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CLEAVAGE STAGE VERSUS BLASTOCYST STAGE EMBRYO TRANSFER IN ASSISTED REPRODUCTIVE TECHNOLOGY

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ABSTRACT

Background

Recent advances in cell culture media have led to a shift in IVF practice from early cleavage embryo transfer to blastocyst stage transfer. The rationale for blastocyst culture is to improve both uterine and embryonic synchronicity and self selection of viable embryos thus resulting in higher implantation rates.

Objective

To determine if blastocyst stage embryo transfers (ETs) affect live birth rate and associated outcomes compared with cleavage stage ETs and to investigate what factors may influence this.

Criteria for considering studies for this review

Cochrane Menstrual Disorders and Subfertility Group Specialised Register of controlled trials, Cochrane Controlled Trials Register (CENTRAL) (The Cochrane Library), MEDLINE, EMBASE and Bio extracts. The last search date was January 2007.

Selection criteria

Trials were included if they were randomised and compared the effectiveness of early cleavage versus blastocyst stage transfers.

Data collection and analysis

Of the 50 trials that were identified, 18 randomised controlled trials (RCTs) met the inclusion criteria and were reviewed. The primary outcome was rate of live birth. Secondary outcomes were rates per couple of clinical pregnancy, multiple pregnancy, high order pregnancy, miscarriage, failure to transfer embryos and cryopreservation. Quality assessment, data extraction and meta-analysis were performed following Cochrane guidelines.

Main results

Evidence of a significant difference in live-birth rate per couple between the two treatment groups was detected in favour of blastocyst culture (9 RCTs; OR 1.35, 95% CI 1.05 to 1.74 (Day 2/3: 29.4% versus Day 5/6: 36.0%)). This was particularly for trials with good prognosis patients, equal number of embryos transferred (including single embryo transfer) and those in which the randomisation took place on Day 3. Rates of embryo freezing per couple was significantly higher in Day 2 to 3 transfers (9 RCTs; OR 0.45, 95% CI 0.36 to 0.56). Failure to transfer any embryos per couple was significantly higher in the Day 5 to 6 group (16 RCTs; OR 2.85, 95% CI 1.97 to 4.11 (Day 2/3: 2.8% versus Day 5/6: 8.9%)) but was not significantly different for good prognosis patients (9 RCTs; OR 1.50, 95% CI 0.79 to 2.84).

Authors' conclusions

This review provides evidence that there is a significant difference in pregnancy and live birth rates in favour of blastocyst transfer with good prognosis patients with high numbers of eight-cell embryos on Day three being the most favoured in subgroup for whom there is no difference in cycle cancellation. There is emerging evidence to suggest that in selected patients, blastocyst culture maybe applicable for single embryo transfer.

PLAIN LANGUAGE SUMMARY

Keeping embryos a few days longer in the laboratory before transfer has not been shown to lead to more pregnancies than regular IVF

In vitro fertilisation (IVF) is fertilisation (egg and sperm creating an embryo) in a laboratory (in a 'test tube'). With regular IVF, embryos are transferred into the woman's uterus two to three days after fertilisation (at the cleavage stage). An alternative technique delays transferal until five to six days after fertilisation (at blastocyst stage). This may be better timing and allow choice of more viable embryos. The review of trials found evidence that more women will have a pregnancy and baby with blastocyst transfer than with regular IVF. There was however, a higher risk that a women would have fewer embryos to freeze and no embryos available for transfer.

WHAT'S NEW

What's new

Last assessed as up-to-date: 22 July 2007.

Date	Event	Description
20 September 2010	Amended	Contact details updated.

BACKGROUND

The fledgling era of in vitro fertilisation (IVF) from 1980 to the mid 1990s, was characterised by relatively static success rates of around 20% pregnancy rates. The past decade however, has given rise to exciting advances in ovarian stimulation, cell culture and embryo transfer techniques that have culminated in significant overall improvements in successful pregnancies. This is evident in the annual statistical reports from different areas of the globe. One such report for example, has demonstrated a doubling of pregnancy rate per embryo transfer cycle from 1994 to 2003 despite a decrease in the mean number of embryos transferred (Waters 2006).

The contribution of embryo culture to these improvements is the focus of this Cochrane review of cleavage stage versus blastocyst stage transfer. With the introduction of a variety of commercial preparations of sequential media in the late 1990s, the IVF industry witnessed an explosion of worldwide interest in blastocyst culture, with most clinics conducting research into its application in their own setting. As a result a substantial volume of publications followed. These included conflicting trials and debates about the merits and drawbacks of extended culture. A lack of strong consensus about the best practice for blastocyst culture has not aided by the fact that many of the trials were not prospectively randomised and/or were underpowered. The need for an evidence-based approach using meta-analysis of small trials was, therefore, required to assist in deciphering the overall affect of blastocyst culture to help identify patient subsets and practices that might best benefit from this approach.

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 because of: a) the low developmental rate of embryos cultured past this stage and b) unlike other primates, human embryos have the 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 (Menezo 1990 ; Van Blerkom 1993 ; Yeung 1992). The most dramatic was the understanding that the in vitro environment in which an early cleavage stage embryo grows best in is different from that of 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 RNA messages of maternal origin (Braude 1998). With the application of stage-specific media, there have been reports of blastocyst development and implantation rates as high as 60% to 65% (Schoolcraft 2001).

There are two central arguments why blastocyst culture has purported advantages over traditional cleavage stage transfer. Firstly, it has long been recognised that it is 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 this may cause stress on the embryo and result in reduced implantation potential (Gardner 1996). There is also evidence of a significant reduction in uterine pulsatility at the time when blastocysts are transferred and therefore less chance that embryos can be expelled (Fanchin 2001).

The second argument for blastocyst culture is in their innately higher implantation potential compared with early cleavage 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 is limited. Many published studies that debate the correlation of morphological features with pregnancy rates can be found in the literature (Sjoblom 2006 ; Palmstierna 1998 ; Puissant 1987 ; Rijnders 1998 ;

Roseboom 1995 ; Steer 1992 ; Scott 2000). It is now understood that a disturbingly large proportion of morphologically normal Day 3 embryos are chromosomally abnormal, 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 embryos cannot ensure the absence of chromosomal abnormality (Magli 2000), Staessen 2004 have demonstrated that, at least in women older than 36y years , the incidence can be reduced from 59% on Day 3 to 35% in Day 5 blastocysts.

Arguments against blastocyst culture are largely related to this process of self selection. Couples undergoing blastocyst culture are expected to have a higher incidence of: a) being cancelled due to failed embryo development (Marek 1999) and b) having 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. While this approach presents information about the comparative number of pregnancy opportunities that each treatment approach can provide a couple, it does not take into account the implantation potential for fresh and thawed embryos. An alternative efficacy formula was developed by Schoolcraft (Schoolcraft 2001) that takes this into account. Using the formula (mean number of embryos transferred multiplied by implantation rate) + (mean number of embryos cryopreserved multiplied by implantation rate) - (1 minus cancellation rate), this group of researchers were able to demonstrate a 19% greater efficiency in blastocyst culture compared with early cleavage stage transfers. Disappointingly, such utilisation and efficiency analysis is not possible in the majority of RCTs due to the lack of thaw cycle outcomes within a reasonable time frame for trials.

