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The merits of blastocyst versus cleavage stage embryo transfer: a Cochrane review

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This paper is based on a Cochrane review published in The Cochrane Library, issue 2, 2002 (see www.CochraneLibrary.net for information) with permission from The Cochrane Collaboration and John Wiley and Sons. Cochrane reviews are regularly updated as new evidence emerges and in response to comments and criticisms, and The Cochrane Library should be consulted for the most recent version of the review.

BACKGROUND: The aim of this study was to determine the relative merits of blastocyst versus cleavage stage embryo transfer, concerning the chance of pregnancy, live birth, multiple pregnancy and the factors contributing to these primary outcomes, from the best available evidence. METHODS: A systematic review employing the principles of the Cochrane Menstrual Disorders and Subfertility Group was undertaken. Fourteen randomized controlled trials, all comparing day 2/3 with day 5/6 embryo transfer, were included in a meta-analysis. RESULTS: For day 2/3 versus day 5/6 transfer, there was no significant difference in the odds of pregnancy [odds ratio (OR) = 0.91, 95% confidence interval (CI) 0.71–1.17] nor of live birth (OR = 0.83, 95% CI 0.48–1.42) per treated couple. These results were similar whether all trials, only trials with transfer of equal numbers of day 2/3 versus day 5/6, or only trials with transfer of fewer day 5/6 than day 2/3 embryos, were pooled. There was no significant difference in the odds of multiple pregnancy for day 2/3 versus day 5/6 transfer overall (OR 0.77, 95% CI 0.52–1.13) nor where fewer day 5/6 than day 2/3 embryos were transferred (day 2/3 versus day 5/6 OR 0.69, 95% CI 0.42–1.12). CONCLUSION: The current evidence fails to support a widespread change of practice from cleavage stage to blastocyst stage embryo transfer in couples undergoing IVF.

Key words: blastocyst/cleavage stage/embryo/IVF/randomized controlled trial

Introduction

In the late 1990s and since the turn of the millennium, improvement in IVF success rates has led to speculation over possible reasons for improved outcomes, including restriction of sperm–oocyte exposure time, bench-top incubator technology and the use of sequential media (Jansen, 2003). Products and methods have been developed to enable embryos to be viably grown *in vitro* for extended periods (Gardner and Lane, 1998), although it has remained unclear what benefits may accrue from this technical advance. Assessment of the efficacy and cost effectiveness of any change in practice is essential because IVF is relatively inaccessible in most communities and the cost to individuals and state-owned healthcare systems is high. This review aims to evaluate the proposed merits of a change in practice from cleavage stage embryo transfer (2–8 cells on day 2–3 post fertilization), to blastocyst stage transfer (>64 cells on day 5–6 post fertilization).

There are two central reasons why an alternative to the cleavage embryo transfer system has been proposed. First, it has long been recognized that it is physiologically premature to expose early stage embryos to the uterine environment. *In vivo*, embryos travel through the Fallopian tubes and do not reach the uterus before the morula stage (Croxatto *et al.*, 1972), which equates to at least day 4 of *in vitro* culture. The uterus provides a different nutritional milieu from the oviduct, therefore it is postulated that this may cause homeostatic stress on the embryo, resulting in a reduced implantation potential (Gardner *et al.*, 1996). The second reason is the widely acknowledged shortcomings of the morphological criteria used for selection of cleavage stage embryos for day 2/3 for transfers, with much debate over the correlation of morphological features with pregnancy rates (Puissant *et al.*, 1987; Steer *et al.*, 1992; Roseboom *et al.*, 1995; Palmstierna *et al.*, 1998; Rijnders and Jansen, 1998). Prior to day 3 of culture, when genomic

activation and then compaction begins, embryonic development is primarily controlled by transcripts and stored RNA messages of maternal origin (Braude *et al.*, 1998). It is not until after this transitional stage that development proceeds under the control of an activated embryonic genome, resulting in the expression of a plethora of growth factors and receptors. In addition, it is suspected that a large proportion of morphologically normal day 3 embryos are chromosomally abnormal, thus contributing to the 80–90% rate of implantation failure post-embryo transfer observed in some cleavage stage protocols (Magli *et al.*, 1998). It has been theorized that extending embryo culture until day 5/6 (blastocyst stage) may provide advantages over traditional protocols by, first, allowing transfer of embryos into a synchronized uterine environment, and second, providing the ability to select only those embryos that have demonstrated the potential for continued development under embryonic genomic control.

