Continued)*

Participants	• Number of participants randomised: 59 • Age: BS 30 and CS 29 • Number of previous treatments: not stated • Infertility duration: BS 4.1 and CS 3.8 years • Prognosis: good prognosis • Inclusion criteria: participants under 38 years of age, 3 or fewer previous cycles • Exclusion criteria: not described

Interventions	• BS group: N = 30
	• CS group (day 3): N = 29
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ All embryos were placed in media(Sydney IVF sequential)
	  ○ Fertilised oocytes were transferred to culture dish with cleavage medium (Sydney IVF cleavage medium, Cook Medical)
	  ○ To support luteal phase, all participants received progesterone suppository.

Outcomes	Live birth rate Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate
Notes	Prognosis: good prognosis Data from thesis 2002 and not abstract 2001 Low fertilisation rate Aim to reduce twinning

Motta 1998

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Brazil
Participants	• Number of participants randomised 83, in 116 cycles • Age: not stated • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: unselected participants • Inclusion criteria: unselected • Exclusion criteria: not described

Interventions	• BS group: N = 58
	• CS group (day 2): N = 58
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ The media used was sequential media P1 or Irvines Blast.

Outcomes	Clinical pregnancy rate Multiple pregnancy rate

Motta 1998 *(Continued)*

	Embryo freezing rate Failure to transfer
Notes	Prognosis: moderate to good. No letter sent

Pantos 2004

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Greece
Participants	• Number of participants randomised: 243 • Age: BS 33.1 and CS D2 32.4; D3 31.3 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: unselected participants • Inclusion criteria: primary infertility, fewer than 4 previous unsuccessful ART attempts. Female age under 41 years of age. • Exclusion criteria: not described

Interventions	• BS group: N = 81
	• CS group (day 3) N = 81 D2; N = 81 D3
	• Description of the cycles:
	  ○ Ovarian stimulation with long or short protocol, using GnRH agonist and rFSH.
	  ○ All oocytes and embryos were cultured in sequential media from Vitrolife (IVF-20, G1.2, and G2.2; Scandinavian IVF Science AB, Goteborg, Sweden).

Outcomes	Clinical pregnancy rate Embryo freezing rate
Notes	

Papanikolaou 2005

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Belgium
Participants	• Number of participants randomised: 164 • Age: BS 29.9 and CS 29.6 • Number of previous treatments: BS 1.4 and CS 1.3 • Infertility duration: BS 2.9 and CS 2.7 years • Prognosis: good prognosis • Inclusion criteria: female age ≤ 37 years; three or fewer previous treatments, FSH on day 3 of the cycle ≤ 12IU/ml, ejaculated sperm origin, IVF or ICSI cycles with having at least four good-quality embryos on day 3 of embryo culture.

Papanikolaou 2005 *(Continued)*

	• Exclusion criteria: oocyte donation cycles; non-ejaculated sperm (testicular sperm aspiration, fine needle aspiration, micro-epididymal sperm aspiration, percutaneous epididymal sperm aspiration); and pre genetic screening.

Interventions	• BS group: N = 80
	• CS group (day 3): N = 84
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH), or short protocol (stimulation with rFSH and start with GnRH antagonist)
	  ○ Oocytes and embryos were cultured in sequential media of Vitrolife Sweden AB, Kungsbacka, Sweden, (GII or GIII series). On the morning of day 3, embryos were transferred from cleavage medium to blastocyst medium.

Outcomes	Live birth rate Clinical pregnancy rate Miscarriage rate Multiple pregnancy rate Failure to transfer
Notes	Prognosis: high, 4 high-quality embryos D3, young women Letter sent regarding concealment 100% ET rate

Papanikolaou 2006

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Belgium
Participants	• Number of participants randomised: 351 • Age: BS 30.5 and CS 30.4 • Number of previous treatments: not stated • Infertility duration: BS 3.7 and CS 3.5 years • Prognosis: good prognosis • Inclusion criteria: women under 36 years of age who were undergoing a first or second trial of in vitro fertilization or intracytoplasmic sperm injection, whose serum follicle-stimulating hormone level on day 3 of the menstrual cycle was 12 IU per liter or less, and who were undergoing transfer of one embryo. • Exclusion criteria: the use of preimplantation genetic diagnosis

Interventions	• BS group: N = 176
	• CS group (day 3): N = 175
	• Description of the cycles:
	  ○ Short protocol with GnRH antagonist and stimulation with rFSH)
	  ○ On the morning of day 3, the embryos were removed from cleavage medium and placed in blastocyst medium (sequential medium).
	  ○ To support luteal phase, all participants received progesterone suppository.

Outcomes	Live birth rate Clinical pregnancy rate

Papanikolaou 2006 *(Continued)*

	Multiple pregnancy rate
	Miscarriage rate
	Embryo freezing rate
	Failure to transfer embryos
Notes	Prognosis: high, young women Letter sent regarding concealment, media and freezing

Rienzi 2002

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Italy
Participants	• Number of participants randomised: 98 • Age: BS 32.2 and CS 31.6 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: couples with female age of <38 years who were treated by ICSI and who had ≥ 8 two-pronucleated zygotes on the day following ICSI. • Exclusion criteria: not described

Interventions	• BS group: N = 50
	• CS group (day 3): N = 48
	• Description of the cycles:
	  ○ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ○ Normally fertilized oocytes (zygotes) were cultured in G1.2 medium up to day 3 after ICSI and in G.2.2 medium (both media purchased from Vitrolife) from day 3 to day 5 where applicable.
	  ○ Day 3 and day 5 embryo cryopreservation was performed with freeze-kit 1 and freeze-kit 2 (both purchased from Vitrolife) respectively, according to the manufacturer's instructions.
	  ○ To support luteal phase, all participants received progesterone suppository.

Outcomes	Live birth rate Clinical pregnancy rate Miscarriage rate Multiple pregnancy rate Embryo freezing rate Failure to transfer embryos
Notes	Prognosis: good; > 8 zygotes Letter sent and reply received

Schillaci 2002

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Italy
Participants	• Number of participants randomised: 110 • Age: not stated • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: unselected participants • Inclusion criteria: indication for ICSI treatment, with eight or more metaphase II oocytes and at least three zygotes • Exclusion criteria: not described
Interventions	• BS group: N = 60 • CS group (day 3): N = 60 • Description of the cycles: ○ The media used in the CS group was IVF-20, media used in the BS group was G1-G2(Vitrolife)
Outcomes	Clinical pregnancy rate
Notes	Prognosis: unclear Abstract only

Singh 2017

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: NA
Participants	• Number of participants randomised: 438 • Age: not stated • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: participants under 40 years of age, if more than 6 oocytes were retrieved and three top quality embryos (6-8 blastomeres & less than 20% fragmentation without multinucleation) were observed at day 2 • Exclusion criteria: not described
Interventions	• BS group: N = 243 • CS group (day 3): N = 195 • Description of the cycles: not described
Outcomes	Clinical pregnancy rate
Notes	

Ten 2011

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Spain
Participants	• Number of participants randomised: 55 • Age: BS 33.4 and CS 33.1 • Number of previous treatments: not stated • Infertility duration: not stated • Prognosis: good prognosis • Inclusion criteria: women with on day three after ovarian punction at least one embryo type A and two type B, according to the ASEBIR classification • Exclusion criteria: not described
Interventions	• BS group: N = 28 • CS group (day 3): N = 27 • Description of the cycles: not described
Outcomes	Clinical pregnancy rate but cumulative pregnancy rate later Number of frozen embryos
Notes	Abstract only

Van der Auwera 2002

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: Belgium
Participants	• Number of participants randomised: 136 • Age: BS 31.5 and CS 31.7 • Number of previous treatments: BS 1.7 and CS 1.7 • Infertility duration: BS 3.4 and CS 3.3 years • Prognosis: unselected participants • Inclusion criteria: IVF and ICSI patients • Exclusion criteria: not described

Interventions	• BS group: N = 70
	• CS group (day 2): N = 66
	• Description of the cycles:
	  ◦ Long protocol (down-regulation with GnRH agonist and stimulation with rFSH)
	  ◦ Oocytes and embryos were cultured in either sequential media from Cook (Fertilization, Cleavage and Blastocyst medium; Cook IVF, Queensland, Australia) or sequential media from Vitrolife (IVF-500, G1.2 and G2.2, Scandinavian IVF Science AB, Göteborg, Sweden)
	  ◦ Luteal supplementation was given either by 1500 IU HCG, every 3 days, starting on the third day after oocyte retrieval, or by vaginal progesterone (600 mg/day, Utrogestan®; Piette International, Drogenbos, Belgium)

Outcomes	Live birth rate Cumulative clinical pregnancy rate

Van der Auwera 2002 *(Continued)*

	Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate Embryo freezing rate Failure to transfer embryos
Notes	Prognosis: mixed - unselected Aim to reach highest cryoaugmented pregnancy rate Smaller cohort of embryos to choose from in D2 group due to freezing on day 1 New policy women with > 5 zygotes go to D5 transfer with 79% pregnancy rate

Yang 2018

Study characteristics	
Methods	• Study type: randomised controlled trial in parallel groups • Country: China
Participants	• Number of participants randomised: 600 • Age: BS 28.0 and CS 28.3 • Number of previous treatments: not stated • Infertility duration: BS 3.8 and CS 3.8 years • Prognosis: good prognosis • Inclusion criteria: participants under 37 years of age, who were undergoing their first or second fresh IVF cycle using their own oocytes, and who had FSH levels ≤ 12 IU/mL on Day 3 of the cycle and 10 or more oocytes retrieved. IVF and ICSI patients • Exclusion criteria: underlying uterine conditions including endometriosis, untreated unilateral or bilateral hydrosalpinx, and uterine myoma (multiple, submucous or intramural myoma > 3 cm), or had cycles planned for oocyte donation or PGD, or had recurrent pregnancy loss. Also people with significantly abnormal oocytes, or < 6 normally fertilised embryos (2PN) or who were considered unlikely to complete the study based on the investigator's judgement.

Interventions	• Conventional blastocyst stage: N = 300
	• TLS cleavage stage (day 3): N = 300
	• Description of the cycles:
	  ○ Ovarian stimulation protocols were carried out according to the subject's ovarian reserve.
	  ○ All the oocytes were placed in fertilisation medium (G-IVF; Vitrolife, Goteborg, Sweden)

Outcomes	• Clinical pregnancy rate • Multiple pregnancy rate • Miscarriage rate
Notes	

2PN: two-pronuclear zygote
AHA: assisted hatching
ART: assisted reproductive technology
Blast: blastocyst
Blastocyst rate: number of blastocysts developed divided by number of 2PN embryos available
BMI: body mass index
BS: blastocyst stage

CS: cleavage stage
D2: embryo transfer on day 2 post-OPU (i.e. early cleavage stage)
D3: embryo transfer on day 3 post-OPU
D5: embryo transfer on day 5 post-OPU (i.e. blastocyst stage)
ET: embryo transfer
FCS: foetal chord serum
FSH: follicle stimulating hormone
G1/G2: sequential media from Vitrolife
GnRH: gonadotropin-releasing hormone
h: hour(s)
high-order: high-order multiple pregnancy
HCG: human chorionic gonadotropin (trigger injection that initiates ovulation and maturation of oocytes)
ICSI: intracytoplasmic sperm injection
IM: intramuscular injection
IU/L: international units per litre
IVF: in vitro fertilisation
MII: metaphase II
mIU/m: milli international units per millilitre
morula: embryonic stage prior to blastocysts (usually embryos with delayed development on day 5)
NS: not stated
OPU: oocyte pick up
Ov stim: ovarian stimulation regimen
PGD: preimplantation genetic diagnosis
rFSH: recombinant follicle stimulating hormone (fertility ovarian stimulation drug)
#: number
US: ultrasound

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

Study	Reason for exclusion
Bungum 2002	Used co-culture
Cornelisse 2018	Non-RCT; letter to the editor
Green 2016	Ineligible study design: a retrospective cohort
Guerin 1991	Used co-culture
Holden 2017	Ineligible study design: retrospective review
Levron 2001	Quasi-randomised RCT
Loup 2009	Included transfer of embryos on two separate days within the same cycle
Menezo 1992	Used co-culture
Utsonomiya 2004	Non-randomised study (sequentially numbered)
Zech 2007	Non-randomised study; according to even or odd year of birth

RCT: randomised controlled trial

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

Clua Obrado 2020

Methods	Single center randomised controlled trial
Participants	Recipients between 18 and 50 years old in their first or second synchronous cycle Exclusion criteria: implantation failure and PGT-A
Interventions	D3 or D5 embryo transfer
Outcomes	Clinical pregnancy rate and cumulative live birth rate
Notes	March 2017 - August 2018 NCT 03088735 Barcelona, Spain The authors were contacted but they did not want to provide the missing information until the study was published in a peer-reviewed journal.

PGT-A: preimplantation genetic testing for aneuploidies

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

ChiCTR-ICR-15006184

Study name	Cumulative live birth rates after cleavage-stage versus blastocyst-stage embryo transfer: a multi-center, prospective, randomized controlled trial
Methods	• Study type: Randomised controlled trial in parallel groups • Country: China
Participants	• Number of participants to be randomised: 600 • Age: 20 to 39 years old • Number of previous treatments: NA • Infertility duration: NA • Prognosis: NA • Inclusion criteria: infertility, ≥ 4 top-quality embryos (≥ 8-cell, < 20% fragments) • Exclusion criteria:NA
Interventions	Blastocyst transfer group: extending embryo culture 2-3 days in vitro to the blastocyst stage Cleavage transfer group: no intervention
Outcomes	Cumulative birth rates within one year; embryo freezing rate; cumulative pregnancy rate per woman; miscarriage rate; neonatal outcomes; monozygotic twinning; multiple pregnancy rate per transfer; live birth rate/per embryo
Starting date	May 2015
Contact information	Telephone:+86 025 68302608 Email: jyliu_nj@126.com
Notes	ChiCTR-ICR-15006184 Recruitment: From2018-09-29To 2019-08-31 Jiangsu, China

Cornelisse 2021

Study name	Three or Five Trial (ToF Trial)
Methods	For randomisation, all cases that subsequently meet all inclusion criteria will be randomised by the local laboratory staff, on the second day after fertilisation using the online software program Castor (V.2018.3.11, Castor Electronic Data Capture, Amsterdam, the Netherlands). Laboratory staff can access the online randomisation program using a unique password for this study. The laboratory staff is unable to access forthcoming random assignments prior to randomisation. Allocation to the cleavage-stage embryo transfer arm or the blastocyst-stage embryo transfer arm transfer will be based on a 1:1 randomisation with randomly selected block sizes of 2, 4 and 6 and stratification for age (≥ 36 years or < 36 years). Laboratory staff, clinicians and the participants cannot be blinded, due to the nature of the intervention. Participating clinicians, laboratory staff and investigators will not be able to access the randomisation sequence.
Participants	Women between 18 and 43 years of age, aiming to start an IVF treatment, are being selected for inclusion in this study. For inclusion and randomisation, at least four embryos should be available on culture day 2 (an embryo is defined as an oocyte with cell division on day 2 after insemination; ≥ three pronucleus embryos are excluded). A woman can participate in the study in her first, second or third IVF treatment, and can participate in only one treatment cycle. Women are excluded if they meet any of the following criteria: use of preimplantation genetic diagnosis or use of vitrified oocytes. No cycles with preimplantation genetic testing for aneuploidy will be part of this study as this procedure is not allowed in the Netherlands.
Interventions	(1) The control group, with embryo transfer on day 3 after oocyte retrieval and with cryopreservation of supernumerary good-quality embryos on day 3 or 4 according to the local protocol and criteria, or (2) the intervention group, with embryo transfer on day 5 after oocyte retrieval with cryopreservation of supernumerary good-quality embryos on day 5 or 6. Cryopreserved embryos on day 6 will only be transferred after all frozen–thawed embryo transfer(s) on day 5 have been transferred without an ongoing pregnancy.
Outcomes	The primary outcome is the cLBR per oocyte retrieval, which includes the results of the fresh and frozen–thawed embryo transfers. Endpoints of the study are live birth, no pregnancy leading to live birth after transfer of all available embryos or after a follow-up time of 12 months after the oocyte retrieval.
Starting date	28 August 2018
Contact information	Ms Simone Cornelisse; simone.cornelisse@ radboudumc.nl
Notes	Netherlands Inclusions completed, results expected in 2023

ISRCTN48090543

Study name	Trial comparing blastocyst transfer with cleavage stage transfer in women with increased maternal age
Methods	Prospective randomised double-blinded study
Participants	Inclusion criteria • Female participants aged between 37 and 42 years undergoing an IVF/ICSI attempt at the GENERA Centre for Reproductive Medicine in Rome

ISRCTN48090543 *(Continued)*

	• History of less than 3 failed IVF/ICSI cycles • ≥ 6 MII retrieved • Signed consent form
Interventions	Participants will be randomised on the day of ovum pick up by an independent operator to: 1. Blastocyst transfer: 1.1. Ovarian stimulation by agonist or antagonist protocol 1.2. Ovum pick up performed 36 hours after human chorionic gonadotropin (HCG) administration 1.3. In vitro fertilization performed by intracytoplasmic sperm injection (ICSI) 1.4. In vitro culture performed with sequential media in 6% CO2 and 5% O2 atmosphere 1.5. Embryo transfer on day 5 two best quality blastocyst. Remaining blastocyst preserved by vitrification procedure 1.6. Luteal support by progesterone 200 mg vaginally three times a day from oocyte retrieval plus one day 2. Cleavage stage transfer: 2.1. Ovarian stimulation by agonist or antagonist protocol 2.2. Ovum pick up performed 36 hours after human chorionic gonadotropin (HCG) administration 2.3. In vitro fertilization performed by intra-cytoplasmic sperm injection (ICSI) 2.4. In vitro culture performed with sequential media in 6% CO2 and 5% O2 atmosphere 2.5. Embryo transfer on day 3 two best quality embryos. Remaining embryos preserved by vitrification procedure 2.6. Luteal support by progesterone 200 mg vaginally three times a day from oocyte retrieval plus one day
Outcomes	Cumulative live birth rate after blastocyst or cleavage stage strategy including pregnancies from fresh + cryoembryos transferred within 6 months after the end of the treatment
Starting date	01 November 2011
Contact information	Dr. Laura Rienzi: rienzi@generaroma.it
Notes	

NCT01107002

Study name	Comparison of 5 day embryo transfer with 2-3 day transfer in patients who failed to conceive in two or more day 2-3 embryo transfer cycle in Royan Institute
Methods	Prospective randomised clinical trial divided into two groups. Random permuted blocks with a block size of 4 was used and complete allocation concealment Single-blind (outcomes assessor)
Participants	200 participants with infertility Inclusion criteria: two or more previous failed IVF/ICSI cycles; 18 to 40 years
Interventions	Experimental: day 5 embryo transfer group Other group (day 2-3) not described (only mentioned in the title and objectives)
Outcomes	Clinical pregnancy rate; live birth rate; implantation rate; miscarriage rate
Starting date	July 2008
Contact information	www.royaninstitute.org

NCT01107002 *(Continued)*

Notes	Sponsor: Royan Institute Study completion date: August 2010 ClinicalTrials.gov ID: NCT01107002

NCT02639000

Study name	Effects of blastocyst stage embryo transfer compared with cleavage stage embryo transfer in women ≤ 38 years
Methods	• Study type: randomised controlled trial in parallel groups • Country: Italy
Participants	• Number of participants to be randomised: 388 • Age: 18 to 37 years • Inclusion criteria: women with infertility undergoing an IVF cycle, with at least 4 fertilised eggs
Interventions	Experimental: blastocyst: embryo transfer of at maximum 2 embryos at blastocyst stage Active comparator: cleavage: embryo transfer of at maximum 2 embryos at cleavage stage
Outcomes	Pregnancy rate; multiple pregnancy rate; implantation rate
Starting date	July 2010
Contact information	Paolo Emanuele Levi-Setti +39-0282244505 paolo.levi_setti@humanitas.it
Notes	Study completion: April 2016 Sponsor: Istituto Clinico Humanitas ClinicalTrials.gov ID: NCT02639000