There is also the question of how scientists can be so certain that any given Day 3 embryo has the ability to become a viable blastocyst in vivo, but not in vitro. Based on the very wide range of basculation rates reported, there is evidence that not all clinical and laboratory environments are equal, despite identical sequential media being used. This is an obvious compounding factor when performing a meta-analysis. Variables such as number of incubators, gas mix, culture ware quality control (Gardenr 2003b), and the superovulation regimen (Bukulmez 2007 ; Schoolcraft 2001) have all been reported to have an impact of blastocyst culture outcomes. For this reason there maybe an argument for introducing a minimum Day 2 to 3 implantation rate (that is approximately 20%) for trial inclusion criteria, but this may differ depending on the overall patient prognosis for each trial (for example Devreker 2000).

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). Monozygotic twinning is frequently reported above 1% in assisted reproductive (ART) cycles (Sills 2000), while the background rate of MZ 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 embryo has been implicated as one of the interventions associated with an increase in MZ 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 it's incidence (Moayeri 2007). Similarly, as the underlying mechanisms that lead to an altered sex ratio is elucidated, whether it be media constituents or simply the morphological selection criteria (Luna 2007), the imbalance may also be rectified.

The niche of blastocyst culture is unfolding against a backdrop of evolving regulatory and community pressures. Until relatively recently it was been widely accepted that in order to achieve acceptable pregnancy rates, several embryos were required to be replaced in the uterus (Edwards 1983). However, pressure on the assisted reproductive technology (ART) industry to reduce the multiple-birth rate and high order birth rates (more than two fetal sacs) over the past decade has seen a steady decline in the number of embryos transferred. 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 for improving the assessment of the quality of embryos. Performing blastocyst culture may offer one of those mechanisms (Gardner 2004 ; Milki 2004).

Advocates of blastocyst culture are confident that only the most viable embryos will survive the extended culture to Day 5 to 6. This would result in a higher probability of implantation and require fewer embryos to be transferred, thereby lowering the costly multiple-birth rate (Jones 1999 , Gardner 1998b). Critics of the approach express concern at the increased incidence of women failing to have embryos available for transfer (Marek 1999), although the day of patient recruitment into the blastocyst program is crucial to this argument. 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, eight-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. And most importantly, does blastocyst culture achieve the primary aim of providing the subfertile couple with a normal, healthy baby?

OBJECTIVES

The primary aim of this review was to compare the outcomes of cleavage stage with blastocyst stage embryo transfers in subfertile couples.

METHODS OF THE REVIEW

CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

Types of studies

All randomised controlled trials (RCTs) comparing early-stage embryo transfers (Day 2 to 3) with blastocyst stage

transfers (Day 5 to 6) were considered. Quasi-randomised controlled trials (trials that stated they used random allocation but allocation was, for example, the day of the week, which is not truly random) were excluded and withdrawn from the previous versions of the review.

Types of participants

Inclusion criteria
Couples undergoing in vitro fertilization (IVF) or (ICSI) for therapeutic reasons or for oocyte donation within all patient prognosis groups.

Patient prognosis groups (patient subsets or populations) is a term used to describe the categories that couples are assigned to based on several factors such as their age, type of infertility, ovarian response to the superovulation drugs and number of previous failed attempts. See the subgroup analysis section in the 'Methods of the review' below for the categories.

Exclusion criteria
Couples whose IVF or ICSI cycle, or both, has involved in vitro matured oocytes or pre-implantation diagnosis.

Types of intervention

Inclusion criteria
Single and sequential media culture methods for IVF and ICSI where the embryos were grown for between 2 to 6 days in vitro prior to embryo transfer and where Day 2 to 3 transfers were compared with Day 5 to 6 transfers.

Exclusion criteria
Co-culture methods.

Types of outcome measures

Primary outcome

Live-birth rate per couple (number of live-births per couple).

Secondary outcomes

Clinical pregnancy rate per couple: number of couples achieving a clinical pregnancy (defined by the demonstration of fetal heart activity on ultrasound scan).

Multiple-pregnancy rate per couple: number of multiple pregnancies per couple.

High order multiple-pregnancy rate per couple: three or more fetal heartbeats per couple.

Miscarriage rate: number of occurrences per couple and per pregnant woman.

Embryo freezing rate: number of couples that had embryos frozen per couple.

Failure to have any embryo transfer rate: percentage of couples that did not have an embryo transfer.

Additional outcomes not appropriate for statistical pooling

Data per cycle or per embryo transfer (ET) or per ovum pick up (OPU) were not able to be pooled (Vail 2003). However, due to the frequency that this form of data is reported in the literature they have been entered into the 'Table of comparisons' for the following outcomes:
i) live births per OPU and ET;
ii) clinical pregnancy rate per OPU and ET;
iii) implantation rate; the number of fetal sacs divided by the number of embryos transferred.

SEARCH METHODS FOR IDENTIFICATION OF STUDIES

Search methods for identification of studies

All reports that described (or might have described) randomised controlled trials comparing early-stage embryo transfer and blastocyst stage transfer in the treatment of subfertility, using IVF or ICSI, were obtained using the search strategy developed by the Menstrual Disorders and Subfertility Group.

We searched the Cochrane Menstrual Disorders and Subfertility Group Specialised Register of controlled trials, the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library), MEDLINE (1966 to Jan 2007), EMBASE (1980 to Jan 2007) and Bio extracts using the Cochrane highly sensitive search strategy and the following keywords: blastocyst/embryo or embryo transfer/cleavage stage, ovum/culture media or embryo culture/sequential culture/co-culture. See Appendix 1 .

The National Research Register (NRR), a register of ongoing and recently completed research projects funded by or of interest to the United Kingdom's National Health Service, entries from the Medical Research Council Clinical Trials Register, and details on reviews in progress collected by the NHS Centre for Reviews and Dissemination, were searched. The Clinical Trials register (clinicaltrials.gov), a registry of both federally and privately funded US and other clinical trials, was also searched.

The search was performed on titles, abstracts and keywords of the listed articles. The citation lists of relevant publications review articles, and included studies were also searched. Relevant conference abstracts were hand searched.

DATA COLLECTION AND ANALYSIS

Data collection and analysis

Two review authors (DB, NJ or CF) performed the selection of trials for inclusion in the review after employing the search strategy described previously. Excluded articles were detailed in the Characteristics of excluded studies and Included trials were analysed for the quality criteria and methodological details outlined below see. This information is presented in a Characteristics of included studies and provides a context for assessing the reliability of results.

Trial characteristics
1. Allocation of concealment:
a) a third party (telephone) or trialist (computer, sealed envelope or register);
b) not stated.