Allowing human embryos to develop to the blastocyst stage in IVF programmes is not novel. What is new, however, is the accessibility and range of reportedly successful media products that has resulted in an exponential rise in the acceptability and use of this approach (Alves da Motta *et al.*, 1998). Initial reports of blastocyst culture involved single media consisting of a mixture of a complex and simple media formulation (Scholtes *et al.*, 1996) or co-culture (Ménézo *et al.*, 1990; Yeung *et al.*, 1992; Van Blerkom, 1993). More recently, the development of stage-specific sequential media has been claimed to allow 36–66% of embryos to develop to blastocysts with a high viability of up to 50% implantation rate (Jones *et al.*, 1998a; Gardner *et al.*, 1998b).

Advocates of blastocyst culture maintain that the increased implantation rate, in conjunction with a policy to replace fewer embryos, may allow maintenance of the overall chance of pregnancy, but reduce the costly multiple birth rate (Jones and Trounson, 1999). Critics of the approach express concern at the increased incidence of women failing to have embryos for transfer (Marek *et al.*, 1999), although the day of patient recruitment into the blastocyst programme 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. Other concerns include a possible increased incidence of monozygotic twinning (Behr *et al.*, 2000), an altered sex ratio in favour of male births (Ménézo *et al.*, 1999), the sensitivity of the system to suboptimal conditions and the reduced proportion of supernumerary embryos for freezing (Tsirgotis, 1998). It is also yet to be clarified if there are patient groups for whom blastocyst culture is disadvantageous. And finally, does blastocyst culture achieve the primary aim of providing the subfertile couple with a normal healthy baby?

This review is based on a Cochrane Review (Blake *et al.*, 2003), for which the original search was undertaken in August 2001. The search has now been updated in April 2003.

Objective

The primary aim of this review was to determine if the intervention of blastocyst stage embryo transfer offers any benefit, in terms of increasing clinical pregnancy and live birth, or reducing multiple pregnancy, compared to cleavage stage embryo transfer. The patient population was couples undergoing IVF or ICSI for therapeutic reasons or oocyte donation. The primary outcomes assessed were pregnancy, live birth and multiple pregnancy per woman/couple as the key denominator. Studies reporting outcomes as rates per cycle only (oocyte retrieval and embryo transfer), were assessed but not combined as a meta-analysis, as the apparent confidence limits may be incorrect (Daya, 2003; Vail and Gardener, 2003). For primary outcomes, it was determined *a priori* to separately pool, first, trials where it was planned to transfer equal numbers of cleavage stage and blastocyst embryos and, second, trials where it was planned to transfer fewer blastocysts than cleavage stage embryos. A secondary aim was to assess the factors that contribute to the primary outcome (including rates of implantation, miscarriage, ectopic pregnancy and cancellation) and to compare the overall embryo utilization of both embryo culture approaches. Subgroup analyses were performed to assess the effects of a policy of transfer of a different number of blastocysts compared to cleavage stage embryos and the effects of selection of good or poor prognosis patients. Sensitivity analyses were planned to assess the effects of co-interventions such as different culture media or culture conditions for the two groups and assisted hatching.

Materials and methods

Search strategy

All reports describing a comparison of cleavage stage embryo transfer and blastocyst stage transfer using IVF or ICSI were obtained using the search strategy developed by the Menstrual Disorders and Subfertility Group.

The Cochrane Menstrual Disorders & Subfertility Group's specialized register of controlled trials was searched. The Cochrane Controlled trials register, MEDLINE (1966 to April 2003), EMBASE (1980 to April 2003) and Bio extracts were also searched, using the Cochrane Highly Sensitive Search Strategy and the following key words: blastocyst/embryo or embryo transfer/cleavage stage, ovum/culture media or embryo culture/sequential culture/co-culture. The National Research Register (NRR), a register of ongoing and recently completed research projects funded by, or of interest to, the UK National Health Service, as well as entries from the Medical Research Council's Clinical Trials Register, and details on reviews in progress collected by the NHS Centre for Reviews and Dissemination, were also searched. The Clinical Trials Register (clinicaltrials.gov), a registry of both federally and privately funded US clinical trials, was also searched.