NCT04210414

Study name	Cleave-stage transfer on day 3 versus day 5 transfer when only one embryo available (Cleave-blast)
Methods	Study type: interventional (clinical trial) Estimated enrollment: 1100 participants Allocation: randomised Intervention model: parallel assignment Masking: triple (care provider, investigator, outcomes assessor)
Participants	Inclusion criteria: infertile couple with only one embryo available for transfer
Interventions	Experimental: day 3 transfer Transfer on day 3 when only one embryo is available Experimental: day 5 transfer

NCT04210414 *(Continued)*

	Transfer on day 5 when only one embryo is available
Outcomes	Ongoing pregnancy rate [Time frame: within 20 weeks of gestation]
Starting date	10 January 2020
Contact information	Muhammad Fawzy drfawzy001@gmail.com
Notes	

Neuhausser 2020

Study name	Day 3 vs Day 5 Embryo Transfer for Patients With Low Embryo Numbers Going Through in Vitro Fertilization
Methods	Phase 2. Allocation: dandomized Intervention model: parallel assignment Intervention model description: non-inferiority Masking: none (open label) Primary purpose: treatment
Participants	18 to 44 years old Inclusion criteria • First autologous IVF cycle • Written, informed consent Exclusion Criteria • Planned gestational carrier • Planned donor egg • Morbid obesity: BMI > 40 • History of recurrent pregnancy loss (≥ 2 spontaneous abortions) • Presence of uterine factor infertility • Treatment plan includes embryos cultured 'out of protocol' • Planned preimplantation genetic testing
Interventions	- uterine transfer of embryo on day 3 after fertilisation (cleavage stage) - uterine transfer of embryo on day 5 after fertilisation (blastocyst stage) • Procedure: day 3 uterine transfer • Procedure: day 5 uterine transfer
Outcomes	Live birth [Time frame: 9 months] defined as delivery of a live born infant ≥ 22 weeks of gestation
Starting date	1 January 2021
Contact information	Werner Neuhausser, MD PhD (646) 510-4825 wneuhaus@bidmc.harvard.edu
Notes	

PACTR201402000773124

Study name	Blastocyst versus day 2 transfer in low responders
Methods	Parallel: different groups receive different interventions at same time during study Randomised: permuted block randomisation (block size = 4) and the block size was not variable Sealed opaque envelopes
Participants	Inclusion criteria: poor response to ovarian stimulation as defined by European Society of Human Reproduction and Embryology (ESHRE) consensus (the Bologna criteria) (2011) Exclusion criteria: only one immature oocyte retrieve
Interventions	Blastocyst versus day 2 embryo transfer
Outcomes	Clinical pregnancy with cardiac pulsation
Starting date	25 June 2013
Contact information	elsayedamr@yahoo.com
Notes	

PACTR201709002592834

Study name	A randomized controlled trial of pregnancy outcome of sequential versus day 3 and day 5 embryo transfer in cases with recurrent implantation failure
Methods	RCT: simple randomisation using a randomised table created by a computer software program Sealed opaque envelopes
Participants	Age 35 years or less Normal endometrial cavity on hysteroscopy Recurrent (2 or more) implantation failure Negative thrombophilia screening No hydrosalpinx No endometriosis Day 3 FSH < 10 IU/L and Estradiol < 80 pg/mL Antimullerian hormone 1-3 ng/mL Availability of at least 5 embryos on postfertilisation check
Interventions	Day 3 vs day 5 embryo transfer
Outcomes	Live birth rate
Starting date	15 April 2015
Contact information	haithamtorky@yahoo.com
Notes	

cLBR: cumulative live birth rate
FSH: follicle-stimulating hormone
HCG: human chorionic gonadotropin
ICSI: intracytoplasmic sperm injection
IU: international units

IVF: in vitro fertilisation
LBR: live birth rate
MII: metaphase II
NA: not available

RISK OF BIAS

Legend: Low risk of bias | High risk of bias | Some concerns

Risk of bias for analysis 1.1 Live birth per couple

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Brugnon 2010	~	✗	✗	✓	~	✗
Devreker 2000	✗	✗	✓	✓	~	✗
Elgindy 2011	~	~	✓	✓	~	~
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hatirnaz 2017	~	~	✓	✓	~	~
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 1.2 Live birth per couple: grouped by number of embryos transferred

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 1.2.1 More cleavage-stage than blastocyst embryos transferred						
Devreker 2000	✗	✗	✓	✓	~	✗
Elgindy 2011	~	~	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Levitas 2004	✓	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Subgroup 1.2.2 Single embryo transfer						
Brugnon 2010	~	✗	✗	✓	~	✗
Papanikolaou 2006	~	✓	✓	✓	~	~
Subgroup 1.2.3 Equal number of embryos transferred						
Brugnon 2010	~	✗	✗	✓	~	✗
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Hatirnaz 2017	~	~	✓	✓	~	~
Levi-Setti 2018	~	✓	✓	✓	✓	~
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 1.3 Live birth rate per couple: grouped by prognosis

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 1.3.1 good prognostic factors						
Brugnon 2010	~	✗	✗	✓	~	✗
Elgindy 2011	~	~	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Rienzi 2002	~	✗	✗	✓	~	✗
Subgroup 1.3.2 poor prognostic factors						
Devreker 2000	✗	✗	✓	✓	~	✗
Levitas 2004	✓	✗	✓	✓	~	✗
Subgroup 1.3.3 unselected group						
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Hatirnaz 2017	~	~	✓	✓	~	~
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 1.4 Live birth rate: grouped by day of randomisation

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 1.4.1 randomisation at start of cycle						
Brugnon 2010	~	✗	✗	✓	~	✗
Emiliani 2003	~	✓	✓	✓	~	~
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Papanikolaou 2006	~	✓	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Van der Auwera 2002	+	~	+	+	~	~
Subgroup 1.4.2 randomised on day of OPU and day 1 after OPU						
Fernandez-Shaw 2015	~	+	+	+	~	~
Frattarelli 2003	+	-	+	+	~	-
Hatirnaz 2017	~	~	+	+	~	~
Levron 2002	~	+	+	+	~	~
Rienzi 2002	~	-	-	+	~	-
Subgroup 1.4.3 randomised day 2 to 3 post-OPU						
Elgindy 2011	~	~	+	+	~	~
Papanikolaou 2005	~	~	+	+	~	~
Subgroup 1.4.4 day of randomisation unstated						
Devreker 2000	-	-	+	+	~	-
Livingstone 2002	+	-	+	+	~	-

Risk of bias for analysis 2.1 Cumulative pregnancy rate from fresh and frozen transfers

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Brugnon 2010	~	-	-	+	~	-
Emiliani 2003	~	+	+	+	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Fernandez-Shaw 2015						
Rienzi 2002						
Van der Auwera 2002						

Risk of bias for analysis 2.2 Cumulative pregnancy rate per couple: grouped by number of embryos transferred

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 2.2.1 single embryo transfer						
Brugnon 2010						
Subgroup 2.2.2 equal number of embryos transferred						
Brugnon 2010						
Emiliani 2003						
Fernandez-Shaw 2015						
Rienzi 2002						
Van der Auwera 2002						

Risk of bias for analysis 2.3 Cumulative pregnancy rate per couple: grouped by prognosis

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 2.3.1 good prognostic factors						
Brugnon 2010	~	✗	✗	✓	~	✗
Rienzi 2002	~	✗	✗	✓	~	✗
Subgroup 2.3.2 unselected group						
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 2.4 Cumulative pregnancy rate: grouped by day of randomisation

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 2.4.1 randomisation at start of cycle						
Brugnon 2010	~	✗	✗	✓	~	✗
Emiliani 2003	~	✓	✓	✓	~	~
Van der Auwera 2002	✓	~	✓	✓	~	~
Subgroup 2.4.2 randomised on day of OPU and day 1 after OPU						
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗

Risk of bias for analysis 2.5 Cumulative pregnancy rate from fresh and frozen transfers: grouped by vitrification or slow freezing

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 2.5.1 slow freezing						
Brugnon 2010	~	✗	✗	✓	~	✗
Emiliani 2003	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~
Subgroup 2.5.2 vitrification						
Fernandez-Shaw 2015	~	✓	✓	✓	~	~

Risk of bias for analysis 3.1 Clinical pregnancy rate per couple

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Aziminekoo 2015	~	~	✓	✓	~	~
Brugnon 2010	~	✗	✗	✓	✓	✗
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Devreker 2000	✗	✗	✓	✓	✗	✗
Elgindy 2011	~	~	✓	✓	~	~
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Fisch 2007	~	~	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Gaafar 2015	✓	~	✗	✓	~	✗
Gardner 1998a	~	✗	✓	✓	✓	✗
Hatirnaz 2017	~	~	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kaser 2017	✓	~	✓	✓	✓	~
Kaur 2014	~	✓	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗
Pantos 2004	~	~	✓	✓	~	~
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Schillaci 2002	~	✗	✓	✓	~	✗
Singh 2017	~	✗	✗	✓	~	✗
Ten 2011	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 3.2 Clinical pregnancy rate per couple: grouped by number of embryos transferred

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 3.2.1 equal number of embryo transfers						
Aziminekoo 2015	~	~	✓	✓	~	~
Brugnon 2010	~	✗	✗	✓	✓	✗
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Fisch 2007	~	~	✓	✓	~	~
Gaafar 2015	✓	~	✗	✓	~	~
Hatirnaz 2017	~	~	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Kaser 2017	✓	~	✓	✓	✓	~
Kaur 2014	~	✓	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Singh 2017	~	✗	✗	✓	~	✗
Ten 2011	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~
Subgroup 3.2.2 more cleavage stage than blastocyst embryos transferred						
Devreker 2000	✗	✗	✓	✓	✓	✗
Elgindy 2011	~	~	✓	✓	~	~
Emiliani 2003	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Gardner 1998a	~	✗	✓	✓	✓	✗
Karaki 2002	✓	✗	✓	✓	~	✗
Levitas 2004	✓	✗	✓	✓	~	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗
Pantos 2004	~	~	✓	✓	~	~
Schillaci 2002	~	✗	✓	✓	~	✗
Subgroup 3.2.3 single embryo transfer						
Brugnon 2010	~	✗	✗	✓	✓	✗
Fisch 2007	~	~	✓	✓	~	~
Kaser 2017	✓	~	✓	✓	✓	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 3.3 Clinical pregnancy rate per couple: grouped by prognosis

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 3.3.1 good prognostic factors						
Brugnon 2010	~	✗	✗	✓	✓	✗
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Elgindy 2011						
Fisch 2007						
Frattarelli 2003						
Gardner 1998a						
Hreinsson 2004						
Kaser 2017						
Kaur 2014						
Levi-Setti 2018						
Levron 2002						
Livingstone 2002						
Papanikolaou 2005						
Papanikolaou 2006						
Rienzi 2002						
Singh 2017						
Ten 2011						
Yang 2018						
Subgroup 3.3.2 poor prognostic factors						
Aziminekoo 2015						
Devreker 2000						

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Levitas 2004						
Subgroup 3.3.3 unselected group						
Emiliani 2003						
Fernandez-Shaw 2015						
Gaafar 2015						
Hatirnaz 2017						
Karaki 2002						
Kolibianakis 2004						
Motta 1998						
Pantos 2004						
Schillaci 2002						
Van der Auwera 2002						

Risk of bias for analysis 3.4 Clinical pregnancy rate per couple: grouped by day of randomisation

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 3.4.1 randomised start of cycle						
Brugnon 2010						
Emiliani 2003						

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Gardner 1998a	~	✗	✓	✓	✓	✗
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Papanikolaou 2006	~	✓	✓	✓	~	~
Van der Auwera 2002	✓	~	✓	✓	~	~
Subgroup 3.4.2 randomised on day of OPU or day 1						
Aziminekoo 2015	~	~	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Fisch 2007	~	~	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hatirnaz 2017	~	~	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kaser 2017	✓	~	✓	✓	✓	~
Levron 2002	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Schillaci 2002	~	✗	✓	✓	~	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Yang 2018	✓	~	✓	✓	✓	~
Subgroup 3.4.3 randomised on day 2 to 3						
Bungum 2003	✓	✓	✓	✓	~	~
Elgindy 2011	~	~	✓	✓	~	~
Kaur 2014	~	✓	✓	✓	~	~
Papanikolaou 2005	~	~	✓	✓	~	~
Singh 2017	~	✗	✗	✓	~	✗
Ten 2011	~	✗	✗	✓	~	✗
Subgroup 3.4.4 day of randomisation unstated						
Devreker 2000	✗	✗	✓	✓	✓	✗
Gaafar 2015	✓	~	✗	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗
Pantos 2004	~	~	✓	✓	~	~

Risk of bias for analysis 3.5 Clinical pregnancy rate per couple: TLS (with algorithm) cleavage stage versus conventional blastocyst stage

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Kaser 2017	✓	~	✓	✓	✓	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 3.6 Clinical pregnancy rate per couple: TLS (with algorithm) cleavage stage versus TLS (with algorithm) blastocyst stage

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Kaser 2017	✓	~	✓	✓	✓	~

Risk of bias for analysis 4.1 Multiple pregnancy rate per couple

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Aziminekoo 2015	~	~	✓	✓	~	~
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Elgindy 2011	~	~	✓	✓	~	~
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hatirnaz 2017	~	~	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Karaki 2002	✓	✗	✓	✓	~	✗
Kaur 2014	~	~	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 4.2 Multiple pregnancy rate per couple: grouped by number of embryos transferred

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 4.2.1 equal number of embryos transferred						
Aziminekoo 2015	~	~	✓	✓	~	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Hatirnaz 2017	~	~	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~
Kaur 2014	~	~	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~
Subgroup 4.2.2 more cleavage stage than blastocyst embryos transferred						
Elgindy 2011	~	~	✓	✓	~	~
Emiliani 2003	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Karaki 2002	✓	✗	✓	✓	~	✗
Levitas 2004	✓	✗	✓	✓	~	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗
Subgroup 4.2.3 single embryo transfer						
Papanikolaou 2006	~	✓	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 4.3 Multiple pregnancy rate per couple: grouped by prognosis

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 4.3.1 good prognostic factors						
Aziminekoo 2015	~	~	✓	✓	~	~
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Elgindy 2011	~	~	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hreinsson 2004	✓	✓	✓	✓	~	~
Kaur 2014	~	~	✓	✓	~	~
Levi-Setti 2018	~	✓	✓	✓	✓	~

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Levron 2002	~	✓	✓	✓	~	~
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Yang 2018	✓	~	✓	✓	✓	~
Subgroup 4.3.2 poor prognostic factors						
Levitas 2004	✓	✗	✓	✓	~	✗
Subgroup 4.3.3 unselected						
Emiliani 2003	~	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Hatirnaz 2017	~	~	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 4.4 Multiple pregnancy rate per couple: grouped by day of randomisation

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Subgroup 4.4.1 randomised start of cycle						
Emiliani 2003	~	✓	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Papanikolaou 2006	~	✓	✓	✓	~	~
Van der Auwera 2002	✓	~	✓	✓	~	~
Subgroup 4.4.2 randomised on day of OPU or day 1						
Aziminekoo 2015	~	~	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hatirnaz 2017	~	~	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Yang 2018	✓	~	✓	✓	✓	~
Subgroup 4.4.3 randomised on day 2 to 3						

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Bungum 2003	✓	✓	✓	✓	~	~
Elgindy 2011	~	~	✓	✓	~	~
Kaur 2014	~	~	✓	✓	~	~
Papanikolaou 2005	~	~	✓	✓	~	~
Subgroup 4.4.4 day of randomisation unstated						
Livingstone 2002	✓	✗	✓	✓	~	✗
Motta 1998	~	✗	✓	✓	~	✗

Risk of bias for analysis 4.5 High-order pregnancies (more than 2 gestational sacs) per couple

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hreinsson 2004	✓	✓	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kaur 2014	~	~	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levitas 2004	✓	✗	✓	✓	~	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Levron 2002	~	✓	✓	✓	~	~
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 4.6 Multiple pregnancy rate per couple: TLS (with algorithm) cleavage stage versus conventional blastocyst stage

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 5.1 Miscarriage rate per couple

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Aziminekoo 2015	~	~	✓	✓	~	~
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Devreker 2000	✗	✗	✓	✓	✓	✗

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Elgindy 2011	~	~	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Gaafar 2015	✓	~	✗	✓	~	✗
Hatirnaz 2017	~	~	✓	✓	~	~
Hreinsson 2004	✓	✓	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kaur 2014	~	✓	✓	✓	~	~
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levi-Setti 2018	~	✓	✓	✓	✓	~
Levitas 2004	✓	✗	✓	✓	~	✗
Livingstone 2002	✓	✗	✓	✓	~	✗
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~
Yang 2018	✓	~	✓	✓	✓	~

Risk of bias for analysis 6.1 Embryo freezing per couple

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Brugnon 2010	~	✗	✗	✓	~	✗
Bungum 2003	✓	✓	✓	✓	~	~
Fernandez-Shaw 2015	~	✓	✓	✓	~	~
Gardner 1998a	~	✗	✓	✓	✓	✗
Hreinsson 2004	✓	✓	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Motta 1998	~	✗	✓	✓	~	✗
Pantos 2004	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Ten 2011	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~

Risk of bias for analysis 7.1 Failure to transfer any embryos (per couple)

	Bias					
Study	**Randomisation process**	**Deviations from intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported results**	**Overall**
Gardner 1998a	~	✗	✓	✓	✓	✗
Aziminekoo 2015	~	~	✓	✓	~	~
Bungum 2003	✓	✓	✓	✓	~	~
Coskun 2000	✓	~	✓	✓	~	~
Devreker 2000	✗	✗	✓	✓	~	✗
Emiliani 2003	~	✓	✓	✓	~	~
Frattarelli 2003	✓	✗	✓	✓	~	✗
Hreinsson 2004	✓	✓	✓	✓	~	~
Karaki 2002	✓	✗	✓	✓	~	✗
Kolibianakis 2004	✗	✗	✓	✓	~	✗
Levitas 2004	✓	✗	✓	✓	~	✗
Levron 2002	~	✓	✓	✓	~	~
Motta 1998	~	✗	✓	✓	~	✗
Papanikolaou 2005	~	~	✓	✓	~	~
Papanikolaou 2006	~	✓	✓	✓	~	~
Rienzi 2002	~	✗	✗	✓	~	✗
Van der Auwera 2002	✓	~	✓	✓	~	~

DATA AND ANALYSES

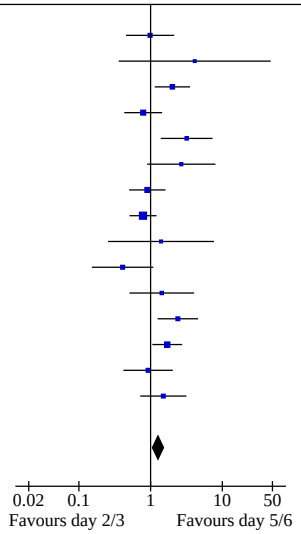
Comparison 1. Blastocyst- versus cleavage-stage transfer: live birth rate following fresh transfer