2. Method of randomisation:
a) computer generated;
b) random numbers table;
c) not stated; and
d) time of randomisation

3. Study design:
a) presence or absence of blinding;
b) duration of follow up;
c) type of follow up.

4. Size of study, number of women:
a) recruited;
b) randomised;
c) excluded;
d) analysed;
e) lost to follow up.

5. Study setting:
a) single-centre or multi-centre;
b) location;
c) timing.

6. Analyses:
a) power calculation;
b) whether or not by intention to treat.

7. Indication or criteria for blastocyst culture:
a) diagnostic and therapeutic;
b) therapeutic.

Characteristics of the study participants
1. Baseline characteristics:
a) age;
b) primary or secondary infertility;
c) cause and duration of infertility;
d) previous treatment.

2. Other subgroup criteria, women:
a) undergoing IVF or ICSI, or both;
b) over the age of 37 undergoing IVF or ICSI, or both;
c) with high basal follicle stimulating hormone (FSH) (more than 15 on Day 3) undergoing IVF or ICSI, or both;
d) with greater than 10 follicles one day prior to egg retrieval;
e) with less than five oocytes on the day of egg retrieval;
f) with repeated implantation failure (more than two failed stimulation cycles with ET).

3. Treatment characteristics:
a) fertilisation rate;
b) blastocysts rate;
c) embryo transfer policy;
d) mean number of embryos transferred;
e) pregnancy determination.

Interventions used
1. Ovarian stimulation
2. Luteal support

3. Culture medium: a) single medium; b) sequential media.
4. Culture method: a) oil overlay; b) open system; c) communal culture; d) individual culture.
5. Assisted hatching: a) enzyme; b) Tyrodes; c) laser.

Outcomes
1. Primary:
a) live birth (per couple randomised);

2. Secondary:
a) clinical pregnancy (per couple randomised);
b) multiple pregnancy (per couple randomised);
c) high order pregnancy rate (three or more fetal heart beats per couple randomised). a) miscarriage;
d) embryo freezing;
e) failure to transfer any embryos;
f) implantation

3. Additional outcomes not appropriate for statistical pooling:
a) cycle data per ovum pick up (OPU) and embryo transfer (ET)

Subgroup analysis
The following subgroup analyses were planned.

Subgroup A: studies where the policy for the number of embryos replaced was equal in both Day 2 to 3 and Day 5 to 6 groups (includes studies where there was a policy of single embryo transfer) versus studies where fewer Day 5 to 6 than Day 2 to 3 embryos were replaced.

Subgroup B: 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.

Subgroup C: studies that randomised at the start of the cycle (that is prior to ovarian stimulation) were compared with the days immediately prior and post OPU (that is day of final ultrasound scan and prior to HCG trigger up to and including the day of fertilisation check, when numbers of oocytes are anticipated).

Sensitivity analysis
The following sensitivity analyses were planned: studies that used concealment of allocation, reported were the randomisation method and the day of randomisation was considered.

Information was independently extracted on methodological quality and outcome data by two review authors (DB, NJ) using forms designed according to Cochrane guidelines. Another co-author (CF) was available to resolve any discrepancies. Additional information on trial methodology or actual original trial data were sought from the principal author of trials that appeared to meet eligibility criteria but were unclear in aspects of methodology, or where the data was in a form unsuitable for meta-analysis. Reminder correspondence was sent when a reply was not received within three weeks. Replies were received from 12 contact authors (Bungum 2003 ; Frattarelli 2003 ; Hreinsson 2004 ; Karaki 2002 ; Levitas 2004 ; Levron 2002 ; Livingstone 2002 ; Papanikolaou 2005 ; Papanikolaou 2006 ; Plachot 1999 ; Rienzi 2002 ; Utsunomiya 2004) who provided information regarding methodology and outcome data.

Statistical analyses were performed in accordance with the guidelines for statistical analysis developed by the Cochrane Menstrual Disorders and Subfertility Group. Heterogeneity between the results of different studies was examined by inspecting the scatter of data points, the overlap in their confidence intervals and more formally by checking the results of the chi squared tests. A priori, it was planned to look at the possible contribution of differences in trial design to the heterogeneity identified. Where possible, the outcomes were pooled statistically.

Where possible the data were analysed using an intention to treat analysis. We used the number of women randomised as the denominator even if the authors did not.

For dichotomous data (for example clinical pregnancy rate), results for each study were expressed as odds ratios (OR) with 95% confidence intervals and combined for meta-analysis with RevMan software using the Peto-modified Mantel-Haenzel method. The data were entered on the graphs so that in positive outcomes (for example pregnancy) points to the left of the line of no effect favour Day 5 to 6 transfer, and in negative outcomes (for example miscarriage) points to the right of the line of no effect favour Day 5 to 6 transfer.

A search for new trials is conducted bi-annually and the review updated as and when new trials to be incorporated are found.

METHODOLOGICAL QUALITY

RESULTS

Results

Description of studies

See: Characteristics of included studies ; Characteristics of excluded studies .

Fifty trials were identified as providing data comparing early cleavage stage and blastocyst stage embryo transfer outcomes, dating back to 1991. Eighteen trials met the inclusion criteria and were fully reviewed.

Excluded studies
Thirty-two studies failed to meet the inclusion criteria for reasons outlined in the table 'Characteristics of excluded studies'. In accordance with the policy of the Cochrane Menstrual Disorders and Subfertility Group, all quasi-randomised trials that had previously been included in the original Cochrane Review in 2000 have now been excluded from this update (Demylle 2000 ; Gudmundsson 1998 ; Huisman 2000 ; Levran 1999 ; Plachot 2000 ; Scholtes 1996). Janny 1993 was excluded as co-culture with animal-derived Vero cells is no longer current practice and differs significantly from the methodology in all the remaining studies. Boyarsky 2001 was excluded as more than 50% of the experimental group was allocated to Day 3 transfer. All other excluded trials failed to use a random design. Most of these trials were also either retrospective, used retrospective or self-selecting controls or lacked a control group. Where possible, data about participant selection, pregnancy rates, implantation rate, blastulation rate and the number of embryos transferred have been extracted and added to the table.

Included studies
Eighteen studies met the inclusion criteria. One of the studies had been published or presented on separate dates and both sets of data appear in the table 'Characteristics of included studies' within single entries. Motta 1998 A & B are two conference abstracts presenting different aspects of data from the same trial. The review consists of a total of 2616 couples. The size of trials ranged from 23 (Devreker 2000) to 460 couples (Kolibianakis 2004) including both Day 2 to 3 and Day 5 to 6 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 or university-based clinics. Nine countries were represented in the included studies with Belgium being the most prolific, with six studies. The countries represented were: Brazil (Motta 1998 A & B), Belgium (Devreker 2000 ; Emiliani 2003 ; Kolibianakis 2004 ; Van der Auwera 2002 ; Papanikolaou 2005 ; Papanikolaou 2006), Australia (Livingstone 2002), Israel (Coskun 2000 ; Levitas 2004 ; Levron 2002), Jordan (Karaki 2002), Sweden (Hreinsson 2004), Italy (Rienzi 2002 ; Schillaci 2002), Denmark (Bungum 2003) and USA (Frattarelli 2003 ; Gardner 1998a).