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

Study selection

Eligibility criteria for inclusion were as follows: randomized controlled trials only; pre-specified criteria of population, interventions and primary outcomes (see Objective) included in trial. Exclusion criteria included trials where the IVF/ICSI cycles involved *in vitro*-matured oocytes or preimplantation diagnosis.

Selection of trials for inclusion in this review, quality assessment, data extraction and statistical analysis were performed in accordance

with the policy of the Cochrane Menstrual Discorders and Subfertility Group (Blake *et al.*, 2003). Additional information was sought, where necessary, from authors of included trials. Replies were received from Plachot *et al.* (2000) and Huisman *et al.* (2000), who provided information regarding methodology and outcome data.

Forty-seven trials were identified as providing data comparing early cleavage stage and blastocyst stage embryo transfer outcomes, dating back to 1991. Seventeen trials met the inclusion criteria and were fully reviewed; 14 of these had data suitable for inclusion in the meta-analysis; four were quasi-randomized studies and excluded from the main meta-analysis (Scholtes and Zeilmaker, 1996; Gudmundsson *et al.*, 1998; Huisman *et al.*, 2000; Plachot *et al.*, 2000). It was determined to consider evidence from the quasi-randomized trials only in the absence of randomized data or in support of randomized data where the latter were minimal. Thirty-three studies were excluded from the meta-analysis for reasons outlined in Table I. Where possible, relevant outcome data have been included in Table I.

Two of the 14 included trials had been published or presented on separate dates. Motta *et al.* (1998a,b) are two conference abstracts presenting different aspects of data from the same trial. Levitas *et al.* (2001) is a more recent publication (with a little more data) of a previously presented abstract (Levitas *et al.*, 2000). Only the most recent data from these trials have been included in the analysis.

All except two studies implied that the number of cycles also represented the number of women in the studies. Boyarsky *et al.* (2001) categorically stated that the number of women in the trial represented 'different' women undergoing a single cycle—there was no such categorical statement in any other trial, thus raising the possibility of misinterpretation. Motta *et al.* (1998a,b) reported 33 repeat cycles by some women (116 cycles carried out by 83 women). Janny *et al.* (1993) refer only to the number of cycles and not the number of women.

Description and quality assessment of included trials

All trials, where the time-frame was specified, appear to have completed recruitment within 24 months. Six studies did not state the time frame (Motta *et al.*, 1998a,b; Coskun *et al.*, 2000; Levitas *et al.*, 2001; Levron *et al.*, 2002; Rienzi *et al.*, 2002; Schillaci *et al.*, 2002). All studies are reported to have been performed at single private or university-based clinics. Ten countries were represented in the included studies: Australia (Livingstone and Bowman, 2001); Brazil (Motta *et al.*, 1998a,b); Belgium (Demylle *et al.*, 2000; Van der Auwera *et al.*, 2002), France (Janny *et al.*, 1993); Israel (Coskun *et al.*, 2000; Levitas *et al.*, 2001; Levron *et al.*, 2002); Italy (Schillaci *et al.*, 2002); Jordan (Karaki *et al.*, 2002); Russia (Boyarsky *et al.*, 2001), Spain (Rienzi *et al.*, 2002) and USA (Gardner *et al.*, 1998a).