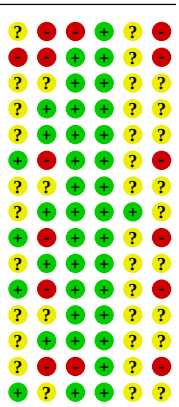
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Live birth per couple	15	2219	Odds Ratio (M-H, Fixed, 95% CI)	1.27 [1.06, 1.51]
1.2 Live birth per couple: grouped by number of embryos transferred	15	2677	Odds Ratio (M-H, Fixed, 95% CI)	1.30 [1.10, 1.53]
1.2.1 More cleavage-stage than blastocyst embryos transferred	6	483	Odds Ratio (M-H, Fixed, 95% CI)	1.51 [1.03, 2.22]
1.2.2 Single embryo transfer	2	458	Odds Ratio (M-H, Fixed, 95% CI)	1.47 [0.98, 2.20]
1.2.3 Equal number of embryos transferred	9	1736	Odds Ratio (M-H, Fixed, 95% CI)	1.21 [0.99, 1.47]
1.3 Live birth rate per couple: grouped by prognosis	15		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.3.1 good prognostic factors	9	1514	Odds Ratio (M-H, Fixed, 95% CI)	1.28 [1.04, 1.59]
1.3.2 poor prognostic factors	2	77	Odds Ratio (M-H, Fixed, 95% CI)	2.05 [0.53, 7.96]
1.3.3 unselected group	4	628	Odds Ratio (M-H, Fixed, 95% CI)	1.19 [0.86, 1.66]
1.4 Live birth rate: grouped by day of randomisation	15		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.4.1 randomisation at start of cycle	6	1207	Odds Ratio (M-H, Fixed, 95% CI)	1.08 [0.84, 1.38]
1.4.2 randomised on day of OPU and day 1 after OPU	5	566	Odds Ratio (M-H, Fixed, 95% CI)	1.16 [0.82, 1.65]
1.4.3 randomised day 2 to 3 post-OPU	2	364	Odds Ratio (M-H, Fixed, 95% CI)	2.17 [1.42, 3.33]
1.4.4 day of randomisation unstated	2	82	Odds Ratio (M-H, Fixed, 95% CI)	1.71 [0.67, 4.39]

Analysis 1.1. Comparison 1: Blastocyst- versus cleavage-stage transfer: live birth rate following fresh transfer, Outcome 1: Live birth per couple

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Brugnon 2010	22	55	21	52	6.0%	0.98 [0.45 , 2.13]
Devreker 2000	3	11	1	12	0.3%	4.13 [0.36 , 47.30]
Elgindy 2011	52	100	35	100	7.7%	2.01 [1.14 , 3.55]
Emiliani 2003	33	82	41	89	10.8%	0.79 [0.43 , 1.45]
Fernandez-Shaw 2015	25	60	11	60	3.0%	3.18 [1.39 , 7.31]
Frattarelli 2003	15	29	8	28	1.8%	2.68 [0.89 , 8.02]
Hatirnaz 2017	33	101	35	100	10.9%	0.90 [0.50 , 1.62]
Levi-Setti 2018	54	194	64	194	21.3%	0.78 [0.51 , 1.21]
Levitas 2004	3	23	3	31	1.0%	1.40 [0.26 , 7.66]
Levron 2002	8	46	15	44	5.8%	0.41 [0.15 , 1.09]
Livingstone 2002	14	30	11	29	2.8%	1.43 [0.51 , 4.04]
Papanikolaou 2005	38	80	23	84	5.4%	2.40 [1.25 , 4.60]
Papanikolaou 2006	56	175	38	176	11.9%	1.71 [1.06 , 2.76]
Rienzi 2002	24	50	24	48	5.9%	0.92 [0.42 , 2.04]
Van der Auwera 2002	24	70	17	66	5.3%	1.50 [0.72 , 3.15]
Total (95% CI)		**1106**		**1113**	**100.0%**	**1.27 [1.06 , 1.51]**
Total events:	404		347			

Heterogeneity: Chi² = 29.92, df = 14 (P = 0.008); I² = 53%
Test for overall effect: Z = 2.61 (P = 0.009)
Test for subgroup differences: Not applicable

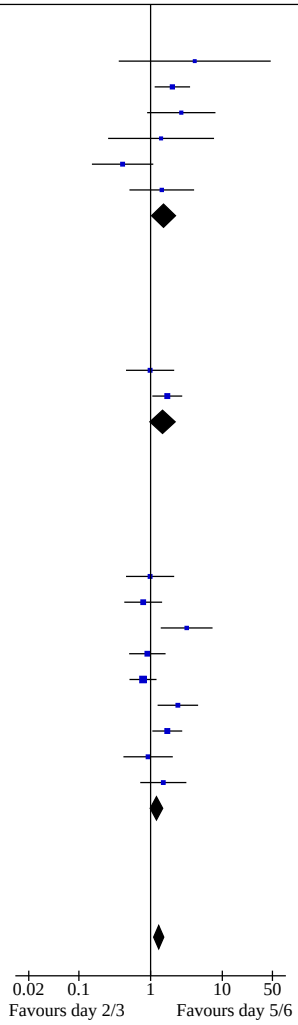


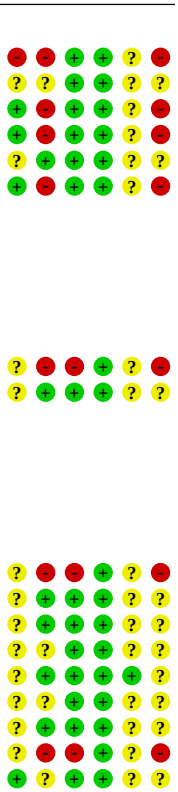


Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 1.2. Comparison 1: Blastocyst- versus cleavage-stage transfer: live birth rate following fresh transfer, Outcome 2: Live birth per couple: grouped by number of embryos transferred

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.2.1 More cleavage-stage than blastocyst embryos transferred						
Devreker 2000	3	11	1	12	0.3%	4.13 [0.36 , 47.30]
Elgindy 2011	52	100	35	100	6.6%	2.01 [1.14 , 3.55]
Frattarelli 2003	15	29	8	28	1.5%	2.68 [0.89 , 8.02]
Levitas 2004	3	23	3	31	0.9%	1.40 [0.26 , 7.66]
Levron 2002	8	46	15	44	5.0%	0.41 [0.15 , 1.09]
Livingstone 2002	14	30	11	29	2.3%	1.43 [0.51 , 4.04]
Subtotal (95% CI)		**239**		**244**	**16.5%**	**1.51 [1.03 , 2.22]**
Total events:	95		73			
Heterogeneity: Chi² = 9.50, df = 5 (P = 0.09); I² = 47%						
Test for overall effect: Z = 2.11 (P = 0.03)						
1.2.2 Single embryo transfer						
Brugnon 2010	22	55	21	52	5.1%	0.98 [0.45 , 2.13]
Papanikolaou 2006	56	175	38	176	10.1%	1.71 [1.06 , 2.76]
Subtotal (95% CI)		**230**		**228**	**15.2%**	**1.47 [0.98 , 2.20]**
Total events:	78		59			
Heterogeneity: Chi² = 1.41, df = 1 (P = 0.23); I² = 29%						
Test for overall effect: Z = 1.85 (P = 0.06)						
1.2.3 Equal number of embryos transferred						
Brugnon 2010	22	55	21	52	5.1%	0.98 [0.45 , 2.13]
Emiliani 2003	33	82	41	89	9.2%	0.79 [0.43 , 1.45]
Fernandez-Shaw 2015	25	60	11	60	2.5%	3.18 [1.39 , 7.31]
Hatirnaz 2017	33	101	35	100	9.3%	0.90 [0.50 , 1.62]
Levi-Setti 2018	54	194	64	194	18.1%	0.78 [0.51 , 1.21]
Papanikolaou 2005	38	80	23	84	4.6%	2.40 [1.25 , 4.60]
Papanikolaou 2006	56	175	38	176	10.1%	1.71 [1.06 , 2.76]
Rienzi 2002	24	50	24	48	5.0%	0.92 [0.42 , 2.04]
Van der Auwera 2002	24	70	17	66	4.5%	1.50 [0.72 , 3.15]
Subtotal (95% CI)		**867**		**869**	**68.3%**	**1.21 [0.99 , 1.47]**
Total events:	309		274			
Heterogeneity: Chi² = 19.25, df = 8 (P = 0.01); I² = 58%						
Test for overall effect: Z = 1.84 (P = 0.07)						
Total (95% CI)		**1336**		**1341**	**100.0%**	**1.30 [1.10 , 1.53]**
Total events:	482		406			
Heterogeneity: Chi² = 31.76, df = 16 (P = 0.01); I² = 50%						
Test for overall effect: Z = 3.13 (P = 0.002)						
Test for subgroup differences: Chi² = 1.47, df = 2 (P = 0.48), I² = 0%						



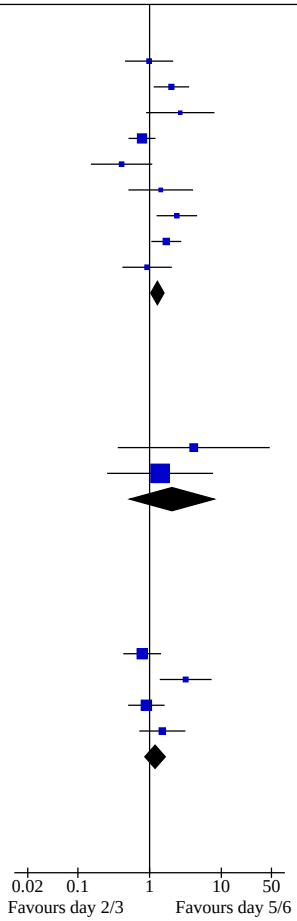


Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 1.3. Comparison 1: Blastocyst- versus cleavage-stage transfer: live birth rate following fresh transfer, Outcome 3: Live birth rate per couple: grouped by prognosis

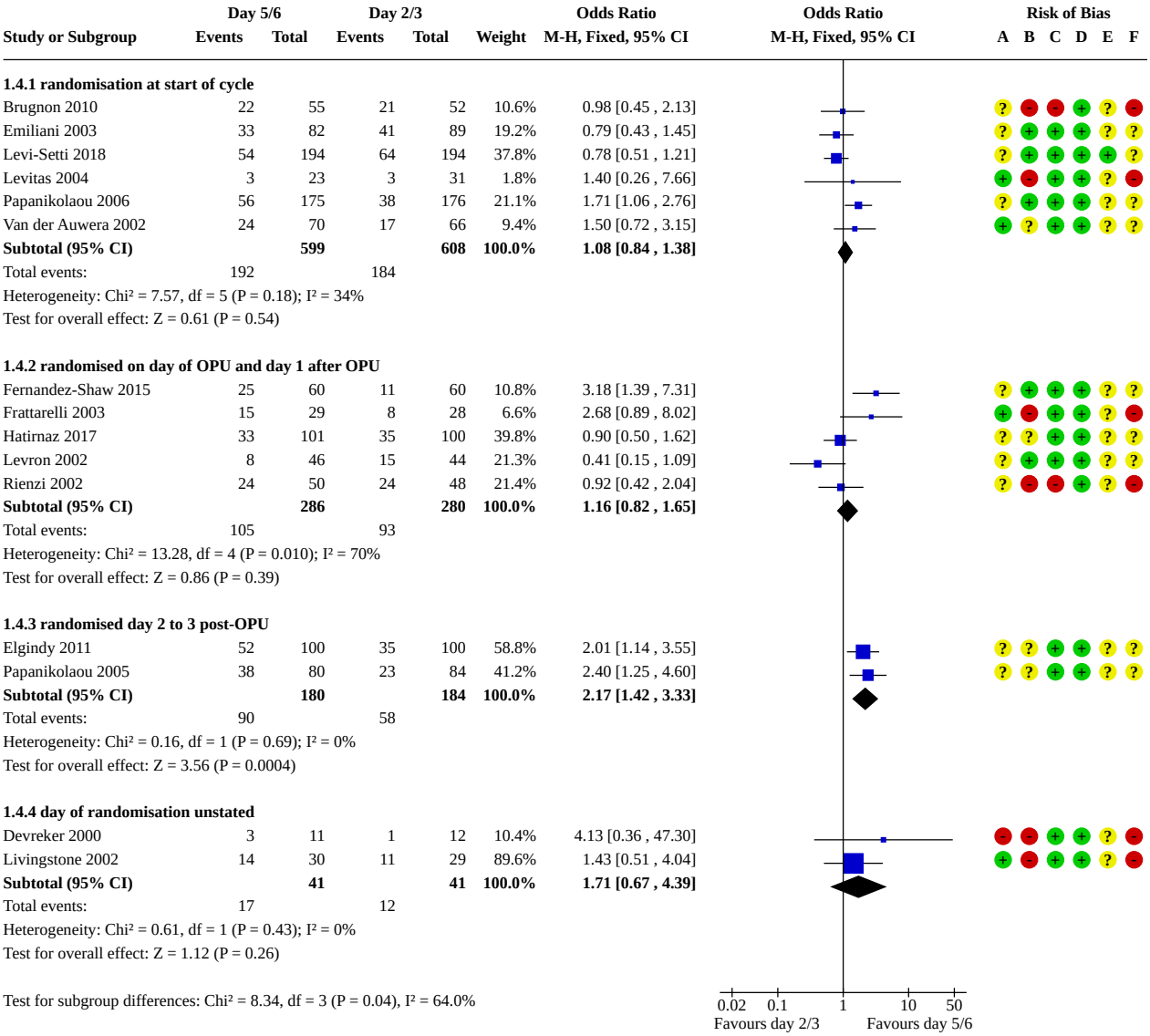
Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
1.3.1 good prognostic factors							
Brugnon 2010	22	55	21	52	8.7%	0.98 [0.45 , 2.13]	? - - + ? -
Elgindy 2011	52	100	35	100	11.3%	2.01 [1.14 , 3.55]	? ? + + ? ?
Frattarelli 2003	15	29	8	28	2.6%	2.68 [0.89 , 8.02]	+ - + + ? -
Levi-Setti 2018	54	194	64	194	31.0%	0.78 [0.51 , 1.21]	? + + + + ?
Levron 2002	8	46	15	44	8.5%	0.41 [0.15 , 1.09]	? + + + ? ?
Livingstone 2002	14	30	11	29	4.0%	1.43 [0.51 , 4.04]	+ - + + ? -
Papanikolaou 2005	38	80	23	84	7.9%	2.40 [1.25 , 4.60]	? ? + + ? ?
Papanikolaou 2006	56	175	38	176	17.3%	1.71 [1.06 , 2.76]	? + + + ? ?
Rienzi 2002	24	50	24	48	8.6%	0.92 [0.42 , 2.04]	? - - + ? -
Subtotal (95% CI)		**759**		**755**	**100.0%**	**1.28 [1.04 , 1.59]**	
Total events:	283		239				
Heterogeneity: Chi² = 20.42, df = 8 (P = 0.009); I² = 61%							
Test for overall effect: Z = 2.29 (P = 0.02)							
1.3.2 poor prognostic factors							
Devreker 2000	3	11	1	12	23.8%	4.13 [0.36 , 47.30]	- - + + ? -
Levitas 2004	3	23	3	31	76.2%	1.40 [0.26 , 7.66]	+ - + + ? -
Subtotal (95% CI)		**34**		**43**	**100.0%**	**2.05 [0.53 , 7.96]**	
Total events:	6		4				
Heterogeneity: Chi² = 0.51, df = 1 (P = 0.48); I² = 0%							
Test for overall effect: Z = 1.04 (P = 0.30)							
1.3.3 unselected group							
Emiliani 2003	33	82	41	89	36.1%	0.79 [0.43 , 1.45]	? + + + ? ?
Fernandez-Shaw 2015	25	60	11	60	9.9%	3.18 [1.39 , 7.31]	? + + + ? ?
Hatirnaz 2017	33	101	35	100	36.4%	0.90 [0.50 , 1.62]	? ? + + ? ?
Van der Auwera 2002	24	70	17	66	17.7%	1.50 [0.72 , 3.15]	+ ? + + ? ?
Subtotal (95% CI)		**313**		**315**	**100.0%**	**1.19 [0.86 , 1.66]**	
Total events:	115		104				
Heterogeneity: Chi² = 8.40, df = 3 (P = 0.04); I² = 64%							
Test for overall effect: Z = 1.05 (P = 0.30)							
Test for subgroup differences: Chi² = 0.63, df = 2 (P = 0.73), I² = 0%							



Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 1.4. Comparison 1: Blastocyst- versus cleavage-stage transfer: live birth rate following fresh transfer, Outcome 4: Live birth rate: grouped by day of randomisation



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 2. Blastocyst- versus cleavage-stage transfer: cumulative pregnancy rate following fresh and frozen transfer

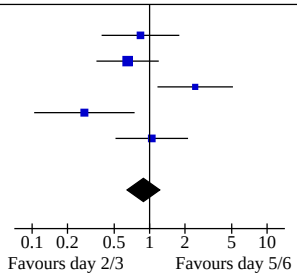
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Cumulative pregnancy rate from fresh and frozen transfers	5	632	Odds Ratio (M-H, Fixed, 95% CI)	0.89 [0.64, 1.22]

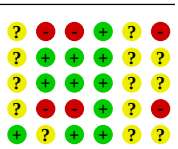
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.2 Cumulative pregnancy rate per couple: grouped by number of embryos transferred	5	739	Odds Ratio (M-H, Fixed, 95% CI)	0.88 [0.65, 1.18]
2.2.1 single embryo transfer	1	107	Odds Ratio (M-H, Fixed, 95% CI)	0.84 [0.39, 1.79]
2.2.2 equal number of embryos transferred	5	632	Odds Ratio (M-H, Fixed, 95% CI)	0.89 [0.64, 1.22]
2.3 Cumulative pregnancy rate per couple: grouped by prognosis	5	632	Odds Ratio (M-H, Fixed, 95% CI)	0.89 [0.64, 1.22]
2.3.1 good prognostic factors	2	205	Odds Ratio (M-H, Fixed, 95% CI)	0.54 [0.30, 0.98]
2.3.2 unselected group	3	427	Odds Ratio (M-H, Fixed, 95% CI)	1.10 [0.74, 1.61]
2.4 Cumulative pregnancy rate: grouped by day of randomisation	5	632	Odds Ratio (M-H, Fixed, 95% CI)	0.89 [0.64, 1.22]
2.4.1 randomisation at start of cycle	3	414	Odds Ratio (M-H, Fixed, 95% CI)	0.81 [0.54, 1.20]
2.4.2 randomised on day of OPU and day 1 after OPU	2	218	Odds Ratio (M-H, Fixed, 95% CI)	1.06 [0.61, 1.83]
2.5 Cumulative pregnancy rate from fresh and frozen transfers: grouped by vitrification or slow freezing	5		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.5.1 slow freezing	4	512	Odds Ratio (M-H, Fixed, 95% CI)	0.69 [0.48, 0.99]
2.5.2 vitrification	1	120	Odds Ratio (M-H, Fixed, 95% CI)	2.44 [1.17, 5.12]

Analysis 2.1. Comparison 2: Blastocyst- versus cleavage-stage transfer: cumulative pregnancy rate following fresh and frozen transfer, Outcome 1: Cumulative pregnancy rate from fresh and frozen transfers

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Brugnon 2010 (1)	24	55	25	52	18.2%	0.84 [0.39 , 1.79]
Emiliani 2003	43	82	56	89	32.0%	0.65 [0.35 , 1.20]
Fernandez-Shaw 2015 (2)	33	60	20	60	11.3%	2.44 [1.17 , 5.12]
Rienzi 2002	31	50	41	48	19.9%	0.28 [0.10 , 0.75]
Van der Auwera 2002	24	70	22	66	18.6%	1.04 [0.51 , 2.12]
Total (95% CI)		**317**		**315**	**100.0%**	**0.89 [0.64 , 1.22]**
Total events:	155		164			

Heterogeneity: Chi² = 13.76, df = 4 (P = 0.008); I² = 71%
Test for overall effect: Z = 0.75 (P = 0.46)
Test for subgroup differences: Not applicable