Patient selection criteria comprised three main groups: unselected patients (Emiliani 2003 ; Karaki 2002 ; Kolibianakis 2004 ; Motta 1998 A & B ; Schillaci 2002 ; Van der Auwera 2002); good prognostic factors where participants were positively selected, that is. those that would be expected to do well with blastocyst culture (Bungum 2003 ; Coskun 2000 ; Frattarelli 2003 ; Gardner 1998a ; Hreinsson 2004 ; Levron 2002 ; Livingstone 2002 ; Rienzi 2002 ; Papanikolaou 2005 ; Papanikolaou 2006); and poor prognostic factors where couples were selected who had experienced multiple failures with conventional treatment or had poor response to ovulation induction (Devreker 2000 ; Levitas 2004). Most studies recruited women aged less than 40 years of age with the exception of Gardner 1998a who had no age limit. The mean age across all the studies varied from 29 years to 34 years.

The trials that provided details on the ovarian stimulation regimen mostly reported using a similar GnRH pituitary down-regulation protocol prior to hMG/FSH administration. However the three most recent trials (Kolibianakis 2004 ; Papanikolaou 2005 ; Papanikolaou 2006) all used a GnRH antagonist to varying degrees.

Fifteen trials used sequential media, of which nine used Vitro life G1/G2 while the remaining media were combinations of brands or made in house. Three did not state the media used (in additional tables).

Freezing of embryos in both experimental groups was reported in 11 of 18 of the included trials (Bungum 2003 ; Gardner 1998a ; Hreinsson 2004 ; Karaki 2002 ; Kolibianakis 2004 ; Levron 2002 ; Motta 1998 A & B ; Papanikolaou 2005 ; Papanikolaou 2006 ; Rienzi 2002 ; 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 practiced assisted hatching but only for the Day 3 ET group.

For the Day 2 to 3 transfer groups, most transfers were on Day 3, with the exception of four trials (Devreker 2000 ; Emiliani 2003 ; Motta 1998 A & B ; Van der Auwera 2002) and Levitas 2004 that a policy of Day 2 or 3.

Risk of bias in included studies

See the 'Additional tables' for a summary of the quality of the included studies.

Allocation concealment
In seven studies the method of concealing allocation was sealed envelopes (Bungum 2003 ; Coskun 2000 ; Karaki 2002 ; Levitas 2004 ; Levron 2002 ; Livingstone 2002 ; Van der Auwera 2002). Frattarelli 2003 stated that the allocation was concealed although no details were provided. Kolibianakis 2004 stated the allocation was not concealed while Papanikolaou 2006 stated that couples were identified to physicians as group A or B. In the remaining study the method was unknown.

Method of randomisation
Eight studies used computer generated randomisation (Frattarelli 2003 ; Gardner 1998a ; Kolibianakis 2004 ; Levitas 2004 ; Livingstone 2002 ; Papanikolaou 2005 ; Papanikolaou 2006 ; Rienzi 2002), Emiliani 2003 and Hreinsson 2004 used a list and the remaining studies did not state their method of randomisation.

Blinding

The length of culture and the day of embryo transfer was different for each of the experimental groups making it impossible to blind which group a participant was in for the doctor, scientist, nurse and participant. There was no evidence to suggest that the statistician in any trial was blinded to the assignment status.

Intention to treat, withdrawals and dropouts
Only the two most recent trials stated that they performed an intention-to-treat analysis (Papanikolaou 2005 ; Papanikolaou 2006). The latter trial also performed an interim analysis and was terminated after 50% of the intended patients were enrolled due to a significant difference being detected. Identification of participants failing to have an embryo transfer was not stated, or unclear, in some trials. Coskun 2000 implied that a 100% embryo transfer rate was achieved in both Day 2 to 3 and Day 5 to 6 groups, which is unexpectedly high and is possibly explained by transferring embryos of a lesser stage when blastocysts were not available. Where the number of couples and the number of ETs were different, the number of couples was used as the denominator even when exclusions took place post randomisation, assuming no pregnancies occurred. For example, Frattarelli 2003 excluded eight couples including four for embryo quality. These eight couples were able to be added to the denominator and, therefore, an intention-to-treat analysis was possible. Emiliani 2003 excluded 10 women because of protocol violations. Livingstone 2002 excluded 20 women post randomisation on the basis of study quality and no further data on these were available. Van der Auwera 2002 excluded seven women post-randomisation as three couples randomised to Day 2 requested blastocyst transfer and four couples requested Day 2 transfer. These numbers were added to the denominator, assuming they did not conceive. For the remaining studies, the Day 5 to 6 ET rate ranged from 71% to 96%.

Power and interim analysis
Nine trials reported having performed a power analysis (Bungum 2003 ; Emiliani 2003 ; Frattarelli 2003 ; Hreinsson 2004 ; Kolibianakis 2004 ; Livingstone 2002 ; Papanikolaou 2005 ; Papanikolaou 2006 ; Van der Auwera 2002) but most were unable to achieve the level of statistical significance required with the number of couples recruited. Two exceptions were the two most recent trials (Papanikolaou 2005 ; Papanikolaou 2006) where the results of an interim analysis led to termination of the trials at the halfway point due to a significant difference being detected.

Timing of randomisation
Six trials randomised women before their response to superovulation was known, that is either prior to or early in the treatment cycle (Emiliani 2003 ; Gardner 1998a ; Kolibianakis 2004 ; Levitas 2004 ; Papanikolaou 2005 ; Van der Auwera 2002). Gardner 1998a performed randomisation on Day 8 of treatment, then accepted women into the trial on the day of the HCG trigger if they had greater than 10 follicles. Seven trials performed randomisation immediately prior or post OPU (day of trigger up to day of fertilisation check), when the number of oocytes could be anticipated or was known (Coskun 2000 ; Frattarelli 2003 ; Hreinsson 2004 ; Karaki 2002 ; Levron 2002 ; Rienzi 2002 ; Schillaci 2002). Only two trials randomised women on Day 3 when the number of high quality embryos is known (Bungum 2003 ; Papanikolaou 2006). In three trials, the timing of randomisation was not clear (Devreker 2000 ; Motta 1998 A & B ; Livingstone 2002).

Attempts were made to obtain additional information regarding all aspects of randomisation, blinding, power analysis and intention to treat from all trial authors.

Effects of interventions

Live birth per couple
Evidence of a significant difference was detected between the two treatment groups for live-birth rate per couple (9 RCTs; OR 1.35, 95% CI 1.05 to 1.74 (Day 2/3: 29.4% versus Day 5/6: 36.0%)). There was no heterogeneity detected and the I2 was 49.7%. Separate analyses showed that there was also no heterogeneity for trials where equal numbers of embryos were transferred (including single embryo transfers), with good prognosis patients and where trials were randomised on Day 3 of culture; but not for trials where more cleavage stage than blastocysts embryos were transferred, that involved unselected or poor prognosis patients and cycles were randomised prior to Day 3 of culture. Sensitivity analysis excluding the studies which did not report concealment of allocation (Grade B and C) did not affect the conclusions for live-birth rates (OR 1.40, 95% CI 0.88 to 2.23).