Patient selection criteria comprise three main groups: unselected patients (Janny *et al.*, 1993; Motta *et al.*, 1998a,b; Karaki *et al.*, 2002; Van der Auwera *et al.*, 2002), positively selected patients—those who would be expected to do well with blastocyst culture (Gardner *et al.*, 1998a; Coskun *et al.*, 2000; Demylle *et al.*, 2000; Boyarsky *et al.*, 2001; Livingstone and Bowman, 2001; Levron *et al.*, 2002; Rienzi *et al.*, 2002; Schillaci *et al.*, 2002) and negatively selected patients—couples who had experienced multiple failures with conventional treatment (Levitas *et al.*, 2001). Methods of positive selection included: >10 follicles on the day of hCG trigger (Gardner *et al.*, 1998a); ≥8 collected oocytes (Boyarsky *et al.*, 2001; Schillaci *et al.*, 2002); ≥4 (Coskun *et al.*, 2000; Demylle *et al.*, 2000), ≥5 (Levron *et al.*, 2002) or ≥8 (Rienzi *et al.*, 2002) fertilized oocytes; age <38 years and ≤3 previous IVF cycles or a previous live birth (Livingstone and Bowman, 2001).

All trials except four (Demylle *et al.*, 2000; Boyarsky *et al.*, 2001; Rienzi *et al.*, 2002; Schillaci *et al.*, 2002) provided baseline information about the included patient population. Most studies reported that the mean age was in the range of 33–35 years, with the exception of the two reports from Israel (Coskun *et al.*, 2000; Demylle *et al.*, 2000) where the mean age of women was substantially younger (30 years). One trial selected for women having either their first or second cycle (Demylle *et al.*, 2000). Gardner *et al.* (1998a), while exercising no related selection criteria, had mean previous cycles for each group of 0.2 for day 3 and 0.6 for day 5 embryo transfer women. Most trials provided details about the number of oocytes retrieved: all but one had relatively high mean yields of >10 oocytes per patient in each group; Schillaci *et al.* (2002) had a mean of 9 oocytes. Some trials published the mix of causes of infertility in each group to demonstrate that they were similar. Over half of the trials included male factor patients treated with ICSI in addition to patients treated with standard IVF; one included only patients treated with ICSI (Rienzi *et al.*, 2002).

The trials that provided details on the ovarian stimulation regimen (Janny *et al.*, 1993; Demylle *et al.*, 2000; Boyarsky *et al.*, 2001; Levron *et al.*, 2002; Schillaci *et al.*, 2002) reported using a similar GnRH pituitary down-regulation protocol prior to hMG or recombinant FSH administration. Luteal phase support consisted of progesterone administration via i.m. injection or vaginal suppository. Two studies reported on the additional administration of hCG during the luteal phase (Livingstone and Bowman, 2001; Van der Auwera *et al.*, 2002).

Sequential media was the most commonly used method of embryo culture. However, the source of media in these trials did originate from at least five different brands or products, with the G2 from Vitrolife (Sweden or in-house made) for culture between day 3 and day 5/6 being the most widely used (Gardner *et al.*, 1998a; Coskun *et al.*, 2000; Demylle *et al.*, 2000; Boyarsky *et al.*, 2001; Levitas *et al.*, 2001; Karaki *et al.*, 2002; Rienzi *et al.*, 2002; Schillaci *et al.*, 2002; Van der Auwera *et al.*, 2002). Other brands included Medicult (Denmark), Cook (Australia), Irvine Scientific (USA) and in-house-prepared solutions of Ham's F-10/Earle's (Gibco). Only three studies used the same brand of media for both day 2/3 and day 5/6 consistently throughout the trial (Motta *et al.*, 1998a,b; Levron *et al.*, 2002; Rienzi *et al.*, 2002). The remaining studies using sequential media either used different products for each group or a variety of brands throughout the trial. This may have been due either to a belief that some products offered advantages in certain situations or to problems with supply during the trial. Janny *et al.* (1993) was the only study that used co-culture of embryos on Vero cells. The method of embryo culture was reported as microdrops under oil in two studies (Demylle *et al.*, 2000; Livingstone and Bowman, 2001) and culture tubes were specified in one study (Gardner *et al.*, 1998a).

Cryopreservation of embryos in both experimental groups was common practice in at least half of the included trials, but not reported on in five studies (Demylle *et al.*, 2000; Boyarsky *et al.*, 2001; Levitas *et al.*, 2001; Livingstone and Bowman, 2001; Schillaci *et al.*, 2002). Coskun *et al.* (2000) reported no provision for day 5 freezing. The only trial reporting the use of assisted hatching was Gardner *et al.* (1998a); however, as this was performed only in the day 3 embryo transfer group, it could be considered a co-intervention.