Footnotes

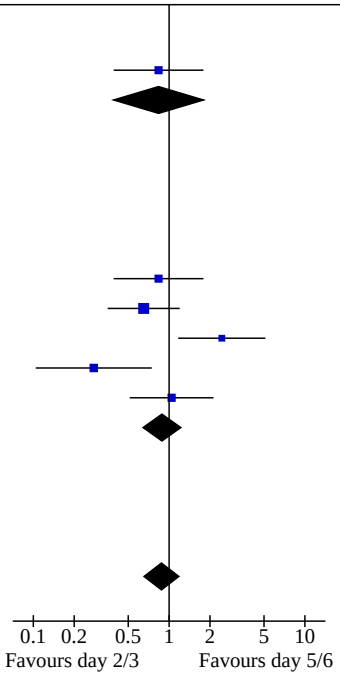
(1) Study had policy of single embryo transfer
(2) Both cumulative pregnancy and live birth rates given, same numbers except for one voluntary termination in blastocyst group due to anomaly after VET

Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 2.2. Comparison 2: Blastocyst- versus cleavage-stage transfer: cumulative pregnancy rate following fresh and frozen transfer, Outcome 2: Cumulative pregnancy rate per couple: grouped by number of embryos transferred

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.2.1 single embryo transfer						
Brugnon 2010 (1)	24	55	25	52	15.4%	0.84 [0.39 , 1.79]
Subtotal (95% CI)		**55**		**52**	**15.4%**	**0.84 [0.39 , 1.79]**
Total events:	24		25			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.46 (P = 0.65)						
2.2.2 equal number of embryos transferred						
Brugnon 2010 (1)	24	55	25	52	15.4%	0.84 [0.39 , 1.79]
Emiliani 2003	43	82	56	89	27.1%	0.65 [0.35 , 1.20]
Fernandez-Shaw 2015 (2)	33	60	20	60	9.5%	2.44 [1.17 , 5.12]
Rienzi 2002	31	50	41	48	16.9%	0.28 [0.10 , 0.75]
Van der Auwera 2002	24	70	22	66	15.8%	1.04 [0.51 , 2.12]
Subtotal (95% CI)		**317**		**315**	**84.6%**	**0.89 [0.64 , 1.22]**
Total events:	155		164			
Heterogeneity: Chi² = 13.76, df = 4 (P = 0.008); I² = 71%						
Test for overall effect: Z = 0.75 (P = 0.46)						
Total (95% CI)		**372**		**367**	**100.0%**	**0.88 [0.65 , 1.18]**
Total events:	179		189			
Heterogeneity: Chi² = 13.78, df = 5 (P = 0.02); I² = 64%						
Test for overall effect: Z = 0.87 (P = 0.39)						
Test for subgroup differences: Chi² = 0.02, df = 1 (P = 0.89), I² = 0%						



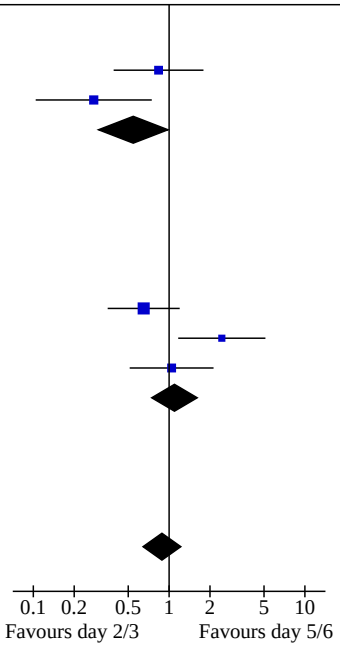
Footnotes

(1) Study had policy of single embryo transfer

(2) Both cumulative pregnancy and live birth rates given, same numbers except for one voluntary termination in blastocyst group due to anomaly after \

Analysis 2.3. Comparison 2: Blastocyst- versus cleavage-stage transfer: cumulative pregnancy rate following fresh and frozen transfer, Outcome 3: Cumulative pregnancy rate per couple: grouped by prognosis

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.3.1 good prognostic factors						
Brugnon 2010 (1)	24	55	25	52	18.2%	0.84 [0.39 , 1.79]
Rienzi 2002	31	50	41	48	19.9%	0.28 [0.10 , 0.75]
Subtotal (95% CI)		**105**		**100**	**38.1%**	**0.54 [0.30 , 0.98]**
Total events:	55		66			
Heterogeneity: Chi² = 3.00, df = 1 (P = 0.08); I² = 67%						
Test for overall effect: Z = 2.03 (P = 0.04)						
2.3.2 unselected group						
Emiliani 2003	43	82	56	89	32.0%	0.65 [0.35 , 1.20]
Fernandez-Shaw 2015 (2)	33	60	20	60	11.3%	2.44 [1.17 , 5.12]
Van der Auwera 2002	24	70	22	66	18.6%	1.04 [0.51 , 2.12]
Subtotal (95% CI)		**212**		**215**	**61.9%**	**1.10 [0.74 , 1.61]**
Total events:	100		98			
Heterogeneity: Chi² = 7.36, df = 2 (P = 0.03); I² = 73%						
Test for overall effect: Z = 0.46 (P = 0.64)						
Total (95% CI)		**317**		**315**	**100.0%**	**0.89 [0.64 , 1.22]**
Total events:	155		164			
Heterogeneity: Chi² = 13.76, df = 4 (P = 0.008); I² = 71%						
Test for overall effect: Z = 0.75 (P = 0.46)						
Test for subgroup differences: Chi² = 3.79, df = 1 (P = 0.05), I² = 73.6%						



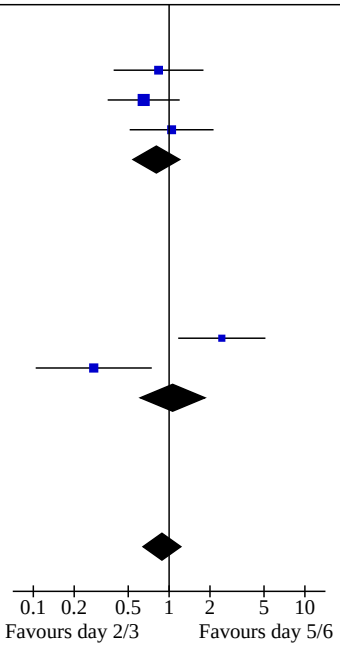
Footnotes

(1) Study had policy of single embryo transfer

(2) Both cumulative pregnancy and live birth rates given, same numbers except for one voluntary termination in blastocyst group due to anomaly after \

Analysis 2.4. Comparison 2: Blastocyst- versus cleavage-stage transfer: cumulative pregnancy rate following fresh and frozen transfer, Outcome 4: Cumulative pregnancy rate: grouped by day of randomisation

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.4.1 randomisation at start of cycle						
Brugnon 2010 (1)	24	55	25	52	18.2%	0.84 [0.39 , 1.79]
Emiliani 2003	43	82	56	89	32.0%	0.65 [0.35 , 1.20]
Van der Auwera 2002	24	70	22	66	18.6%	1.04 [0.51 , 2.12]
Subtotal (95% CI)		**207**		**207**	**68.8%**	**0.81 [0.54 , 1.20]**
Total events:	91		103			
Heterogeneity: Chi² = 0.99, df = 2 (P = 0.61); I² = 0%						
Test for overall effect: Z = 1.07 (P = 0.28)						
2.4.2 randomised on day of OPU and day 1 after OPU						
Fernandez-Shaw 2015 (2)	33	60	20	60	11.3%	2.44 [1.17 , 5.12]
Rienzi 2002	31	50	41	48	19.9%	0.28 [0.10 , 0.75]
Subtotal (95% CI)		**110**		**108**	**31.2%**	**1.06 [0.61 , 1.83]**
Total events:	64		61			
Heterogeneity: Chi² = 11.99, df = 1 (P = 0.0005); I² = 92%						
Test for overall effect: Z = 0.21 (P = 0.83)						
Total (95% CI)		**317**		**315**	**100.0%**	**0.89 [0.64 , 1.22]**
Total events:	155		164			
Heterogeneity: Chi² = 13.76, df = 4 (P = 0.008); I² = 71%						
Test for overall effect: Z = 0.75 (P = 0.46)						
Test for subgroup differences: Chi² = 0.64, df = 1 (P = 0.42), I² = 0%						



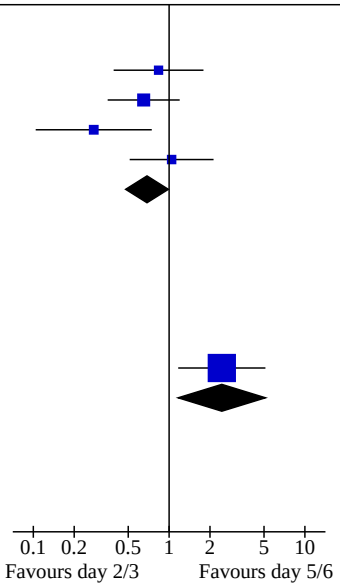
Footnotes

(1) Study had policy of single embryo transfer

(2) Both cumulative pregnancy and live birth rates given, same numbers except for one voluntary termination in blastocyst group due to anomaly after \

Analysis 2.5. Comparison 2: Blastocyst- versus cleavage-stage transfer: cumulative pregnancy rate following fresh and frozen transfer, Outcome 5: Cumulative pregnancy rate from fresh and frozen transfers: grouped by vitrification or slow freezing

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.5.1 slow freezing						
Brugnon 2010 (1)	24	55	25	52	20.5%	0.84 [0.39 , 1.79]
Emiliani 2003	43	82	56	89	36.1%	0.65 [0.35 , 1.20]
Rienzi 2002	31	50	41	48	22.5%	0.28 [0.10 , 0.75]
Van der Auwera 2002	24	70	22	66	21.0%	1.04 [0.51 , 2.12]
Subtotal (95% CI)		**257**		**255**	**100.0%**	**0.69 [0.48 , 0.99]**
Total events:	122		144			
Heterogeneity: Chi² = 4.85, df = 3 (P = 0.18); I² = 38%						
Test for overall effect: Z = 2.03 (P = 0.04)						
2.5.2 vitrification						
Fernandez-Shaw 2015 (2)	33	60	20	60	100.0%	2.44 [1.17 , 5.12]
Subtotal (95% CI)		**60**		**60**	**100.0%**	**2.44 [1.17 , 5.12]**
Total events:	33		20			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.37 (P = 0.02)						
Test for subgroup differences: Chi² = 9.12, df = 1 (P = 0.003), I² = 89.0%						



Footnotes

(1) Study had policy of single embryo transfer

(2) Both cumulative pregnancy and live birth rates given, same numbers except for one voluntary termination in blastocyst group due to anomaly after \

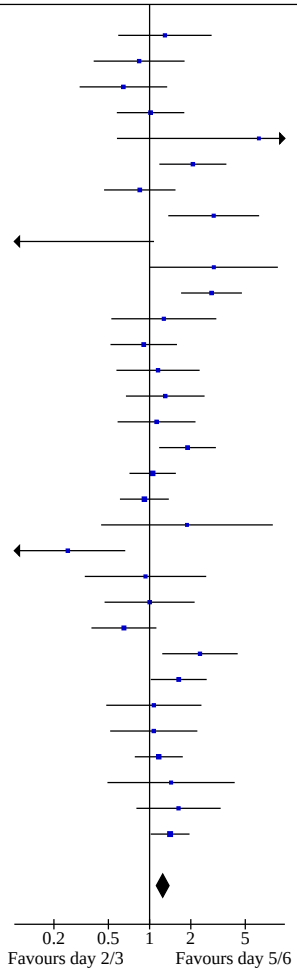
Comparison 3. Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 Clinical pregnancy rate per couple	32	5821	Odds Ratio (M-H, Fixed, 95% CI)	1.25 [1.12, 1.39]
3.2 Clinical pregnancy rate per couple: grouped by number of embryos transferred	32	7062	Odds Ratio (M-H, Fixed, 95% CI)	1.26 [1.14, 1.38]
3.2.1 equal number of embryo transfers	20	4434	Odds Ratio (M-H, Fixed, 95% CI)	1.31 [1.16, 1.48]
3.2.2 more cleavage stage than blastocyst embryos transferred	12	1387	Odds Ratio (M-H, Fixed, 95% CI)	1.07 [0.86, 1.33]
3.2.3 single embryo transfer	5	1241	Odds Ratio (M-H, Fixed, 95% CI)	1.31 [1.04, 1.65]
3.3 Clinical pregnancy rate per couple: grouped by prognosis	32	5821	Odds Ratio (M-H, Fixed, 95% CI)	1.25 [1.12, 1.39]
3.3.1 good prognostic factors	19	3645	Odds Ratio (M-H, Fixed, 95% CI)	1.25 [1.09, 1.43]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.3.2 poor prognostic factors	3	195	Odds Ratio (M-H, Fixed, 95% CI)	1.62 [0.84, 3.10]
3.3.3 unselected group	10	1981	Odds Ratio (M-H, Fixed, 95% CI)	1.22 [1.01, 1.46]
3.4 Clinical pregnancy rate per couple: grouped by day of randomisation	32	5821	Odds Ratio (M-H, Fixed, 95% CI)	1.25 [1.12, 1.39]
3.4.1 randomised start of cycle	8	1759	Odds Ratio (M-H, Fixed, 95% CI)	1.12 [0.92, 1.37]
3.4.2 randomised on day of OPU or day 1	13	2094	Odds Ratio (M-H, Fixed, 95% CI)	1.19 [1.00, 1.42]
3.4.3 randomised on day 2 to 3	6	1275	Odds Ratio (M-H, Fixed, 95% CI)	1.50 [1.19, 1.88]
3.4.4 day of randomisation unstated	5	693	Odds Ratio (M-H, Fixed, 95% CI)	1.33 [0.98, 1.80]
3.5 Clinical pregnancy rate per couple: TLS (with algorithm) cleavage stage versus conventional blastocyst stage	2	709	Odds Ratio (M-H, Fixed, 95% CI)	1.41 [1.04, 1.90]
3.6 Clinical pregnancy rate per couple: TLS (with algorithm) cleavage stage versus TLS (with algorithm) blastocyst stage	1	110	Odds Ratio (M-H, Fixed, 95% CI)	0.91 [0.43, 1.96]

Analysis 3.1. Comparison 3: Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer, Outcome 1: Clinical pregnancy rate per couple

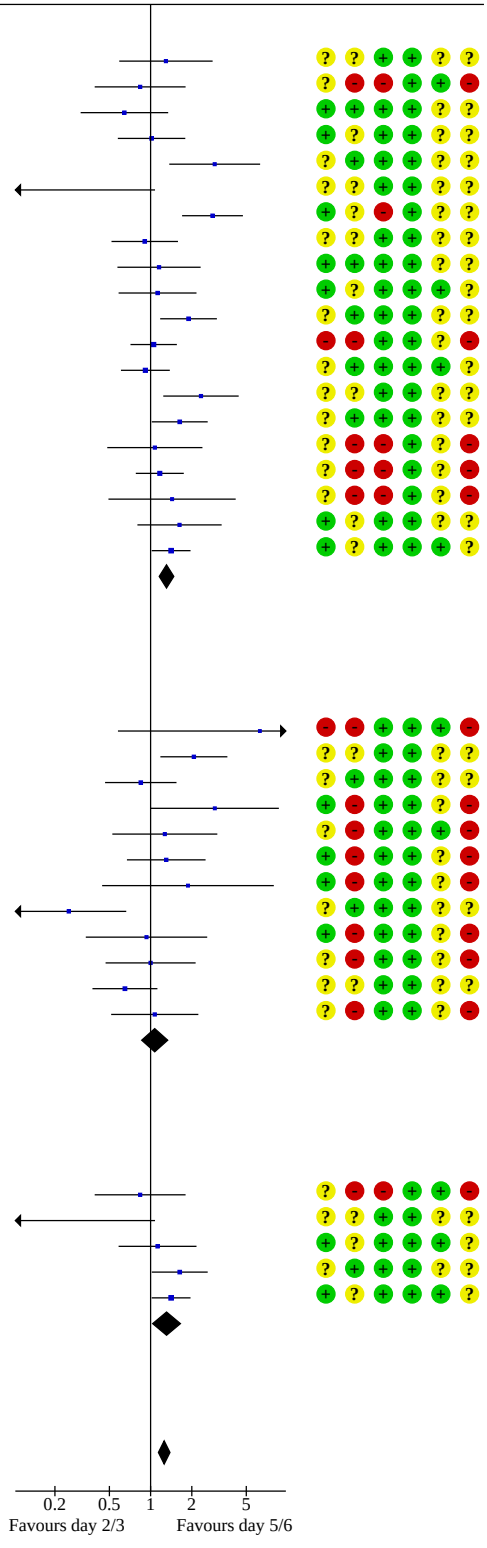
Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Risk of Bias A	B	C	D	E	F
Aziminekoo 2015	19	57	17	61	1.8%	1.29 [0.59 , 2.84]	?	?	+	+	?	?
Brugnon 2010	23	55	24	52	2.4%	0.84 [0.39 , 1.80]	?	-	-	+	+	-
Bungum 2003	32	61	36	57	2.9%	0.64 [0.31 , 1.34]	+	+	+	+	?	?
Coskun 2000	39	100	39	101	3.9%	1.02 [0.58 , 1.79]	+	?	+	+	?	?
Devreker 2000	4	11	1	12	0.1%	6.29 [0.58 , 68.42]	-	-	+	+	-	-
Elgindy 2011	59	100	41	100	2.8%	2.07 [1.18 , 3.64]	?	?	+	+	?	?
Emiliani 2003	39	82	46	89	3.8%	0.85 [0.47 , 1.55]	?	+	+	+	?	?
Fernandez-Shaw 2015	31	60	16	60	1.3%	2.94 [1.37 , 6.31]	?	+	+	+	?	?
Fisch 2007	4	8	11	12	0.7%	0.09 [0.01 , 1.08]	?	?	+	+	?	?
Frattarelli 2003	18	29	10	28	0.6%	2.95 [1.00 , 8.65]	+	-	+	+	?	-
Gaafar 2015	76	126	44	126	2.9%	2.83 [1.70 , 4.72]	+	?	-	+	?	-
Gardner 1998a	32	45	31	47	1.5%	1.27 [0.53 , 3.07]	?	-	+	+	+	-
Hatirnaz 2017	42	101	44	100	4.3%	0.91 [0.52 , 1.58]	?	?	+	+	?	?
Hreinsson 2004	22	64	25	80	2.4%	1.15 [0.57 , 2.32]	+	+	+	+	?	?
Karaki 2002	28	80	24	82	2.6%	1.30 [0.67 , 2.52]	+	-	+	+	?	-
Kaser 2017	47	107	23	56	2.8%	1.12 [0.58 , 2.16]	+	?	+	+	+	?
Kaur 2014	66	150	44	150	4.1%	1.89 [1.17 , 3.05]	?	+	+	+	?	?
Kolibianakis 2004	75	226	75	234	8.2%	1.05 [0.71 , 1.56]	-	-	+	+	?	-
Levi-Setti 2018	71	194	75	194	7.9%	0.92 [0.61 , 1.38]	?	+	+	+	+	?
Levitas 2004	5	23	4	31	0.4%	1.88 [0.44 , 7.94]	+	-	+	+	?	-
Levron 2002	8	46	20	44	2.8%	0.25 [0.10 , 0.66]	?	+	+	+	?	?
Livingstone 2002	15	30	15	29	1.3%	0.93 [0.34 , 2.59]	+	-	+	+	?	-
Motta 1998	21	58	21	58	2.2%	1.00 [0.47 , 2.13]	?	-	+	+	?	-
Pantos 2004	30	81	77	162	5.4%	0.65 [0.38 , 1.12]	?	?	+	+	?	?
Papanikolaou 2005	42	80	27	84	2.1%	2.33 [1.24 , 4.40]	?	?	+	+	?	?
Papanikolaou 2006	58	175	41	176	4.5%	1.63 [1.02 , 2.61]	?	+	+	+	?	?
Rienzi 2002	29	50	27	48	1.9%	1.07 [0.48 , 2.39]	?	-	-	+	?	-
Schillaci 2002	24	60	23	60	2.3%	1.07 [0.52 , 2.23]	?	-	+	+	?	-
Singh 2017	83	243	60	195	7.3%	1.17 [0.78 , 1.75]	?	-	-	+	?	-
Ten 2011	17	28	14	27	0.9%	1.44 [0.49 , 4.18]	?	-	-	+	?	-
Van der Auwera 2002	29	70	20	66	2.0%	1.63 [0.80 , 3.30]	+	?	+	+	?	?
Yang 2018	189	300	164	300	10.0%	1.41 [1.02 , 1.96]	+	?	+	+	+	?
Total (95% CI)		**2900**		**2921**	**100.0%**	**1.25 [1.12 , 1.39]**						
Total events:	1277		1139									