Clinical pregnancy rate per couple
Evidence of a significant difference was detected between the two treatment groups was detected for clinical pregnancy rate per couple (17 RCTs: OR 1.17, 95% CI 1.00 to 1.38 (Day 2/3 36.0% vs 40.0%)) . There was no heterogeneity detected and the I2 was 44.7%. Separate analyses showed that this was true for trials where equal numbers of embryos were transferred (including single embryo transfers), but not for trials where more cleavage stage than blastocysts were transferred, or for prognosis or timing of randomisation subgroups. Sensitivity analysis excluding the studies which did not report concealment of allocation (Grades B and C) did not affect the conclusions of clinical pregnancy rates (OR 1.20 95% CI 0.90 to 1.59). Exclusion of the studies (Coskun 2000 ; Karaki 2002 ; Levitas 2004 ; Motta 1998 A & B) where different media were used in each arm of the study did not affect the conclusions on the pregnancy rates (7 RCTs; OR 1.02 95% CI 0.82 to 1.27).

Multiple-pregnancy rate
There was no evidence of a difference in multiple pregnancy rate per couple between the two treatment groups (14 RCTs: OR 0.94, 95% CI 0.72 to 1.23). There was no heterogeneity detected and the I2 was 12.9%. Separate analyses by embryo transfer policy or prognosis did not suggest any subgroup differences in the relative effect of blastocyst versus cleavage stage ET. Sensitivity analysis: Excluding the studies which did not report concealment of allocation (Grade B and C) did not effect the significance of multiple pregnancy rates (OR 0.95 95% CI 0.65 to 1.39). Exclusion of the studies (Coskun 2000 ; Karaki 2002 ; Levitas 2004 ; Motta 1998 A & B) where different media was used in each arm of the study did not effect the conclusions of the multiple pregnancy rates (OR 1.02, 95% CI 0.82 to 1.27).

High order multiple-pregnancy rate
There was no evidence of a difference in high order pregnancy rate per couple between the two treatment groups in 12 RCTs (OR 0.44, 95% CI 0.15 to 1.33). There was no heterogeneity detected and the I2 was 0%. Separate analyses by embryo transfer policy did not suggest any subgroup differences in the relative effect of blastocyst versus cleavage stage ET.

Miscarriage rate
There was no evidence of a difference in miscarriage rate per couple between the two groups (12 RCTs, OR 1.21, 95% CI 0.88 to 1.66). There was no heterogeneity detected and the I2 was 0%.

Monozygotic twinning rate
Seven RCTs reported if any of the multiple pregnancies were monozygotic; there was one set in Day 2 to 3 transfer (Frattarelli 2003), two sets in Day 2 to 3 transfers (Papanikolaou 2006) and one in Day 5 to 6 transfer (Levitas 2004).

Embryo freezing rate
Rates of embryo freezing per couple showed a significant increase for the Day 2 to 3 transfers compared to Day 5 to 6 (9 RCTs, OR 0.45, 95% CI 0.36 to 0.56). Separate analyses by embryo transfer policy and prognosis also showed a significant difference in favour of more embryos frozen with early cleavage stage transfers. There was however significant heterogeneity detected in all subgroups with I2 values of greater than 79%. Four trials reported cumulative pregnancy rates following the transfer of fresh and frozen embryos (Emiliani 2003 ; Gardner 1998a ; Rienzi 2002 ; Van der Auwera 2002)

Failure to transfer any embryos
Failure to transfer any embryos per couple was significantly higher in the Day 5 to 6 group than in the Day 2 to 3 transfer (16 RCTs, OR 2.85, 95% CI 1.97 to 4.11 (Day 2/3 2.8% versus Day 5/6 8.9%)). There was, no heterogeneity detected and the I2 was 19.7%. This finding was also true for subgroups analysed by number of embryos transferred policy, and unselected patient prognosis. However, no significant difference was detected in separate subgroup analysis of both good (9 RCTs, OR 1.50, 95% CI 0.79 to 2.84) and poor (2 RCTs, OR 5.12, 95% CI 0.93 to 28.26) prognosis patients.

Per cycle data
Per cycle data were often reported in clinical trials so were included here as additional information, however; they cannot be included in the meta-analysis as they would not generate valid estimates or confidence intervals due to the unit of analysis used. Reported numbers are given for information in the graphs.

Blastocyst rates
Reported in of the 'Additional tables', blastocyst rates (Day 5 to 6 transfer only) ranged from 28% (Coskun 2000) to 89.9% (Emiliani 2003).

Implantation data
For day 2 to 3 transfer, the implantation rate varied from 3% to 69% and for Day 5 to 6 the implantation rate varied from 20% to 50%.

DISCUSSION

Discussion

This review has, for the first time, provided evidence of a significant difference in live-birth rates between cleavage and blastocyst stage transfers. The recent addition of two new trials from the same group in Belgium was sufficient to change the result from no evidence of benefit to evidence of benefit favouring blastocyst transfer. This IVF group has in the past three years contributed to a remarkable one third of the 2616 couples included in this current review. There are now a total of nine studies reporting live-birth data that fit the inclusion data, which is a significant advancement on the first Cochrane review of 2000 that was only able to include one RCT with this outcome. However, while subgroup analysis shows that this effect is true for good prognosis patients, trials where equal numbers of embryos were transferred and randomisation occurred on Day 3, it is not true for unselected and poor prognosis patients, for trials where more cleavage stage embryos than blastocysts were transferred and randomisation occurred prior to Day 3. For all remaining categories the outcomes remain unchanged from previous updates. In favour of cleavage stage, these outcomes include, evidence of a significant difference in favour of cleavage stage for cryopreservation. While there remains no evidence of a difference between blastocyst and cleavage stage transfers for rates of miscarriage, multiple pregnancies, and high order multiples. Moreover, sensitivity analysis excluding those studies where different media were used for the two arms of the study showed that this did not influence the results.

Implantation rates
Extended culture provides an opportunity to select those embryos that have proven ability to survive and develop to an advanced stage in vitro with subsequent implantation success in vivo. The transfer of Day 5 to 6 embryos also offers the opportunity to replace embryos into a uterine environment that is possibly more synchronised than at Day 2 to 3. For these reasons, blastocyst culture is expected to result in higher implantation rates. In this review approximately half of the included trials reported higher rates for blastocysts while the remaining (with three exceptions that reported a decrease) reported no difference between the groups. Note that pooling of implantation data cannot 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). Nevertheless it is interesting to observe that the higher implantation potential of blastocysts in this review did translate into higher pregnancy rates per couple randomised. This is also despite a significantly higher failure to transfer any embryos in the Day 5 to 6 group. The increased rates of failure to transfer with Day 5 to 6 is largely the result of patients whose embryos had arrested development prior to the day of embryo transfer. Indeed, many of the studies that transferred fewer blastocysts than cleavage stage, did so out of a lack of options rather than by policy. This selection process should yield a higher pregnancy rate per ET in the Day 5 to 6 group, however, only the two most recent trials showed a statistical difference in this category (note that it is inappropriate to pool those outcomes Vail 2003). Another point of consideration is the widely variable policy for minimal quality of embryos for transfer that may have existed amongst trials. Some trials accepted transfer of developmentally delayed embryos on Day 5 to 6, whilst other trials were more selective and refused to transfer embryos that were anything less than a late morula or early blastocyst.