In the day 5/6 groups, blastocyst rates ranged from 28% (Coskun *et al.*, 2000) to 46.5% (Gardner *et al.*, 1998a). Gardner *et al.* (1998a) reported the highest percentage of couples with ≥2 blastocysts for transfer (85%).

The majority of trials replaced cleavage stage embryos on day 3; day 2 replacement was employed by Janny *et al.* (1993), Schillaci *et al.* (2002) and Van der Auwera *et al.* (2002); two trials replaced embryos on a mixture of day 2 and 3 (Levitas *et al.*, 2001; Livingstone and Bowman, 2001). Transfer policies varied between the trials—for the

Table I. Characteristics of excluded studies

Study	Summary details
Abdelmassih *et al.* (1998)	RCT of day 2 versus day 3 transfer–blastocyst patients (only six) were included as a non-randomized comparison to the two randomized groups
Abdelmassih *et al.* (1999)	Non-randomized comparison of day 3 and 5 transfer Pregnancy per oocyte retrieval: day 2, 38%; day 5, 51.7% Implantation rate: day 2, 11.5%; day 5, 20%
Bolton *et al.* (1991)	Non-randomized comparison of day 2/3 and day 5/6 transfer Pregnancy per ET: day 2/3, 24%; day 5/6, 10% Implantation rate: day 2/3, 9%; day 5/6, 7%
Bongso *et al.* (1999)	No clear control group identified; consisted of sequential transfers on day 2 and day 5
Bungum *et al.* (2002)	RCT but data in abstract uninterpretable
Cruz *et al.* (1999)	Not randomized—multiple failure patients chose their group of allocation Pregnancy per ET: day 2/3, 9.1%; day 5/6, 40% Low implantation rate: day 2/3, 3.4%; day 5/6, 11.3%
El Sadek and Amer (2002)	Non-randomized comparison of day 3 and day 5 transfer Clinical pregnancy rate per ET day 3, 41.3%; day 5, 30%
Fong and Bongso (1998)	Non-randomized comparison of co-culture and sequential media—both systems had blastocyst rate 68%
Frattarelli *et al.* (2003)	Survey of opinions of a proposed RCT of cleavage stage versus blastocyst transfer
Gorrill *et al.* (1999)	Non-randomized comparison of cleavage stage frozen embryos, thawed and replaced at cleavage or blastocyst stage Pregnancy rate: cleavage 33%, blastocyst 36% Implantation rate: cleavage 15.2%, blastocyst 16.7%
Gudmundsson *et al.* (1998)	Quasi-randomized trial Pregnancy per couple: day 2/3, 27/118; day 5/6, 36/150
Huisman *et al.* (2000)	Quasi-randomized trial Pregnancy per couple: day 2/3, 128/590; day 5/6, 157/709
Jones *et al.* (1998b)	Non-controlled study of sequential media with assisted hatching: pregnancy per day 5/6/ET, 43%; implantation rate, 25%; blastocyst rate, 51%
Kettel *et al.* (1999)	Non-randomized comparison of day 2/3 and day 5/6 transfer in donor oocyte programme Pregnancy per ET: day 2/3, 41%; day 5/6, 93% Implantation rate: day 2/3, 11%; day 5/6, 50%
Kovacic *et al.* (2002)	Non-randomized comparison of day 2 and day 5 transfer in patients with 1 or 2 embryos Pregnancy rate day 2, 23%; day 5, 21%
Letterie *et al.* (2000)	Non-randomized comparison of day 2/3 and day 5/6 transfer Pregnancy per ET: day 2/3, 52%; day 5/6, 71% Multiple pregnancy rate: day 2/3, 62%; day 5/6, 58%
Levran *et al.* (1999)	RCT of day 2/3 ZIFT versus day 5/6 transfer Pregnancy per ET: day 2/3, ZIFT 12.8%; day 5/6, zero
Levran *et al.* (2002)	Non-randomized comparison of day 2/3 ZIFT versus day 5/6 transfer Clinical pregnancy rate: day 2/3 ZIFT, 40.6%; day 5/6, 3.1%
Marek *et al.* (1999)	Non-randomized comparison of day 2/3 and day 5/6 transfer Pregnancy per oocyte retrieval: day 2/3, 35.9%; day 5/6, 43.8% Implantation rate: day 2/3, 23.3%; day 5/6, 32.4%
Milki *et al.* (1999)	Non-randomized comparison of day 2/3 and day 5/6 transfer Pregnancy per ET: day 2/3, 49%; day 5/6, 70% Implantation rate: day 2/3, 23%; day 5/6, 49%
Milki *et al.* (2000)	Non-randomized comparison of day 2/3 and day 5/6 transfer Pregnancy per ET: day 2/3, 46%; day 5/6, 68% Implantation rate: day 2/3, 20%; day 5/6, 47%
Milki *et al.* (2002)	Non-randomized comparison of day 3 assisted hatched and blastocyst transfer Viable pregnancy rate: day 3/assisted hatched, 26.3%; blastocyst, 29.2%
Olivenness *et al.* (1994)	No control group—four different patient groups with poor to medium prognosis. Day 5/6 pregnancy per ET, 37.2%; implantation rate 20%
Patton *et al.* (1999)	Non-randomized comparison of day 2/3 and day 5/6 transfer Pregnancy per ET: day 2/3, 31%; day 5/6, 47% Implantation rate: day 2/3, 17%; day 5/6, 31%
Plachot *et al.* (2000)	Quasi-randomized trial Pregnancy per couple: day 2/3, 25/60; day 5/6, 19/50
Racowsky 2000	Retrospective analysis of implantation indicators in embryo morphology
Rijnders and Jansen (1998)	Uncontrolled study of day 5/6 transfers—pregnancy per ET 45.8%; implantation rate 24.1%; blastocyst rate 39%
Scholtes and Zeilmaker (1996)	Quasi-randomized trial Pregnancy per ET: day 2/3, 60/223; day 5/6, 102/410
Shapiro *et al.* (2000)	Non-randomized
Simon *et al.* (1999)	Non-randomized comparison of day 2/3 and day 5/6 transfers in multiple failure patients Pregnancy per ET: day 2/3, 35%; day 5/6, 20.2% Implantation rate: day 2/3, 10.7%; day 5/6, 11.8% Blastocyst rate 49.2%
Urman *et al.* (2002)	RCT of zona-intact versus zona-free blastocyst transfer in patients with poor quality blastocysts—concluded that zona-free blastocyst transfer improves the outcome
Van Langendonckt *et al.* (2001)	RCT of two different embryo culture media
Wilson *et al.* (2002)	Non-randomized comparison of day 3 and day 5 transfer—increased clinical and ongoing pregnancy rate with day 5