Heterogeneity: Chi² = 63.32, df = 31 (P = 0.0005); I² = 51%
Test for overall effect: Z = 4.04 (P < 0.0001)
Test for subgroup differences: Not applicable

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.2. Comparison 3: Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer, Outcome 2: Clinical pregnancy rate per couple: grouped by number of embryos transferred



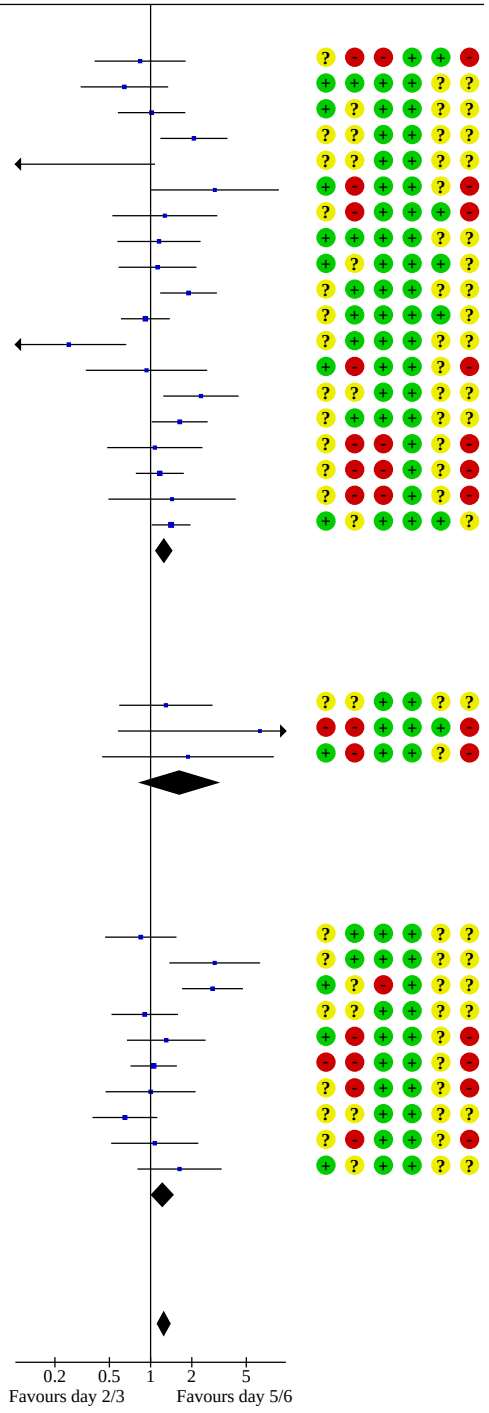
Analysis 3.2. (Continued)

Test for subgroup differences: Chi² = 2.55, df = 2 (P = 0.28), I² = 21.5%

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.3. Comparison 3: Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer, Outcome 3: Clinical pregnancy rate per couple: grouped by prognosis

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.3.1 good prognostic factors						
Brugnon 2010	23	55	24	52	2.4%	0.84 [0.39 , 1.80]
Bungum 2003	32	61	36	57	2.9%	0.64 [0.31 , 1.34]
Coskun 2000	39	100	39	101	3.9%	1.02 [0.58 , 1.79]
Elgindy 2011	59	100	41	100	2.8%	2.07 [1.18 , 3.64]
Fisch 2007	4	8	11	12	0.7%	0.09 [0.01 , 1.08]
Frattarelli 2003	18	29	10	28	0.6%	2.95 [1.00 , 8.65]
Gardner 1998a	32	45	31	47	1.5%	1.27 [0.53 , 3.07]
Hreinsson 2004	22	64	25	80	2.4%	1.15 [0.57 , 2.32]
Kaser 2017	47	107	23	56	2.8%	1.12 [0.58 , 2.16]
Kaur 2014	66	150	44	150	4.1%	1.89 [1.17 , 3.05]
Levi-Setti 2018	71	194	75	194	7.9%	0.92 [0.61 , 1.38]
Levron 2002	8	46	20	44	2.8%	0.25 [0.10 , 0.66]
Livingstone 2002	15	30	15	29	1.3%	0.93 [0.34 , 2.59]
Papanikolaou 2005	42	80	27	84	2.1%	2.33 [1.24 , 4.40]
Papanikolaou 2006	58	175	41	176	4.5%	1.63 [1.02 , 2.61]
Rienzi 2002	29	50	27	48	1.9%	1.07 [0.48 , 2.39]
Singh 2017	83	243	60	195	7.3%	1.17 [0.78 , 1.75]
Ten 2011	17	28	14	27	0.9%	1.44 [0.49 , 4.18]
Yang 2018	189	300	164	300	10.0%	1.41 [1.02 , 1.96]
Subtotal (95% CI)		**1865**		**1780**	**62.8%**	**1.25 [1.09 , 1.43]**
Total events:	854		727			
Heterogeneity: Chi² = 36.42, df = 18 (P = 0.006); I² = 51%						
Test for overall effect: Z = 3.23 (P = 0.001)						
3.3.2 poor prognostic factors						
Aziminekoo 2015	19	57	17	61	1.8%	1.29 [0.59 , 2.84]
Devreker 2000	4	11	1	12	0.1%	6.29 [0.58 , 68.42]
Levitas 2004	5	23	4	31	0.4%	1.88 [0.44 , 7.94]
Subtotal (95% CI)		**91**		**104**	**2.4%**	**1.62 [0.84 , 3.10]**
Total events:	28		22			
Heterogeneity: Chi² = 1.59, df = 2 (P = 0.45); I² = 0%						
Test for overall effect: Z = 1.44 (P = 0.15)						
3.3.3 unselected group						
Emiliani 2003	39	82	46	89	3.8%	0.85 [0.47 , 1.55]
Fernandez-Shaw 2015	31	60	16	60	1.3%	2.94 [1.37 , 6.31]
Gaafar 2015	76	126	44	126	2.9%	2.83 [1.70 , 4.72]
Hatirnaz 2017	42	101	44	100	4.3%	0.91 [0.52 , 1.58]
Karaki 2002	28	80	24	82	2.6%	1.30 [0.67 , 2.52]
Kolibianakis 2004	75	226	75	234	8.2%	1.05 [0.71 , 1.56]
Motta 1998	21	58	21	58	2.2%	1.00 [0.47 , 2.13]
Pantos 2004	30	81	77	162	5.4%	0.65 [0.38 , 1.12]
Schillaci 2002	24	60	23	60	2.3%	1.07 [0.52 , 2.23]
Van der Auwera 2002	29	70	20	66	2.0%	1.63 [0.80 , 3.30]
Subtotal (95% CI)		**944**		**1037**	**34.8%**	**1.22 [1.01 , 1.46]**
Total events:	395		390			
Heterogeneity: Chi² = 24.73, df = 9 (P = 0.003); I² = 64%						
Test for overall effect: Z = 2.11 (P = 0.03)						
Total (95% CI)		**2900**		**2921**	**100.0%**	**1.25 [1.12 , 1.39]**
Total events:	1277		1139			
Heterogeneity: Chi² = 63.32, df = 31 (P = 0.0005); I² = 51%						
Test for overall effect: Z = 4.04 (P < 0.0001)						
Test for subgroup differences: Chi² = 0.68, df = 2 (P = 0.71), I² = 0%						



Risk of bias legend

(A) Bias arising from the randomization process

(B) Bias due to deviations from intended interventions

(C) Bias due to missing outcome data

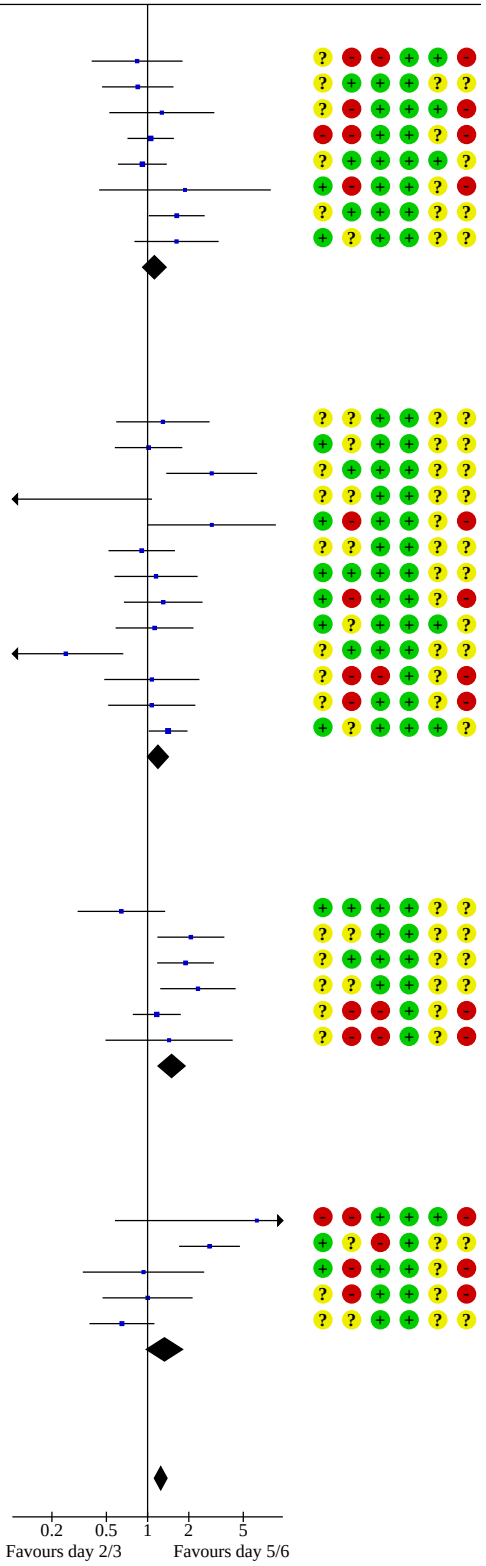
(D) Bias in measurement of the outcome

Analysis 3.3. (Continued)

(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.4. Comparison 3: Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer, Outcome 4: Clinical pregnancy rate per couple: grouped by day of randomisation

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.4.1 randomised start of cycle						
Brugnon 2010	23	55	24	52	2.4%	0.84 [0.39 , 1.80]
Emiliani 2003	39	82	46	89	3.8%	0.85 [0.47 , 1.55]
Gardner 1998a	32	45	31	47	1.5%	1.27 [0.53 , 3.07]
Kolibianakis 2004	75	226	75	234	8.2%	1.05 [0.71 , 1.56]
Levi-Setti 2018	71	194	75	194	7.9%	0.92 [0.61 , 1.38]
Levitas 2004	5	23	4	31	0.4%	1.88 [0.44 , 7.94]
Papanikolaou 2006	58	175	41	176	4.5%	1.63 [1.02 , 2.61]
Van der Auwera 2002	29	70	20	66	2.0%	1.63 [0.80 , 3.30]
Subtotal (95% CI)		**870**		**889**	**30.7%**	**1.12 [0.92 , 1.37]**
Total events:	332		316			
Heterogeneity: Chi² = 6.49, df = 7 (P = 0.48); I² = 0%						
Test for overall effect: Z = 1.13 (P = 0.26)						
3.4.2 randomised on day of OPU or day 1						
Aziminekoo 2015	19	57	17	61	1.8%	1.29 [0.59 , 2.84]
Coskun 2000	39	100	39	101	3.9%	1.02 [0.58 , 1.79]
Fernandez-Shaw 2015	31	60	16	60	1.3%	2.94 [1.37 , 6.31]
Fisch 2007	4	8	11	12	0.7%	0.09 [0.01 , 1.08]
Frattarelli 2003	18	29	10	28	0.6%	2.95 [1.00 , 8.65]
Hatirnaz 2017	42	101	44	100	4.3%	0.91 [0.52 , 1.58]
Hreinsson 2004	22	64	25	80	2.4%	1.15 [0.57 , 2.32]
Karaki 2002	28	80	24	82	2.6%	1.30 [0.67 , 2.52]
Kaser 2017	47	107	23	56	2.8%	1.12 [0.58 , 2.16]
Levron 2002	8	46	20	44	2.8%	0.25 [0.10 , 0.66]
Rienzi 2002	29	50	27	48	1.9%	1.07 [0.48 , 2.39]
Schillaci 2002	24	60	23	60	2.3%	1.07 [0.52 , 2.23]
Yang 2018	189	300	164	300	10.0%	1.41 [1.02 , 1.96]
Subtotal (95% CI)		**1062**		**1032**	**37.5%**	**1.19 [1.00 , 1.42]**
Total events:	500		443			
Heterogeneity: Chi² = 24.70, df = 12 (P = 0.02); I² = 51%						
Test for overall effect: Z = 1.93 (P = 0.05)						
3.4.3 randomised on day 2 to 3						
Bungum 2003	32	61	36	57	2.9%	0.64 [0.31 , 1.34]
Elgindy 2011	59	100	41	100	2.8%	2.07 [1.18 , 3.64]
Kaur 2014	66	150	44	150	4.1%	1.89 [1.17 , 3.05]
Papanikolaou 2005	42	80	27	84	2.1%	2.33 [1.24 , 4.40]
Singh 2017	83	243	60	195	7.3%	1.17 [0.78 , 1.75]
Ten 2011	17	28	14	27	0.9%	1.44 [0.49 , 4.18]
Subtotal (95% CI)		**662**		**613**	**20.1%**	**1.50 [1.19 , 1.88]**
Total events:	299		222			
Heterogeneity: Chi² = 10.60, df = 5 (P = 0.06); I² = 53%						
Test for overall effect: Z = 3.46 (P = 0.0005)						
3.4.4 day of randomisation unstated						
Devreker 2000	4	11	1	12	0.1%	6.29 [0.58 , 68.42]
Gaafar 2015	76	126	44	126	2.9%	2.83 [1.70 , 4.72]
Livingstone 2002	15	30	15	29	1.3%	0.93 [0.34 , 2.59]
Motta 1998	21	58	21	58	2.2%	1.00 [0.47 , 2.13]
Pantos 2004	30	81	77	162	5.4%	0.65 [0.38 , 1.12]
Subtotal (95% CI)		**306**		**387**	**11.8%**	**1.33 [0.98 , 1.80]**
Total events:	146		158			
Heterogeneity: Chi² = 17.64, df = 4 (P = 0.001); I² = 77%						
Test for overall effect: Z = 1.82 (P = 0.07)						
Total (95% CI)		**2900**		**2921**	**100.0%**	**1.25 [1.12 , 1.39]**
Total events:	1277		1139			
Heterogeneity: Chi² = 63.32, df = 31 (P = 0.0005); I² = 51%						
Test for overall effect: Z = 4.04 (P < 0.0001)						
Test for subgroup differences: Chi² = 4.01, df = 3 (P = 0.26), I² = 25.3%						



Analysis 3.4. (Continued)

Test for overall effect: Z = 4.04 (P < 0.0001)
Test for subgroup differences: Chi² = 4.01, df = 3 (P = 0.26), I² = 25.3%

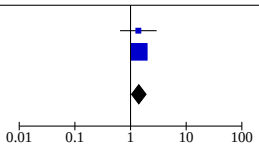
Favours day 2/3 Favours day 5/6

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.5. Comparison 3: Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer, Outcome 5: Clinical pregnancy rate per couple: TLS (with algorithm) cleavage stage versus conventional blastocyst stage

Study or Subgroup	Conventional blastocyst stage Events	Total	TLS (with algorithm) cleavage stage Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Kaser 2017	26	53	23	56	15.8%	1.38 [0.65 , 2.95]	+ ? + + + ?
Yang 2018	189	300	164	300	84.2%	1.41 [1.02 , 1.96]	+ ? + + + ?
Total (95% CI)		**353**		**356**	**100.0%**	**1.41 [1.04 , 1.90]**	
Total events:	215		187				

Heterogeneity: Chi² = 0.00, df = 1 (P = 0.96); I² = 0%
Test for overall effect: Z = 2.23 (P = 0.03)
Test for subgroup differences: Not applicable

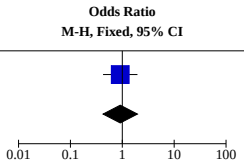


Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 3.6. Comparison 3: Blastocyst- versus cleavage-stage transfer: clinical pregnancy following fresh transfer, Outcome 6: Clinical pregnancy rate per couple: TLS (with algorithm) cleavage stage versus TLS (with algorithm) blastocyst stage

Study or Subgroup	TLS (with algorithm) blastocyst stage Events	Total	TLS (with algorithm) cleavage stage Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
Kaser 2017	21	54	23	56	100.0%	0.91 [0.43 , 1.96]	+ ? + + + ?
Total (95% CI)		**54**		**56**	**100.0%**	**0.91 [0.43 , 1.96]**	
Total events:	21		23				

Heterogeneity: Not applicable
Test for overall effect: Z = 0.23 (P = 0.82)
Test for subgroup differences: Not applicable



Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

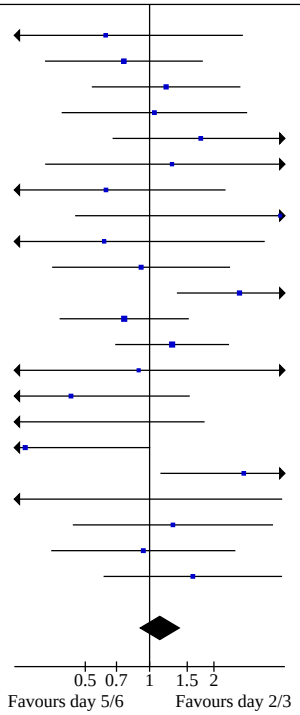
Comparison 4. Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer

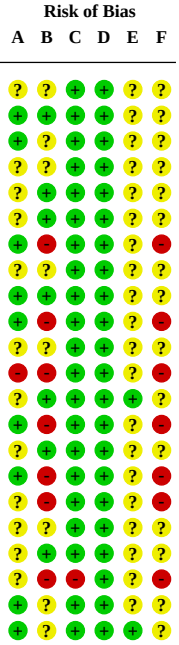
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Multiple pregnancy rate per couple	22	4208	Odds Ratio (M-H, Fixed, 95% CI)	1.12 [0.90, 1.38]
4.2 Multiple pregnancy rate per couple: grouped by number of embryos transferred	22	5159	Odds Ratio (M-H, Fixed, 95% CI)	1.12 [0.91, 1.38]
4.2.1 equal number of embryos transferred	14	3399	Odds Ratio (M-H, Fixed, 95% CI)	1.29 [1.01, 1.65]
4.2.2 more cleavage stage than blastocyst embryos transferred	8	809	Odds Ratio (M-H, Fixed, 95% CI)	0.75 [0.49, 1.13]
4.2.3 single embryo transfer	2	951	Odds Ratio (M-H, Fixed, 95% CI)	1.22 [0.51, 2.91]
4.3 Multiple pregnancy rate per couple: grouped by prognosis	22	4208	Odds Ratio (M-H, Fixed, 95% CI)	1.12 [0.90, 1.38]
4.3.1 good prognostic factors	15	2904	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.89, 1.46]
4.3.2 poor prognostic factors	1	54	Odds Ratio (M-H, Fixed, 95% CI)	0.89 [0.14, 5.81]
4.3.3 unselected	6	1250	Odds Ratio (M-H, Fixed, 95% CI)	1.07 [0.71, 1.59]
4.4 Multiple pregnancy rate per couple: grouped by day of randomisation	22	4208	Odds Ratio (M-H, Fixed, 95% CI)	1.12 [0.90, 1.38]
4.4.1 randomised start of cycle	7	1704	Odds Ratio (M-H, Fixed, 95% CI)	1.02 [0.71, 1.46]
4.4.2 randomised on day of OPU or day 1	9	1647	Odds Ratio (M-H, Fixed, 95% CI)	1.05 [0.73, 1.52]
4.4.3 randomised on day 2 to 3	4	682	Odds Ratio (M-H, Fixed, 95% CI)	1.71 [1.14, 2.56]
4.4.4 day of randomisation unstated	2	175	Odds Ratio (M-H, Fixed, 95% CI)	0.21 [0.06, 0.70]
4.5 High-order pregnancies (more than 2 gestational sacs) per couple	13	2335	Odds Ratio (M-H, Fixed, 95% CI)	0.45 [0.18, 1.15]
4.6 Multiple pregnancy rate per couple: TLS (with algorithm) cleavage stage versus conventional blastocyst stage	1	600	Odds Ratio (M-H, Fixed, 95% CI)	1.59 [0.61, 4.17]