Blastulation rates and medi a
Blastocyst formation rates may also influence the pregnancy rate per ET for each trial. They ranged from 28% in the Coskun 2000 trial to 60% in the Schillaci 2002 trial. In contrast to the initial Cochrane review of this subject, all of the included trials in this update 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. It is highly feasible that as media formulations advance (for example G1/G2 series III) and expertise in blastocyst culture evolves to a critical mass (as in the Belgium clinics (Kolibianakis 2004 ; Papanikolaou 2005 ; Papanikolaou 2006)), blastocyst transfers will continue to emerge as the superior approach.

Viability
In this review couples having blastocyst culture were three times more likely to have a cycle cancelled prior to embryo transfer. Some advocate that it is better for patients to learn that their embryos failed to develop by Day 5 than go through with a transfer on Day 2 to 3 with embryos that had a low potential of success. There has, however, been little research into the emotional status of couples given such choices (Borg 2000). A prerequisite to such a clinical approach firstly requires that the clinic has existing high success rates with Day 2 to 3 and, secondly, great confidence in the culture conditions and protocols for extended culture. There is always the concern that if blastocyst culture is used strictly to select out the most viable embryos, how can it be known that the slow cleaving embryo on Day 3 may not have had a higher chance of pregnancy if replaced into the uterus early than be subjected to extended culture (Racowsky 2000). Studies exploring what key indicators can be detected for selecting which patient group might obtain the most benefit from blastocyst culture include the number of eight-cell embryos on Day 3 (Racowsky 2000), number of pro-nuclear embryos on Day 1, the pro-nuclear grading profile (Scott 2000) and the number of early cleaving embryos. Certainly the Papanikolaou 2005 trial has clearly demonstrated that it is possible to obtain zero cancellation rates and significantly higher live-birth rates with criteria of four good quality embryos on Day 3 (in women under 38 years of age).

Time of randomisation
Studies show that women with a high oocyte yield and good quality eight-cell embryos on Day 3 are more likely to have blastocysts by Day 5 to 6 compared with poor responders and no eight-cell embryos by Day 3. This rationale is supported in this review, where no difference was found in the rate of failure to transfer embryos for the subgroup analysis of good prognosis patients (2.4% for Day 2 to 3 versus 3.5% for Day 5 to 6). Many clinics, therefore, limit blastocyst culture to couples with a minimum number of oocytes collected or the quality of the embryos on Day 3, or both (Boyarsky 2001 ; Papanikolaou 2005 ; Racowsky 2000). Only two trials in this review randomised couples on Day 3, when the selection criteria of three or more eight-cell embryos had been met (Bungum 2003 ; Papanikolaou 2005). The patient population in these two trials were therefore on an equal footing, yet they resulted in opposing results (Bungum 2003 had higher pregnancy rates in Day 2 to 3 transfer while Papanikolaou 2005 had higher pregnancy and live-birth rates in Day 5 to 6 transfer). These trials are in stark contrast to those that randomised couples prior to the start of the treatment cycle, at a time where neither the number of oocytes retrieved nor fertilised, as well as the number of eight-cell embryos, could be anticipated. In some respects the trials can be divided into those that investigated whether outright adoption of blastocyst culture is superior to standard cleavage stage transfers (that is unselected patient populations) or whether blastocyst culture can be incorporated into a clinical setting for enhancement of success in specific patient subgroups (that is poor or high prognosis patients).

Number of Embryos Transferred and multiple pregnancy
See . Perhaps one of the greatest difficulties in drawing conclusions from published blastocyst trials is the variable embryo transfer policies between the two experimental groups. In this meta-analysis, significantly fewer embryos were transferred in the dayversus5 to 6 group than in the dayversus2 to 3 group. There are two primary reasons for this difference. Firstly, many clinics worried about the high incidence of multiple pregnancy with blastocysts will have a policy to transfer no greater than two dayversus5 to 6 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. Regardless of the embryo transfer policy, for many patients there is simply a lack of choice as only one, if any, embryo reaches the blastocyst stage. Only one study had a policy for single blastocyst transfer (Livingstone 2002) but for cleavage stage transfer they transferred two embryos. There is no doubt that with the current global pressure to adopt single embryo transfers so as to reduce the burden of multiple pregnancies on the child and health system, there will be more trials evaluating blastocyst culture for this purpose (Gardner 2004). We are fortunate that this update includes the first trial comparing day2 to 3 and day5 to 6 with a policy of single embryo transfers in both arms of the study (Papanikolaou 2006). The two Papanikolaou papers demonstrate that a greater effect on live birth can be obtained with double embryos transferred in older women (less than 38 years of age) randomised on day3 (Papanikolaou 2005) versus single embryos transferred in younger women (less than 36 years) randomised prior to treatment (Papanikolaou 2006). But remarkably the policy of single embryo

transfer in the former paper reduced the multiple pregnancy rate for blastocyst transfer from 43% to zero.

Miscarriage and monozygotic twinning
Miscarriage rates are a critical factor when evaluating a new mode of treatment and obviously impact on treatment efficiency and live-birth outcomes. Yet only just over half of the included trials provided this data. Theoretically, the rate of miscarriage might be expected to be lowest with the transfer of highly selected embryos that are transferred into a synchronous uterine environment, such as in blastocyst culture. However, the results to date reveal little change from the earlier reviews that show no evidence of a difference in miscarriage rates for couples randomised (10 RCTs; OR 1.36, 95% CI 0.91 to 2.02). Only seven of the included trials reported on the presence or absence of MZ twinning so this analysis remains underpowered to comment meaningfully on MZ twin rates. A total of three sets of MZ twins were reported, two with day2 to 3 embryo transfers and, reassuringly, only one set of MZ twins from blastocyst transfer. Estimations of MZ twin rates in ART are thought to be underestimated, with up to one third being missed without genetic testing.

Embryo freezing
Overall this review found a significant decrease in the number of embryos frozen in the day5 to 6 group. The number of embryos frozen is an important consideration when assessing the effectiveness of a treatment as it offers the patient an additional opportunity to achieve a pregnancy. When considering an alteration in treatment procedure from day2 to 3 versus day5 to 6, the benefits of possible higher implantation rates are weighed up against the disadvantages of not only higher failure to transfer but also lower cryopreservation rates. A total of three trials reported data on pregnancies following transfer of frozen embryos, in both groups (Emiliani 2003 ; Rienzi 2002 ; Van der Auwera 2002). Van der Auwera 2002 used their trial data and results from subsequent thaw cycles after one year to predict a cumulative live-birth rate that was almost identical in both groups (38% versus 39%). That is, the added benefit of a higher cryopreservation rate in the day2 to 3 group cancelled out the higher implantation rates of the fresh day5 to 6 transfers. Similarly, Rienzi 2002 reported no difference in cryo augmented pregnancy rates when at least one thaw cycle was carried out in the day2 to 3 group. Emiliani 2003 , on the other hand, reported significantly higher cumulative pregnancy rates in the day2 to 3, presumably correlating to the much lower cryo survival rate they reported in their blastocyst group (day2 to 3: 46% versus day5 to 6: 27%). However, freezing protocols for early cleavage and blastocyst stage embryos are different and the effectiveness of the latter has yet to be widely accepted, particularly in embryos that have been cultured in sequential media. Recent reports of improved blastocyst freezing techniques may have a positive impact on the cumulative success rates of future blastocyst RCTs (Gardner 2003a).