Analysis 4.1. Comparison 4: Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer, Outcome 1: Multiple pregnancy rate per couple

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Aziminekoo 2015	3	57	5	61	2.8%	0.62 [0.14 , 2.73]
Bungum 2003	13	61	15	57	7.4%	0.76 [0.32 , 1.77]
Coskun 2000	15	100	13	101	6.7%	1.19 [0.54 , 2.66]
Elgindy 2011	12	59	8	41	4.6%	1.05 [0.39 , 2.86]
Emiliani 2003	12	82	8	89	4.0%	1.74 [0.67 , 4.49]
Fernandez-Shaw 2015	5	60	4	60	2.2%	1.27 [0.32 , 4.99]
Frattarelli 2003	5	29	7	28	3.6%	0.63 [0.17 , 2.27]
Hatirnaz 2017	4	101	1	100	0.6%	4.08 [0.45 , 37.18]
Hreinsson 2004	2	64	4	80	2.1%	0.61 [0.11 , 3.46]
Karaki 2002	9	80	10	82	5.3%	0.91 [0.35 , 2.38]
Kaur 2014	32	150	14	150	6.7%	2.63 [1.34 , 5.17]
Kolibianakis 2004	15	226	20	234	11.1%	0.76 [0.38 , 1.53]
Levi-Setti 2018	26	194	21	194	11.0%	1.27 [0.69 , 2.35]
Levitas 2004	2	23	3	31	1.4%	0.89 [0.14 , 5.81]
Levron 2002	4	46	8	44	4.5%	0.43 [0.12 , 1.54]
Livingstone 2002	0	30	4	29	2.7%	0.09 [0.00 , 1.81]
Motta 1998	3	58	10	58	5.7%	0.26 [0.07 , 1.01]
Papanikolaou 2005	18	80	8	84	3.7%	2.76 [1.12 , 6.77]
Papanikolaou 2006	0	175	2	176	1.5%	0.20 [0.01 , 4.17]
Rienzi 2002	9	50	7	48	3.5%	1.29 [0.44 , 3.78]
Van der Auwera 2002	9	70	9	66	4.9%	0.93 [0.35 , 2.52]
Yang 2018	11	300	7	300	4.1%	1.59 [0.61 , 4.17]
Total (95% CI)		**2095**		**2113**	**100.0%**	**1.12 [0.90 , 1.38]**
Total events:	209		188			

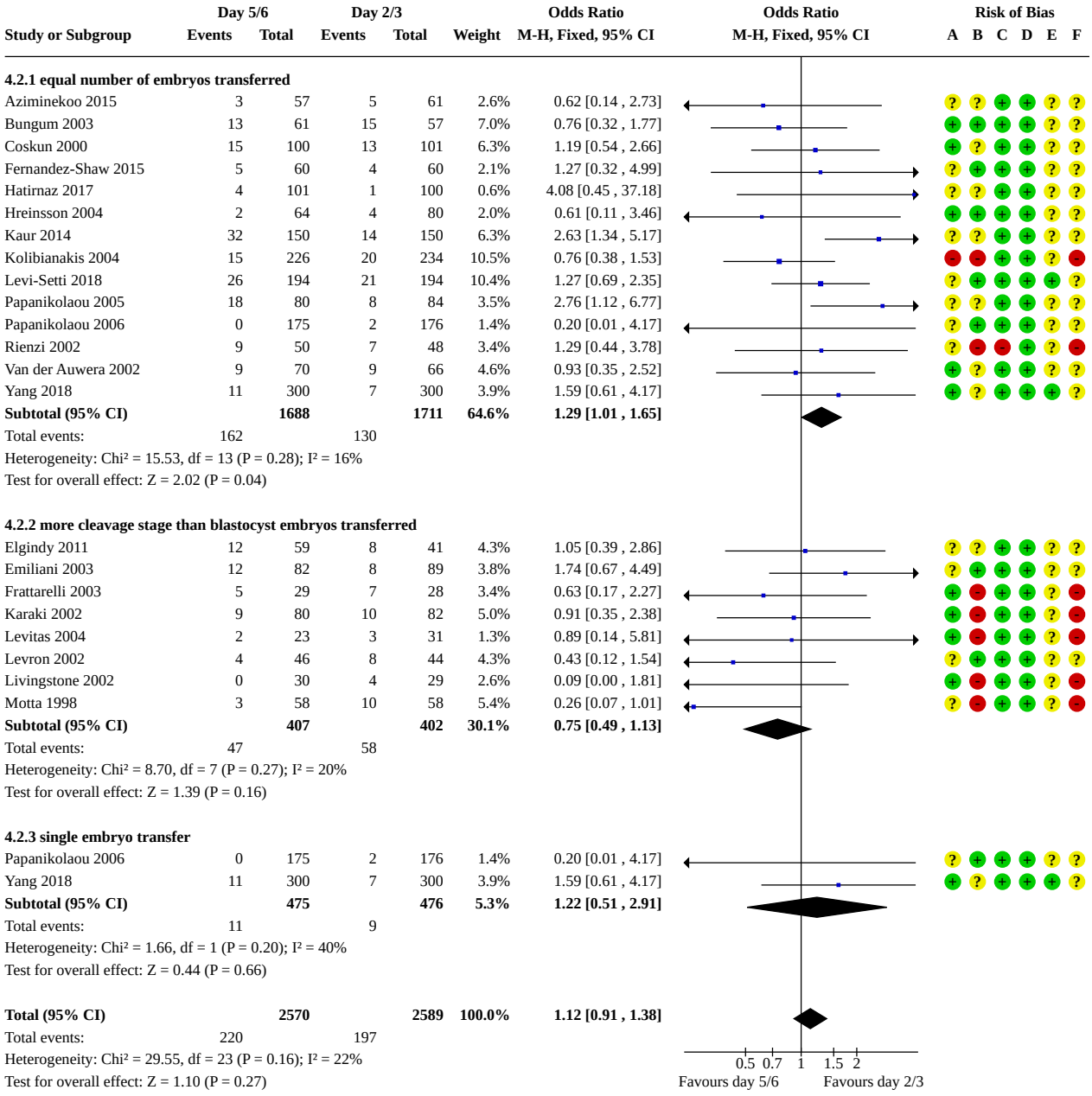
Heterogeneity: Chi² = 27.80, df = 21 (P = 0.15); I² = 24%
Test for overall effect: Z = 1.02 (P = 0.31)
Test for subgroup differences: Not applicable





Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.2. Comparison 4: Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer, Outcome 2: Multiple pregnancy rate per couple: grouped by number of embryos transferred

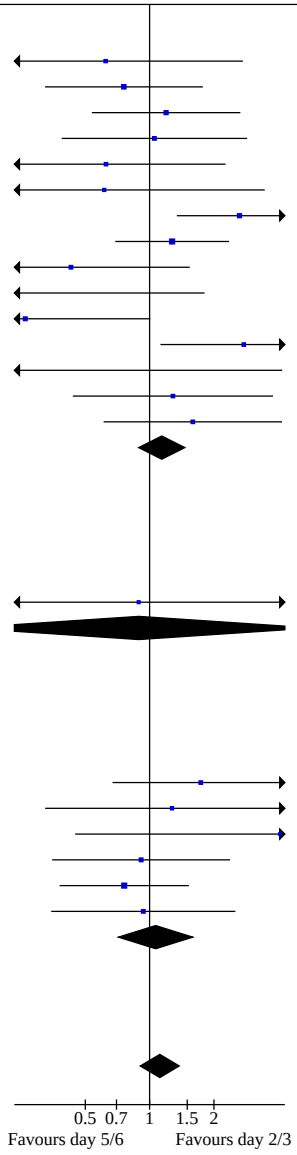


Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.3. Comparison 4: Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer, Outcome 3: Multiple pregnancy rate per couple: grouped by prognosis

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F
4.3.1 good prognostic factors							
Aziminekoo 2015	3	57	5	61	2.8%	0.62 [0.14 , 2.73]	? ? + + ? ?
Bungum 2003	13	61	15	57	7.4%	0.76 [0.32 , 1.77]	+ + + + ? ?
Coskun 2000	15	100	13	101	6.7%	1.19 [0.54 , 2.66]	+ ? + + ? ?
Elgindy 2011	12	59	8	41	4.6%	1.05 [0.39 , 2.86]	? ? + + ? ?
Frattarelli 2003	5	29	7	28	3.6%	0.63 [0.17 , 2.27]	+ - + + ? -
Hreinsson 2004	2	64	4	80	2.1%	0.61 [0.11 , 3.46]	+ + + + ? ?
Kaur 2014	32	150	14	150	6.7%	2.63 [1.34 , 5.17]	? ? + + ? ?
Levi-Setti 2018	26	194	21	194	11.0%	1.27 [0.69 , 2.35]	? + + + + ?
Levron 2002	4	46	8	44	4.5%	0.43 [0.12 , 1.54]	? + + + ? ?
Livingstone 2002	0	30	4	29	2.7%	0.09 [0.00 , 1.81]	+ - + + ? -
Motta 1998	3	58	10	58	5.7%	0.26 [0.07 , 1.01]	? - + + ? -
Papanikolaou 2005	18	80	8	84	3.7%	2.76 [1.12 , 6.77]	? ? + + ? ?
Papanikolaou 2006	0	175	2	176	1.5%	0.20 [0.01 , 4.17]	? + + + ? ?
Rienzi 2002	9	50	7	48	3.5%	1.29 [0.44 , 3.78]	? - - + ? -
Yang 2018	11	300	7	300	4.1%	1.59 [0.61 , 4.17]	+ ? + + + ?
Subtotal (95% CI)		**1453**		**1451**	**70.5%**	**1.14 [0.89 , 1.46]**	
Total events:	153		133				
Heterogeneity: Chi² = 24.01, df = 14 (P = 0.05); I² = 42%							
Test for overall effect: Z = 1.03 (P = 0.30)							
4.3.2 poor prognostic factors							
Levitas 2004	2	23	3	31	1.4%	0.89 [0.14 , 5.81]	+ - + + ? -
Subtotal (95% CI)		**23**		**31**	**1.4%**	**0.89 [0.14 , 5.81]**	
Total events:	2		3				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.12 (P = 0.90)							
4.3.3 unselected							
Emiliani 2003	12	82	8	89	4.0%	1.74 [0.67 , 4.49]	? + + + ? ?
Fernandez-Shaw 2015	5	60	4	60	2.2%	1.27 [0.32 , 4.99]	? + + + ? ?
Hatirnaz 2017	4	101	1	100	0.6%	4.08 [0.45 , 37.18]	? ? + + ? ?
Karaki 2002	9	80	10	82	5.3%	0.91 [0.35 , 2.38]	+ - + + ? -
Kolibianakis 2004	15	226	20	234	11.1%	0.76 [0.38 , 1.53]	- - + + ? -
Van der Auwera 2002	9	70	9	66	4.9%	0.93 [0.35 , 2.52]	+ ? + + ? ?
Subtotal (95% CI)		**619**		**631**	**28.1%**	**1.07 [0.71 , 1.59]**	
Total events:	54		52				
Heterogeneity: Chi² = 3.57, df = 5 (P = 0.61); I² = 0%							
Test for overall effect: Z = 0.32 (P = 0.75)							
Total (95% CI)		**2095**		**2113**	**100.0%**	**1.12 [0.90 , 1.38]**	
Total events:	209		188				
Heterogeneity: Chi² = 27.80, df = 21 (P = 0.15); I² = 24%							
Test for overall effect: Z = 1.02 (P = 0.31)							
Test for subgroup differences: Chi² = 0.13, df = 2 (P = 0.94), I² = 0%							

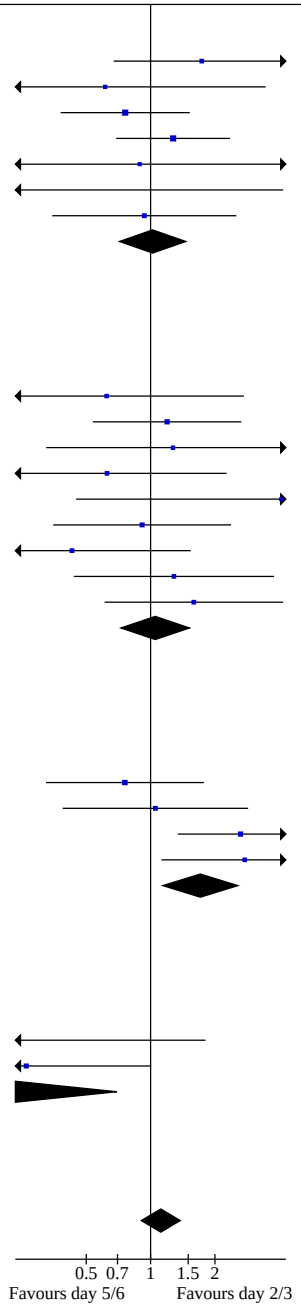


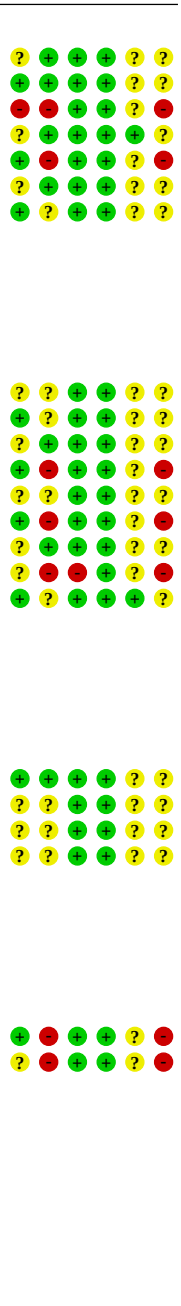
Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.4. Comparison 4: Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer, Outcome 4: Multiple pregnancy rate per couple: grouped by day of randomisation

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
4.4.1 randomised start of cycle						
Emiliani 2003	12	82	8	89	4.0%	1.74 [0.67 , 4.49]
Hreinsson 2004	2	64	4	80	2.1%	0.61 [0.11 , 3.46]
Kolibianakis 2004	15	226	20	234	11.1%	0.76 [0.38 , 1.53]
Levi-Setti 2018	26	194	21	194	11.0%	1.27 [0.69 , 2.35]
Levitas 2004	2	23	3	31	1.4%	0.89 [0.14 , 5.81]
Papanikolaou 2006	0	175	2	176	1.5%	0.20 [0.01 , 4.17]
Van der Auwera 2002	9	70	9	66	4.9%	0.93 [0.35 , 2.52]
Subtotal (95% CI)		**834**		**870**	**36.0%**	**1.02 [0.71 , 1.46]**
Total events:	66		67			
Heterogeneity: Chi² = 3.88, df = 6 (P = 0.69); I² = 0%						
Test for overall effect: Z = 0.12 (P = 0.90)						
4.4.2 randomised on day of OPU or day 1						
Aziminekoo 2015	3	57	5	61	2.8%	0.62 [0.14 , 2.73]
Coskun 2000	15	100	13	101	6.7%	1.19 [0.54 , 2.66]
Fernandez-Shaw 2015	5	60	4	60	2.2%	1.27 [0.32 , 4.99]
Frattarelli 2003	5	29	7	28	3.6%	0.63 [0.17 , 2.27]
Hatirnaz 2017	4	101	1	100	0.6%	4.08 [0.45 , 37.18]
Karaki 2002	9	80	10	82	5.3%	0.91 [0.35 , 2.38]
Levron 2002	4	46	8	44	4.5%	0.43 [0.12 , 1.54]
Rienzi 2002	9	50	7	48	3.5%	1.29 [0.44 , 3.78]
Yang 2018	11	300	7	300	4.1%	1.59 [0.61 , 4.17]
Subtotal (95% CI)		**823**		**824**	**33.3%**	**1.05 [0.73 , 1.52]**
Total events:	65		62			
Heterogeneity: Chi² = 5.55, df = 8 (P = 0.70); I² = 0%						
Test for overall effect: Z = 0.27 (P = 0.79)						
4.4.3 randomised on day 2 to 3						
Bungum 2003	13	61	15	57	7.4%	0.76 [0.32 , 1.77]
Elgindy 2011	12	59	8	41	4.6%	1.05 [0.39 , 2.86]
Kaur 2014	32	150	14	150	6.7%	2.63 [1.34 , 5.17]
Papanikolaou 2005	18	80	8	84	3.7%	2.76 [1.12 , 6.77]
Subtotal (95% CI)		**350**		**332**	**22.3%**	**1.71 [1.14 , 2.56]**
Total events:	75		45			
Heterogeneity: Chi² = 7.08, df = 3 (P = 0.07); I² = 58%						
Test for overall effect: Z = 2.60 (P = 0.009)						
4.4.4 day of randomisation unstated						
Livingstone 2002	0	30	4	29	2.7%	0.09 [0.00 , 1.81]
Motta 1998	3	58	10	58	5.7%	0.26 [0.07 , 1.01]
Subtotal (95% CI)		**88**		**87**	**8.5%**	**0.21 [0.06 , 0.70]**
Total events:	3		14			
Heterogeneity: Chi² = 0.40, df = 1 (P = 0.53); I² = 0%						
Test for overall effect: Z = 2.55 (P = 0.01)						
Total (95% CI)		**2095**		**2113**	**100.0%**	**1.12 [0.90 , 1.38]**
Total events:	209		188			
Heterogeneity: Chi² = 27.80, df = 21 (P = 0.15); I² = 24%						
Test for overall effect: Z = 1.02 (P = 0.31)						
Test for subgroup differences: Chi² = 12.00, df = 3 (P = 0.007), I² = 75.0%						





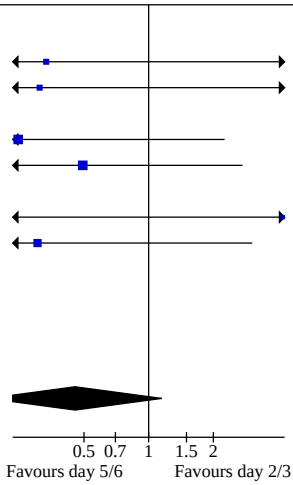
Risk of bias legend

(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.5. Comparison 4: Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer, Outcome 5: High-order pregnancies (more than 2 gestational sacs) per couple

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Bungum 2003	0	61	0	57		Not estimable
Coskun 2000	0	100	1	101	10.4%	0.33 [0.01 , 8.28]
Frattarelli 2003	0	29	1	28	10.5%	0.31 [0.01 , 7.95]
Hreinsson 2004	0	64	0	80		Not estimable
Karaki 2002	1	80	4	82	27.4%	0.25 [0.03 , 2.26]
Kaur 2014	2	150	4	150	27.7%	0.49 [0.09 , 2.73]
Kolibianakis 2004	0	226	0	234		Not estimable
Levitas 2004	1	23	0	31	2.8%	4.20 [0.16 , 107.89]
Levron 2002	1	46	3	44	21.1%	0.30 [0.03 , 3.04]
Papanikolaou 2005	0	80	0	84		Not estimable
Papanikolaou 2006	0	175	0	176		Not estimable
Rienzi 2002	0	50	0	48		Not estimable
Van der Auwera 2002	0	70	0	66		Not estimable
Total (95% CI)		**1154**		**1181**	**100.0%**	**0.45 [0.18 , 1.15]**
Total events:	5		13			

Odds Ratio M-H, Fixed, 95% CI



Risk of Bias: A B C D E F

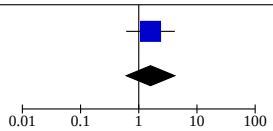
Heterogeneity: Chi² = 2.31, df = 5 (P = 0.80); I² = 0%
Test for overall effect: Z = 1.66 (P = 0.10)
Test for subgroup differences: Not applicable

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Analysis 4.6. Comparison 4: Blastocyst- versus cleavage-stage transfer: multiple pregnancy following fresh transfer, Outcome 6: Multiple pregnancy rate per couple: TLS (with algorithm) cleavage stage versus conventional blastocyst stage

Study or Subgroup	Blastocyst stage Events	Blastocyst stage Total	TLS (with algorithm) cleavage stage Events	TLS (with algorithm) cleavage stage Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Yang 2018	11	300	7	300	100.0%	1.59 [0.61 , 4.17]
Total (95% CI)		**300**		**300**	**100.0%**	**1.59 [0.61 , 4.17]**
Total events:	11		7			

Odds Ratio M-H, Fixed, 95% CI



Risk of Bias: A B C D E F

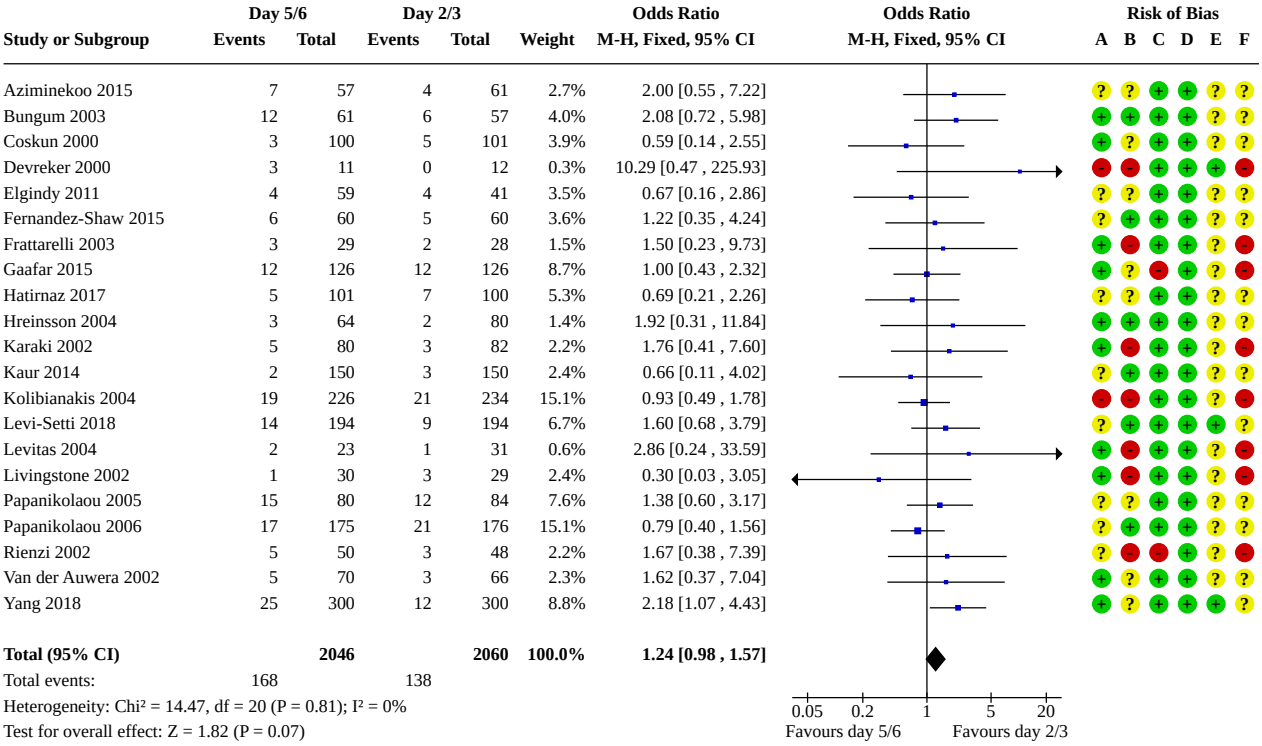
Heterogeneity: Not applicable
Test for overall effect: Z = 0.95 (P = 0.34)
Test for subgroup differences: Not applicable

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 5. Blastocyst- versus cleavage-stage transfer: miscarriage rate following fresh transfer

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.1 Miscarriage rate per couple	21	4106	Odds Ratio (M-H, Fixed, 95% CI)	1.24 [0.98, 1.57]

Analysis 5.1. Comparison 5: Blastocyst- versus cleavage-stage transfer: miscarriage rate following fresh transfer, Outcome 1: Miscarriage rate per couple



Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Aziminekoo 2015	7	57	4	61	2.7%	2.00 [0.55 , 7.22]
Bungum 2003	12	61	6	57	4.0%	2.08 [0.72 , 5.98]
Coskun 2000	3	100	5	101	3.9%	0.59 [0.14 , 2.55]
Devreker 2000	3	11	0	12	0.3%	10.29 [0.47 , 225.93]
Elgindy 2011	4	59	4	41	3.5%	0.67 [0.16 , 2.86]
Fernandez-Shaw 2015	6	60	5	60	3.6%	1.22 [0.35 , 4.24]
Frattarelli 2003	3	29	2	28	1.5%	1.50 [0.23 , 9.73]
Gaafar 2015	12	126	12	126	8.7%	1.00 [0.43 , 2.32]
Hatirnaz 2017	5	101	7	100	5.3%	0.69 [0.21 , 2.26]
Hreinsson 2004	3	64	2	80	1.4%	1.92 [0.31 , 11.84]
Karaki 2002	5	80	3	82	2.2%	1.76 [0.41 , 7.60]
Kaur 2014	2	150	3	150	2.4%	0.66 [0.11 , 4.02]
Kolibianakis 2004	19	226	21	234	15.1%	0.93 [0.49 , 1.78]
Levi-Setti 2018	14	194	9	194	6.7%	1.60 [0.68 , 3.79]
Levitas 2004	2	23	1	31	0.6%	2.86 [0.24 , 33.59]
Livingstone 2002	1	30	3	29	2.4%	0.30 [0.03 , 3.05]
Papanikolaou 2005	15	80	12	84	7.6%	1.38 [0.60 , 3.17]
Papanikolaou 2006	17	175	21	176	15.1%	0.79 [0.40 , 1.56]
Rienzi 2002	5	50	3	48	2.2%	1.67 [0.38 , 7.39]
Van der Auwera 2002	5	70	3	66	2.3%	1.62 [0.37 , 7.04]
Yang 2018	25	300	12	300	8.8%	2.18 [1.07 , 4.43]
Total (95% CI)		**2046**		**2060**	**100.0%**	**1.24 [0.98 , 1.57]**
Total events:	168		138			

Heterogeneity: Chi² = 14.47, df = 20 (P = 0.81); I² = 0%
Test for overall effect: Z = 1.82 (P = 0.07)
Test for subgroup differences: Not applicable

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

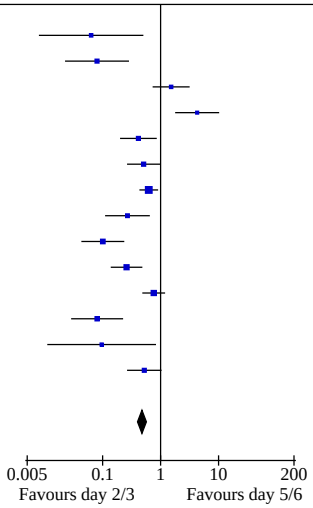
Comparison 6. Blastocyst- versus cleavage-stage transfer: embryo freezing rate per couple

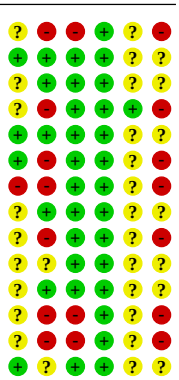
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6.1 Embryo freezing per couple	14	2292	Odds Ratio (M-H, Fixed, 95% CI)	0.48 [0.40, 0.57]

Analysis 6.1. Comparison 6: Blastocyst- versus cleavage-stage transfer: embryo freezing rate per couple, Outcome 1: Embryo freezing per couple

Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Brugnon 2010	42	55	51	52	3.4%	0.06 [0.01 , 0.50]
Bungum 2003	36	61	54	57	6.3%	0.08 [0.02 , 0.28]
Fernandez-Shaw 2015	39	60	33	60	3.2%	1.52 [0.73 , 3.17]
Gardner 1998a	29	45	14	47	1.3%	4.27 [1.78 , 10.24]
Hreinsson 2004	15	64	34	80	6.3%	0.41 [0.20 , 0.86]
Karaki 2002	22	80	35	82	6.9%	0.51 [0.26 , 0.98]
Kolibianakis 2004	114	226	145	234	19.3%	0.62 [0.43 , 0.91]
Levron 2002	12	46	25	44	5.2%	0.27 [0.11 , 0.65]
Motta 1998	15	58	45	58	9.1%	0.10 [0.04 , 0.24]
Pantos 2004	16	81	79	162	11.6%	0.26 [0.14 , 0.48]
Papanikolaou 2006	115	175	126	176	11.8%	0.76 [0.48 , 1.20]
Rienzi 2002	18	50	42	48	7.5%	0.08 [0.03 , 0.23]
Ten 2011	20	28	26	27	2.1%	0.10 [0.01 , 0.83]
Van der Auwera 2002	26	70	35	66	6.2%	0.52 [0.26 , 1.04]
Total (95% CI)		**1099**		**1193**	**100.0%**	**0.48 [0.40 , 0.57]**
Total events:	519		744			

Heterogeneity: Chi² = 82.95, df = 13 (P < 0.00001); I² = 84%
Test for overall effect: Z = 8.25 (P < 0.00001)
Test for subgroup differences: Not applicable



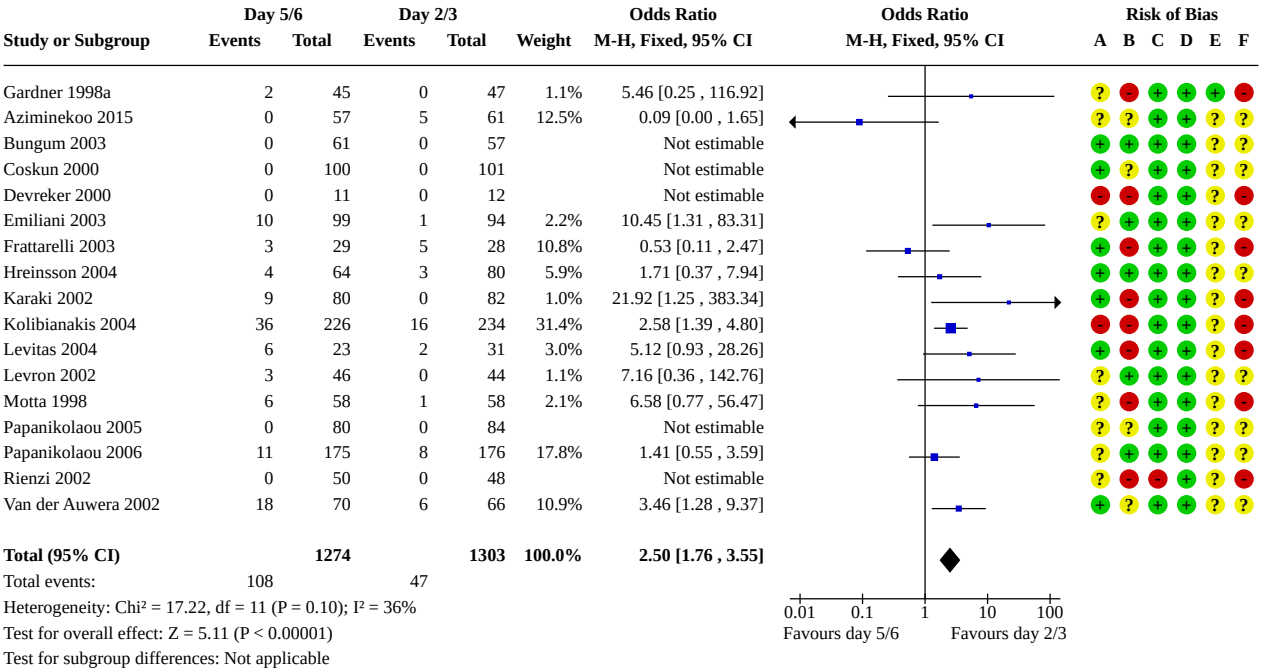


Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

Comparison 7. Blastocyst- versus cleavage-stage transfer: failure rate to transfer embryos (per couple)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
7.1 Failure to transfer any embryos (per couple)	17	2577	Odds Ratio (M-H, Fixed, 95% CI)	2.50 [1.76, 3.55]

Analysis 7.1. Comparison 7: Blastocyst- versus cleavage-stage transfer: failure rate to transfer embryos (per couple), Outcome 1: Failure to transfer any embryos (per couple)



Study or Subgroup	Day 5/6 Events	Day 5/6 Total	Day 2/3 Events	Day 2/3 Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Gardner 1998a	2	45	0	47	1.1%	5.46 [0.25 , 116.92]
Aziminekoo 2015	0	57	5	61	12.5%	0.09 [0.00 , 1.65]
Bungum 2003	0	61	0	57		Not estimable
Coskun 2000	0	100	0	101		Not estimable
Devreker 2000	0	11	0	12		Not estimable
Emiliani 2003	10	99	1	94	2.2%	10.45 [1.31 , 83.31]
Frattarelli 2003	3	29	5	28	10.8%	0.53 [0.11 , 2.47]
Hreinsson 2004	4	64	3	80	5.9%	1.71 [0.37 , 7.94]
Karaki 2002	9	80	0	82	1.0%	21.92 [1.25 , 383.34]
Kolibianakis 2004	36	226	16	234	31.4%	2.58 [1.39 , 4.80]
Levitas 2004	6	23	2	31	3.0%	5.12 [0.93 , 28.26]
Levron 2002	3	46	0	44	1.1%	7.16 [0.36 , 142.76]
Motta 1998	6	58	1	58	2.1%	6.58 [0.77 , 56.47]
Papanikolaou 2005	0	80	0	84		Not estimable
Papanikolaou 2006	11	175	8	176	17.8%	1.41 [0.55 , 3.59]
Rienzi 2002	0	50	0	48		Not estimable
Van der Auwera 2002	18	70	6	66	10.9%	3.46 [1.28 , 9.37]
Total (95% CI)		**1274**		**1303**	**100.0%**	**2.50 [1.76 , 3.55]**
Total events:	108		47			

Heterogeneity: Chi² = 17.22, df = 11 (P = 0.10); I² = 36%
Test for overall effect: Z = 5.11 (P < 0.00001)
Test for subgroup differences: Not applicable

Risk of bias legend
(A) Bias arising from the randomization process
(B) Bias due to deviations from intended interventions
(C) Bias due to missing outcome data
(D) Bias in measurement of the outcome
(E) Bias in selection of the reported result
(F) Overall bias

ADDITIONAL TABLES

Table 1. Culture techniques of included studies

Trial	Culture technique day 2/3	Culture technique day 5/6
Aziminekoo 2015	Sydney IVF cleavage medium, Cook	Sydney IVF blastocyst medium
Brugnon 2010	G series™ medium (Vitrolife, Sweden)	G series™ medium (Vitrolife, Sweden)
Bungum 2003	Sequential G1 Vitrolife	Sequential G1/G2 Vitrolife
Coskun 2000	Sequential MediCult	Sequential G1/G2 Vitrolife
Devreker 2000	NS	NS
Elgindy 2011	NS	NS
Emiliani 2003	In-house sequential (based on G1/G2)	In-house sequential (based on G1/G2)
Fernandez-Shaw 2015	Sequential G1 Vitrolife	Sequential G1/G2 Vitrolife
Fisch 2007	NS	NS

Table 1. Culture techniques of included studies *(Continued)*

Frattarelli 2003	NS	NS
Gaafar 2015	NS	NS
Gardner 1998a	Single Ham's F10 In-house	Sequential G1/G2 In-house
Hatirnaz 2017	Standard culture medium	Sequential G1/G2 Scandinavian IVF Sciences
Hreinsson 2004	Vitrolife IVF	Sequential G1/G2 or CCM Vitrolife
Karaki 2002	MediCult	Sequential G1/G2 Vitrolife
Kaser 2017	Global total with HSA LifeGlobal	Global total with HSA LifeGlobal
Kaur 2014	Cleavage medium	G2 Plus media
Kolibianakis 2004	Sequential G1 Vitrolife	Sequential G1/G2 Vitrolife
Levi-Setti 2018	NS	NS
Levitas 2004	NS	Sequential - G1/G2 Vitrolife
Levron 2002	NS	NS
Livingstone 2002	Sequential - Sydney IVF Cook	Sequential - Sydney IVF Cook
Motta 1998	Sequential - Irvines P1	Sequential - Irvines P1 then Blast media
Pantos 2004		
Papanikolaou 2005	Sequential - Vitrolife G1/G2 GII or GIII	Sequential - Vitrolife G1/G2 GII or GIII
Papanikolaou 2006	Assume sequential - Vitrolife G1/G2	Assume sequential - Vitrolife G1/G2
Rienzi 2002	Sequential G1 Vitrolife	Sequential G1/G2 Vitrolife
Schillaci 2002	NS	NS
Singh 2017	NS	NS
Ten 2011	NS	NS
Van der Auwera 2002	Sequential both Cook and Vitrolife	Sequential both Cook and Vitrolife
Yang 2018	Sequential media (G1.5, Vitrolife) in a Primo Vision time-lapse system (Vitrolife)	Sequential G1/G2 Vitrolife

CCM: a Vitrolife trademarked medium for blastocyst culture
IVF: in vitro fertilisation
G1/G2:sequential media from Vitrolife
NS: not stated

Table 2. Blastocyst formation and implantation rate (in day 5 to 6 transfers)