Costs
Cost comparisons of treatment have not been investigated in this review but are worthy of mention. From the laboratory's perspective, the cost of setting up for blastocyst culture is not insignificant. Often an additional incubator is required due to the extra two to three days that the embryos remain in culture. The extra media costs on the other hand are negligible. Blastocyst culture is however moderately more labor intensive and laboratory staff may be required to perform more weekend work, particularly if embryos from two different stages of development are required to be cryopreserved. For the patient, the higher risk of cancellation due to the more stringent selection process of blastocyst culture, may result in a lower treatment cost. At the end of the day, the cost of the treatment mode must be weighed up against the outcome of a healthy take-home baby.

Risk of bias of studies
The overall risk of bias of studies included in this update has substantially improved from the previous 2000 review, largely due to stricter publication expectations being enforced by journals. This review includes no quasi-randomised trials and almost half of the trials have stated using a sealed envelope to conceal randomization of participants. It is disappointing that so many published trials are excluded from meta-analysis by failing to carry out the CONSORT recommendations for RCTs (Begg 1996), such as randomising couples and not cycles, performing a power analysis and intention-to-treat analysis, concealing allocation and using computer generated or tabulated methods of randomisation. 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. The funnel plot for clinical pregnancy rate however demonstrated that the studies were distributed evenly across the graph, suggesting that publication bias is not present. Ultimately it must be pointed out that despite the magnitude of this meta-analysis, including data from a total of 2616 couples, the number of couples still falls marginally short of the 2832 couples that is required in each arm of the study in order to detect a 5% difference in pregnancy rate (assuming a baseline ongoing pregnancy of 30% at an alpha of level of 0.05 and beta of 0.2). It is, therefore, unlikely that any one clinic will be able to significantly demonstrate a difference between these two approaches to IVF culture.

AUTHORS' CONCLUSIONS

Implications for practice

This review of the best available evidence based on data from randomised controlled trials, suggests that the margin of difference between cleavage stage and blastocyst transfer has begun to show a significant effect on live-birth and pregnancy rates. Blastocyst culture appears to be most favoured in subgroups of good prognosis patients with high numbers of eight cell embryos on day3, for whom there is no difference in cycle cancellation. The recent shift in effect from previous versions of this Cochrane review may reflect an improvement in clinical expertise including media formulation, a raising of reported RCT standards, increased participants nearing statistical significance or a contribution of all of the above.

There is emerging evidence to suggest that in selected patients, blastocyst culture maybe applicable for single embryo

transfer. With this approach, lower rates of cryopreservation maybe the trade off for zero multiple pregnancy rates.

Implications for research

Continuous evaluation of the new generation of sequential media to assess if they are better than existing day2 to 3 methods is necessary. The challenge remains for manufacturers of these products to demonstrate true clinical benefits by carrying out robust multi centre randomised clinical trials. Based on the results of this review, the following recommendations are made to ensure valuable data are produced:

1. adherence to CONSORT recommendations for RCTs especially methods of concealment (Begg 1996);
2. research into best patient selection and inclusion criteria;
3. same media composition and brand for both groups up to the day2 to 3 stage;
4. explicit pre-specified embryo transfer policies for both groups;
5. long-term follow-up reports of cumulative live-birth rates (including embryo thaws) presented as a survival analysis;
6. research into improved blastocyst cryopreservation techniques.
7. application of blastocyst culture for single embryo transfer

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GRAPHS

Graphs and Tables

To view a graph or table, click on the outcome title of the summary table below.

Live birth rate

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth per couple	9	1144	Odds Ratio (M-H, Fixed, 95% CI)	1.35 [1.05, 1.74]
2 Live birth per couple: grouped by number of embryos transferred	9		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
2.1 equal number of embryos transferred	5	920	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.41 [1.07, 1.85]
2.2 more cleavage stage than blastocyst embyros transferred	4	224	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.11 [0.60, 2.07]
2.3 single embryo transfer	1	351	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.70 [1.06, 2.72]
2.4 equal number of multiple embryos transferred (excluding SETs)	4	569	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.28 [0.91, 1.79]
3 Live birth rate per couple: grouped by prognosis	9	1144	Odds Ratio (M-H, Fixed, 95% CI)	1.35 [1.05, 1.74]
3.1 good prognostic factors	5	760	Odds Ratio (M-H, Fixed, 95% CI)	1.49 [1.10, 2.03]
3.2 poor prognostic factors	2	77	Odds Ratio (M-H, Fixed, 95% CI)	2.05 [0.53, 7.96]
3.3 unselected group	2	307	Odds Ratio (M-H, Fixed, 95% CI)	1.02 [0.64, 1.63]
4 Live birth rate: grouped by day of randomisation	9	1144	Odds Ratio (M-H, Fixed, 95% CI)	1.35 [1.05, 1.74]
4.1 randomisation at start of cycle	4	712	Odds Ratio (M-H, Fixed, 95% CI)	1.32 [0.95, 1.83]
4.2 randomised on day of OPU and day 1 after OPU	3	245	Odds Ratio (M-H, Fixed, 95% CI)	0.94 [0.56, 1.57]
4.3 randomised Day 2 to 3 post OPU	1	164	Odds Ratio (M-H, Fixed, 95% CI)	2.40 [1.25, 4.60]
4.4 day of randomisation unstated	1	23	Odds Ratio (M-H, Fixed, 95% CI)	4.13 [0.36, 47.30]
5 Live birth per couple: equal number of embryos transfered	9	1144	Odds Ratio (M-H, Fixed, 95% CI)	1.35 [1.05, 1.74]

Clinical pregnancy rate

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 clinical pregnancy rate per couple	17	2557	Odds Ratio (M-H, Fixed, 95% CI)	1.17 [1.00, 1.38]
2 clinical pregnancy rate per couple: grouped by number of embryos transferred	17		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 equal numbers of ET	8	1672	Odds Ratio (M-H, Fixed, 95% CI)	1.24 [1.02, 1.52]
2.2 more cleavage stage than blastocyst embryos transfered	9	885	Odds Ratio (M-H, Fixed, 95% CI)	1.05 [0.80, 1.38]
2.3 Single embryo transfer	1	351	Odds Ratio (M-H, Fixed, 95% CI)	1.63 [1.02, 2.61]
3 clinical pregnancy rate per couple: grouped by prognosis	17	2557	Odds Ratio (M-H, Fixed, 95% CI)	1.17 [1.00, 1.38]
3.1 Good prognostic factors	9	1315	Odds Ratio (M-H, Fixed, 95% CI)	1.21 [0.96, 1.51]
3.2 Poor prognostic factors	2	77	Odds Ratio (M-H, Fixed, 95% CI)	2.69 [0.81, 8.96]
3.3 Unselected group	6	1165	Odds Ratio (M-H, Fixed, 95% CI)	1.10 [0.86, 1.39]
4 clinical pregnancy rate per couple: grouped by day of randomisation	17	2557	Odds Ratio (M-H, Fixed, 95% CI)	1.17 [1.00, 1.38]
4.1 Randomised start of cycle	6	1264	Odds Ratio (M-H, Fixed, 95% CI)	1.23 [0.97, 1.56]
4.2 Randomised on day of OPU or day 1	7	872	Odds Ratio (M-H, Fixed, 95% CI)	1.04 [0.79, 1.37]
4.3 Randomised on day 2 to 3	2	282	Odds Ratio (M-H, Fixed, 95% CI)	1.34 [0.84, 2.15]
4.4 Day of randomisation unstated	2	139	Odds Ratio (M-H, Fixed, 95% CI)	1.23 [0.61, 2.49]

Multiple-pregnancy rate

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 multiple-pregnancy rate per couple	14	2322	Odds Ratio (M-H, Fixed, 95% CI)	0.94 [0.72, 1.23]
2 multiple-pregnancy rate per couple: grouped by number of embryo transfer	14		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Equal number of embryos transferred	8	1672	Odds Ratio (M-H, Fixed, 95% CI)	1.05 [0.75, 1.46]
2.2 More cleavage stage than blastocyst embryos transferred	6	650	Odds Ratio (M-H, Fixed, 95% CI)	0.76 [0.48, 1.21]
2.3 Single embryo transfer	1	351	Odds Ratio (M-H, Fixed, 95% CI)	0.20 [0.01, 4.17]
3 multiple-pregnancy rate per couple: grouped by prognosis	14	2322	Odds Ratio (M-H, Fixed, 95% CI)	0.93 [0.71, 1.22]
3.1 Good prognostic factors	9	1339	Odds Ratio (M-H, Fixed, 95% CI)	0.92 [0.64, 1.31]
3.2 Poor prognostic factors	1	54	Odds Ratio (M-H, Fixed, 95% CI)	0.89 [0.14, 5.81]
3.3 Unselected	4	929	Odds Ratio (M-H,	0.96 [0.62,

3.3 Unselected	4	929	Fixed, 95% CI)	1.47]
4 high order pregnancies (more than 2 gestational sacs) per couple	12	2035	Odds Ratio (M-H, Fixed, 95% CI)	0.44 [0.15, 1.33]
5 high order pregnancy: grouped by number of embryos transferred	12	2035	Odds Ratio (M-H, Fixed, 95% CI)	0.44 [0.15, 1.33]
5.1 Equal number of embryos transferred	8	1672	Odds Ratio (M-H, Fixed, 95% CI)	0.33 [0.01, 8.28]
5.2 More cleavage stage than blastocyst embryos transferred	4	363	Odds Ratio (M-H, Fixed, 95% CI)	0.46 [0.14, 1.49]
6 high order pregnancies: grouped by prognosis	12	2035	Odds Ratio (M-H, Fixed, 95% CI)	0.44 [0.15, 1.33]
6.1 Good prognostic factors	9	1385	Odds Ratio (M-H, Fixed, 95% CI)	0.29 [0.08, 1.06]
6.2 Poor prognostic factors	1	54	Odds Ratio (M-H, Fixed, 95% CI)	4.2 [0.16, 107.89]
6.3 Unselected	2	596	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable
7 multiple-pregnancy rate per pregnancy	14		Odds Ratio (M-H, Fixed, 95% CI)	Totals not selected
8 high order pregnancies per total pregnancies	13		Odds Ratio (M-H, Fixed, 95% CI)	Totals not selected

Miscarriage rate

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 miscarriage rate per couple	12	1968	Odds Ratio (M-H, Fixed, 95% CI)	1.21 [0.88, 1.66]
2 miscarriage rate per pregnancy	13		Odds Ratio (M-H, Fixed, 95% CI)	Totals not selected

Embryo freezing rate

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 embryo freezing per couple	9	1416	Odds Ratio (M-H, Fixed, 95% CI)	0.45 [0.36, 0.56]
2 Embyro freezing per couple: grouped by number of embryos transferred	9	1416	Odds Ratio (M-H, Fixed, 95% CI)	0.45 [0.36, 0.56]
2.1 equal number of embryos transferred	5	956	Odds Ratio (M-H, Fixed, 95% CI)	0.42 [0.32, 0.55]
2.2 more cleavage stage than blastocyst embryos transferred	4	460	Odds Ratio (M-H, Fixed, 95% CI)	0.51 [0.36, 0.74]
3 Embryo freezing per couple: grouped by prognostic factors	9	1416	Odds Ratio (M-H, Fixed, 95% CI)	0.45 [0.36, 0.56]
3.1 good prognostic factors	5	542	Odds Ratio (M-H, Fixed, 95% CI)	0.41 [0.28, 0.58]
3.2 poor prognostic factors	0	0	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable
3.3 unselected	4	874	Odds Ratio (M-H, Fixed, 95% CI)	0.48 [0.36, 0.62]

Failure to transfer embryos rate per couple

No. of	No. of

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 Failure to transfer any embryos per couple	16	2459	Odds Ratio (M-H, Fixed, 95% CI)	2.85 [1.97, 4.11]
2 Failure to transfer any embryos per couple: grouped by number of embryos transferred	16		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 equal number of embryos transferred	8	1672	Odds Ratio (M-H, Fixed, 95% CI)	2.33 [1.51, 3.61]
2.2 more cleavage stage than blastocyst embryos tranferred	8	787	Odds Ratio (M-H, Fixed, 95% CI)	4.42 [2.18, 8.98]
2.3 single embryo transfer	1	351	Odds Ratio (M-H, Fixed, 95% CI)	1.41 [0.55, 3.59]
3 Failure to transfer any embryos per couple: grouped by prognostic factors	16	2459	Odds Ratio (M-H, Fixed, 95% CI)	2.85 [1.97, 4.11]
3.1 good prognostic factors	9	1315	Odds Ratio (M-H, Fixed, 95% CI)	1.50 [0.79, 2.84]
3.2 poor prognostic factors	2	77	Odds Ratio (M-H, Fixed, 95% CI)	5.12 [0.93, 28.26]
3.3 unselected	5	1067	Odds Ratio (M-H, Fixed, 95% CI)	3.74 [2.32, 6.03]

Cumulative pregnancy rate

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
1 cumulative pregnancy rate from fresh and frozen transfers	3	420	Odds Ratio (M-H, Fixed, 95% CI)	0.58 [0.39, 0.87]

COVER SHEET

Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology

Reviewer(s)	Blake Debbie, Farquhar Cindy, Johnson Neil, Proctor Michelle
Contribution of Reviewer(s)	
Issue protocol first published	2000 issue 2
Issue review first published	2002 issue 2
Date of last minor amendment	Information not supplied by reviewer
Date of last substantive amendment	Information not supplied by reviewer
Most recent changes	
Date new studies sought but none found	Information not supplied by reviewer
Date new studies found but not yet included/excluded	Information not supplied by reviewer
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HISTORY

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

Date	Event	Description
5 May 2008	Amended	Converted to new review format.
23 July 2007	New citation required and conclusions have changed	Substantive amendment

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