Study	Blastocyst formation rate	Implantation D2/3	Implantation D5/6	Other
Aziminekoo 2015	22.4%	21/173; 12.1%	22/152; 14.5%	
Brugnon 2010	Not stated	24/52; 46.2%	23/55; 41.8%	
Bungum 2003	55.2%	50/114; 43.9%	44/120; 36.7%	2/61 participants had only 1 blastocyst
Coskun 2000	28%	50/235; 21.3%	52/218; 23.9%	77% participants had at least 1 blastocyst
Devreker 2000	Not stated	1/34; 2.9%	8/19; 42.1%	
Elgindy 2011	97%	71/197; 36%	53/280; 19%	
Emiliani 2003	48%	57/197; 28.9%	50/168; 29.8%	
Fernandez-Shaw 2015	67.7 %	20/71; 28.1%	36/84; 42.8%	
Fisch 2007	Not stated	11/12; 92%	4/8; 50%	
Frattarelli 2003	Not stated	18/69; 26.1%	23/53; 43.4%	
Gaafar 2015	Not stated	Not stated	Not stated	
Gardner 1998a	46.5%	64/174; 36.8%	53/95; 55.8%	85% women had at least 2 blastocysts
Hatirnaz 2017	52.6%	45/95; 47.4%	43/95; 45.3%	
Hreinsson 2004	33%	29/139; 20.9%	24/114; 21.1%	2 morula replaced (one implanted). 60% pregnancy rate when top-quality blasts transferred
Karaki 2002	33%	37/291 12.7%	37/142; 26.1%	9/80 cancelled due to lack of blastocysts (unselected)
Kaser 2017	Not stated	23/56; 41.1%	26/53; 49.1%	
Kaur 2014	Not stated	66/309; 21.4%	102/290; 35.2%	
Kolibianakis 2004	50.7%	96/234; 41.0%	94/226; 41.6%	
Levi-Setti 2018	Not stated	25.67%	28.37%	
Levitas 2004	43%	4/56; 7.1%	10/24; 4.2%	Day 5-7 26% cancelled due to lack of blastocysts (poor prognosis)
Levron 2002	34.2%	53/137; 38.7%	20/99; 20.2%	6.5% cancelled due to lack of blastocysts (good prognosis)
Livingstone 2002	Not stated			
Motta 1998	Not stated	51/262; 19.5%	36/120; 30.0%	6/58 cycles cancelled D5 no blastocysts
Pantos 2004	44.6%	15.8%	15.8%	

Table 2. Blastocyst formation and implantation rate (in day 5 to 6 transfers) *(Continued)*

Papanikolaou 2005	Not stated	35/170; 20.6%	59/158; 37.3%	4/158 women had only 1 blast transferred due to lack of availability and 1 had it on request
Papanikolaou 2006	Not stated	38/156; 24%	58/149; 38.9%	Number of participants with no embryos available D3: 8 and D5: 11
Rienzi 2002	44.8%	34/96; 35.4%	38/100; 38.0%	Good prognosis
Schillaci 2002	60.3%	23/168; 13.7%	26/110; 23.6%	Unselected population nil cancellations D5
Singh 2017	Not stated	Not stated	Not stated	
Ten 2011	Not stated	21/54; 38.9%	26/56; 46.4%	Good prognosis
Van der Auwera 2002	44.7%	31/106; 29.2%	41/90; 45.6%	27% cancellation D5 (unselected population)
Yang 2018	Not stated	80/290; 62.1%	22/306; 72.5%	

Table 3. Mean number of embryos transferred

Study ID	Day 2/3	Day 5/6
Aziminekoo 2015	2.8 ± 1.1	2.6 ± 0.6
Brugnon 2010	1.0 ± 0	1.0 ± 0
Bungum 2003	2.0 ± NS	2.0 ± NS
Coskun 2000	2.3 ± 0.6	2.2 ± 0.5
Devreker 2000	2.8 ± NS	1.7 ± NS
Elgindy 2011	2.8 ± 0.4	2.0 ± 0.2
Emiliani 2003	2.1 ± 0.4	1.9 ± 0.3
Fernandez-Shaw 2015	1.5 ± 0.5	1.4 ± 0.5
Fisch 2007	1.0 ± 0	1.0 ± 0
Frattarelli 2003	3.0 ± 0.5	2.0 ± 0.2
Gaafar 2015	NS	NS
Gardner 1998a	3.7 ± 0.1	2.2 ± 0.1
Hatirnaz 2017	1.4 ± 0.6	1.4 ± 0.4
Hreinsson 2004	1.8 ± NS	1.9 ± NS
Karaki 2002	3.5 ± 0.6	2.0 ± 0.1

Table 3. Mean number of embryos transferred *(Continued)*

Kaser 2017	1.0 ± 0	1.0 ± 0
Kaur 2014	2.0 ± 0.7	1.9 ± 0.5
Kolibianakis 2004	1.9 ± 0.1	1.8 ± 0.1
Levi-Setti 2018	1.9 ± 0.4	1.8 ± 0.6
Levitas 2004	3.4 ± NS	1.9 ± NS
Levron 2002	3.1 ± 0.6	2.3 ± 0.8
Livingstone 2002	2.0 ± NS	1.0 ± NS
Motta 1998	4.6 ± NS	2.3 ± NS
Pantos 2004	4.0 ± 1.5	3.4 ± 1.1
Papanikolaou 2005	2.0 ± 0	2.0 ± 0.5
Papanikolaou 2006	1.0 ± 0	1.0 ± 0
Rienzi 2002	2.0 ± 0	2.0 ± 0
Schillaci 2002	2.8	1.8
Singh 2017	NS	NS
Ten 2011	2.0 ± NS	2.0 ± NS
Van der Auwera 2002	1.9 ± 0.3	1.9 ± 0.2
Yang 2018	1.0 ± 0	1.0 ± 0

NS - not stated

APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility specialised register search strategy

ProCite platform

Searched 20 October 2021

Keywords CONTAINS "day 2"or"day 3"or "day 3 embryo transfer" or "day 4 embryo transfer" or "cleavage stage" or "cleavage transfer" or "pronuclear morphology" or "early cleavage assessment" or "early cleavage medium" or "early cleavage status" or Title CONTAINS "day 2"or"day 3"or "day 3 embryo transfer" or "day 4 embryo transfer" or "cleavage stage" or "cleavage transfer" or "pronuclear morphology" or "early cleavage assessment" or "early cleavage medium" or "early cleavage status"

AND

Keywords CONTAINS "day 5" or "day 5 transfer" or "day 6 transfer" or "Blastocyst" or "blastocyst culture technique" or "blastocyst media" or "blastocyst stage" or "blastocyst transfer" or "morula" or "morula formation "or Title CONTAINS "day 5" or "day 5 transfer" or "day 6 transfer" or "Blastocyst" or "blastocyst culture technique" or "blastocyst media" or "blastocyst stage" or "blastocyst transfer" or "morula" or "morula formation"

(226 records)

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

Web platform

Searched 20 October 2021

#1 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES 1131

#2 MESH DESCRIPTOR Fertilization in Vitro EXPLODE ALL TREES 2134

#3 MESH DESCRIPTOR Sperm Injections, Intracytoplasmic EXPLODE ALL TREES 564

#4 MESH DESCRIPTOR Oocyte Donation EXPLODE ALL TREES 72

#5 (embryo transfer*):TI,AB,KY 4062

#6 (in vitro fertili?ation):TI,AB,KY 3629

#7 (intracytoplasmic sperm injection*):TI,AB,KY 2073

#8 ((ivf or icsi)):TI,AB,KY 7008

#9 ET:TI,AB,KY 33956

#10 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 41991

#11 (day 2):TI,AB,KY 7060

#12 (day 3):TI,AB,KY 7980

#13 48*:TI,AB,KY 106489

#14 72*:TI,AB,KY 67552

#15 cleav*:TI,AB,KY 1616

#16 pronuclear:TI,AB,KY 78

#17 day2:TI,AB,KY 162

#18 day3:TI,AB,KY 141

#19 ((early adj3 embryo*)):TI,AB,KY 160

#20 ((day two or day three)):TI,AB,KY 1005

#21 MESH DESCRIPTOR Cleavage Stage, Ovum EXPLODE ALL TREES 70

#22 #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 174669

#23 MESH DESCRIPTOR Blastocyst EXPLODE ALL TREES 182

#24 Blastocyst*:TI,AB,KY 1432

#25 ((day 5 or day 6)):TI,AB,KY 7676

#26 ((day5 or day6)):TI,AB,KY 81

#27 ((day five or day six)):TI,AB,KY 520

#28 #23 OR #24 OR #25 OR #26 OR #27 9313

#29 #10 AND #22 AND #28 801

Appendix 3. MEDLINE search strategy

OVID platform

Searched from 1946 to 20 October 2021

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ or exp oocyte donation/ (45499)
2 embryo transfer$.tw. (13414)
3 in vitro fertili?ation.tw. (24970)
4 intracytoplasmic sperm injection$.tw. (7866)
5 (ivf or icsi).tw. (29787)
6 ET.tw. (296630)
7 or/1-6 (353408)
8 day 2.tw. (23962)
9 day3.tw. (83)
10 48$.tw. (766982)
11 72$.tw. (508121)
12 cleav$.tw. (209100)
13 pronuclear.tw. (2442)
14 day 3.tw. (32480)
15 day2.tw. (60)
16 (early adj3 embryo$).tw. (29813)
17 (day two or day three).tw. (3430)
18 exp Cleavage Stage, Ovum/ (2311)
19 or/8-18 (1478615)
20 exp Blastocyst/ (28404)
21 Blastocyst$.tw. (24051)
22 (day 5 or day 6).tw. (37664)
23 (day5 or day6).tw. (46)
24 (day five or day six).tw. (2006)
25 or/20-24 (79108)
26 7 and 19 and 25 (5619)
27 randomized controlled trial.pt. (546666)
28 controlled clinical trial.pt. (94462)
29 randomized.ab. (537227)
30 placebo.tw. (228288)
31 clinical trials as topic.sh. (197761)
32 randomly.ab. (367850)
33 trial.ti. (249362)
34 (crossover or cross-over or cross over).tw. (90920)
35 or/27-34 (1433750)
36 exp animals/ not humans.sh. (4899579)
37 35 not 36 (1318057)
38 26 and 37 (406)

Appendix 4. Embase search strategy

OVID platform

Searched from 1980 to 20 October 2021

1 exp embryo transfer/ (33960)
2 exp fertilization in vitro/ (77081)
3 exp intracytoplasmic sperm injection/ (22837)
4 exp oocyte donation/ (4375)
5 embryo transfer$.tw. (21741)
6 in vitro fertili?ation.tw. (32941)
7 intracytoplasmic sperm injection$.tw. (10530)
8 (ivf or icsi).tw. (51536)
9 ET.tw. (702743)
10 or/1-9 (797670)
11 day 2.tw. (38612)
12 day3.tw. (700)
13 48$.tw. (1191910)
14 72$.tw. (802373)
15 cleav$.tw. (240374)
16 pronuclear.tw. (3034)
17 day 3.tw. (52695)
18 day2.tw. (455)

19 (early adj3 embryo$).tw. (32753)
20 (day two or day three).tw. (6490)
21 exp oocyte cleavage/ (3044)
22 or/11-21 (2197261)
23 exp BLASTOCYST/ (29149)
24 Blastocyst$.tw. (32419)
25 (day 5 or day 6).tw. (57687)
26 (day5 or day6).tw. (455)
27 (day five or day six).tw. (3242)
28 or/23-27 (95325)
29 10 and 22 and 28 (10987)
30 Clinical Trial/ (1006996)
31 Randomized Controlled Trial/ (676265)
32 exp randomization/ (92118)
33 Single Blind Procedure/ (44016)
34 Double Blind Procedure/ (185761)
35 Crossover Procedure/ (68302)
36 Placebo/ (358800)
37 Randomi?ed controlled trial$.tw. (268420)
38 Rct.tw. (43809)
39 random allocation.tw. (2224)
40 randomly allocated.tw. (39286)
41 allocated randomly.tw. (2694)
42 (allocated adj2 random).tw. (831)
43 Single blind$.tw. (27376)
44 Double blind$.tw. (217076)
45 ((treble or triple) adj blind$).tw. (1411)
46 placebo$.tw. (326910)
47 prospective study/ (718257)
48 or/30-47 (2418660)
49 case study/ (81550)
50 case report.tw. (453208)
51 abstract report/ or letter/ (1166106)
52 or/49-51 (1688536)
53 48 not 52 (2360524)
54 29 and 53 (1466)

Appendix 5. PsycINFO search strategy

OVID platform

Searched from 1806 to 20 October 2021

1 exp reproductive technology/ (1991)
2 embryo transfer$.tw. (131)
3 in vitro fertili?ation.tw. (814)
4 intracytoplasmic sperm injection$.tw. (61)
5 (ivf or icsi).tw. (651)
6 ET.tw. (150085)
7 or/1-6 (152389)
8 day 2.tw. (2055)
9 day3.tw. (10)
10 48$.tw. (78525)
11 72$.tw. (48856)
12 cleav$.tw. (4014)
13 pronuclear.tw. (15)
14 day 3.tw. (1826)
15 day2.tw. (11)
16 (early adj3 embryo$).tw. (693)
17 (day two or day three).tw. (397)
18 exp embryo/ (1818)
19 or/8-18 (133288)
20 Blastocyst$.tw. (91)

21 (day 5 or day 6).tw. (2055)
22 (day5 or day6).tw. (2)
23 (day five or day six).tw. (205)
24 or/20-23 (2350)
25 7 and 19 and 24 (16)

Appendix 6. CINAHL search strategy

EBSCO platform

Searched from 1961 to 4 April 2020. Later CINAHL output is included in the CENTRAL 20 October 2021 search output.

#	Query	Results
S20	S9 AND S14 AND S19	373
S19	S15 OR S16 OR S17 OR S18	44,772
S18	TX(day five or day six)	42,508
S17	TX blastocyst*	2,417
S16	TX morula	45
S15	(MM "Blastocyst")	1,015
S14	S10 OR S11 OR S12 OR S13	79,180
S13	TX pronuclear	61
S12	(MM "Cleavage Stage, Ovum")	53
S11	TX (day two or day three)	76,416
S10	TX cleavage	2,862
S9	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8	11,987
S8	TX intracytoplasmic sperm injection*	1,032
S7	TX embryo* N3 transfer*	3,446
S6	TX ovar* N3 hyperstimulat*	963
S5	TX ovari* N3 stimulat*	1,149
S4	TX IVF or TX ICSI	5,682
S3	(MM "Fertilization in Vitro")	3,898
S2	TX vitro fertilization	7,938
S1	TX vitro fertilisation	7,938

Appendix 7. ClinicalTrials.gov search strategy

Web platform

Searched 4 April 2020

"embryo" and "day" and "transfer" (262 hits)

OR

"blastocyst" and "transfer" (87 hits)

Appendix 8. WHO portal (ICTRP) search strategy

Web platform

Searched 4 April 2020

"embryo" and "day" and "transfer" (3 hits)

OR

"blastocyst" and "transfer" (24 hits)

WHAT'S NEW

Date	Event	Description
11 May 2020	New search has been performed	Updated with five new RCTs (Hatirnaz 2017; Kaser 2017; Levi-Setti 2018; Singh 2017; Yang 2018). Cindy Farquhar has been removed from the list of authors and Agustin Ciapponi and Simone Cornelisse have been added to the list of authors for this update.
11 May 2020	New citation required but conclusions have not changed	The addition of five new RCTs has not led to a change in the conclusions of this review.

HISTORY

Protocol first published: Issue 2, 2000
Review first published: Issue 2, 2002

Date	Event	Description
5 May 2016	New citation required and conclusions have changed	Updated May-June 2016
3 May 2016	New search has been performed	Updated with four new RCTs (Aziminekoo 2015; Fernandez-Shaw 2015; Gaafar 2015; Kaur 2014)
28 May 2013	Amended	Correction of author order
21 February 2012	New citation required but conclusions have not changed	5 new studies have been added. There are no changes to the conclusions reported in the 2007 update
21 February 2012	New search has been performed	History of this review: the review was first published in *The Cochrane Library* in 2000 with 10 RCTs, and a journal paper version was published in Human Reproduction in 2003 with an additional four RCTs. The authors were Debbie Blake, Michelle Proctor, Neil Johnson and David Olive. There was no evidence for a

Date	Event	Description
		difference in pregnancy rate and only one trial reported live birth rates In the 2005 update, seven RCTs from the 2000 review were excluded (for quasi-randomisation or per cycle data only or other study design problems) and 13 new RCTs were added. In addition, the outcomes in Metaview were reconfigured. Cindy Farquhar and Quirine Lamberts assisted with the update and were added as authors. Some protocol changes were made to the outcome measures and to the sensitivity analysis (day of randomisation) and subgroup analyses (prognosis). More included trials reported live birth outcomes but there was still no evidence of a difference in success rates The 2007 update had two new trials added, to bring the total to 18. There was a new subcategory for single embryo transfer. This update was performed by Debbie Blake, Neil Johnson and Cindy Farquar. It reported for the first time a significant difference in live birth and pregnancy outcomes in favour of blastocyst culture In the 2012 update led by Demian Glujovsky with the assistance of Cindy Farquhar and Debbie Blake, five new studies were added (Brugnon 2010; Elgindy 2011; Fisch 2007; Pantos 2004; Ten 2011)
29 August 2011	Amended	Plain Language Summary corrected
17 November 2010	Amended	New search strategies performed
11 November 2010	Amended	New author
23 July 2007	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

Debbie Blake: for the initial review and updates to 2005: took the lead in writing the protocol and review; performed initial searches of databases for trials; involved in selecting trials for inclusion; performed independent data extraction and quality assessment of the included trials; and was responsible for statistical analysis and interpretation of the data. Also contributed to the final analysis and text of the 2012, 2016, and 2022 updates.

Demián Glujovsky: for the 2012, 2016 and 2021 updates: took the lead in writing the update of the review; performed new searches of databases for trials; involved in selecting trials for inclusion; performed independent data extraction and quality assessment of the included trials; and was responsible for statistical analysis and interpretation of the data in the update.

Andrea Quinteiro Retamar: for the 2016 and 2021 updates: involved in selecting trials for inclusion; and performed independent data extraction and quality assessment of the included trials.

Cristian Alvarez Sedo: for the final analysis and text of the 2016 update, and for the 2021 update: involved in selecting trials for inclusion; and performed independent data extraction and quality assessment of the included trials.

Simone Cornelisse: for the 2021 update: involved in the risk of bias assessment, data extraction and final analysis and text.

Agustín Ciapponi: for the 2021 update: involved in the risk of bias assessment, and final analysis and text.

DECLARATIONS OF INTEREST

Demián Glujovsky is part of medical staff of a fertility clinic and undertakes private practice within those premises.

Andrea Quinteiro Retamar is the egg donor coordinator of a fertility clinic and undertakes private practice within those premises.

Simone Cornelisse is the lead author of excluded study Cornelisse 2018 and took no part in assessing it for this review.

Cristian Alvarez Sedo, Agustín Ciapponi, and Deborah Blake have no conflicts of interest to declare.

SOURCES OF SUPPORT

Internal sources

- Cindy Farquhar, New Zealand

  University of Auckland

External sources

- None, Other

  None

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

- We included only RCTs and excluded quasi-RCTs (2007 update).
- We excluded couples or women where frozen-thawed cycle results were shown, but no data were available from the fresh cycle (2016 update).
- We added cumulative pregnancy rate to the primary outcomes (previously a secondary outcome) (2007 update).
- We restricted subgroup and sensitivity analyses to specific clinical outcomes: live birth, cumulative pregnancy, clinical pregnancy, and multiple pregnancy (2007 update).
- We added a post hoc subgroup analysis according to freezing technique, to investigate statistical heterogeneity for the outcome cumulative pregnancy rate (2016 update).
- We shortened the objectives to the current wording (2012 update).
- We included a subgroup analysis comparing time-lapse system screening (TLS screening) on cleavage-stage transfer versus blastocyst-stage transfer (with and without time-lapse screening), because TLS was not available when this protocol was originally published and the combination of stage of embryo and TLS could provide a different result (2022 update).
- We assessed the risk of bias with the Cochrane risk of bias 2 (RoB 2) tool (Sterne 2019, 2022 update).
- We excluded cluster-RCTs because these types of trials are not appropriate in this context (2022 update).

NOTES

Conflicts of interest added.

INDEX TERMS

Medical Subject Headings (MeSH)

*Blastocyst; Cleavage Stage, Ovum [*transplantation]; Embryo Transfer [*methods]; Live Birth [*epidemiology]; Pregnancy Outcome; *Pregnancy Rate; Pregnancy, Multiple; Randomized Controlled Trials as Topic

MeSH check words

Female; Humans; Pregnancy