ET = embryo transfer; ZIFT = zygote intra-Fallopian transfer; RCT = randomized controlled trial.

purposes of the meta-analysis the studies were dichotomized *a priori* into those where it was planned to replace fewer blastocysts than cleavage stage embryos (the majority) and the three trials where it was planned to replace equal numbers of blastocyst and cleavage stage embryos (Coskun *et al.*, 2000; Rienzi *et al.*, 2002; Van der Auwera *et al.*, 2002). However, the numbers of embryos transferred varied markedly amongst trials. Discretion of the number of embryos to transfer for each patient in these trials was based on the woman's age (for example, <35 years, only 2 embryos; >38 years, 3–4 embryos) and the quality of the embryos on day 5 (such as 2 blastocysts or 3 less-advanced embryos). The embryo transfer policy for each trial was also affected by the country of origin (Northern European countries are more likely have a maximum of 2 transferred) and historical developments (over time, stricter policies for reducing the number of embryos transferred have been encouraged). In general, transfer of 2–4 embryos for the day 2/3 group and 1–3 embryos for the day 5/6 group was typical. Livingstone and Bowman (2001) compared the policy of the fewest transferred embryos: two cleavage stage embryos versus one blastocyst. Gardner *et al.* (1998a) described a necessity for a policy change mid-trial, reducing the number of embryos to be transferred for the day 5 group from 3 to 2, owing to the unacceptably high multiple pregnancy rate.

Randomization and allocation concealment

Three included trials were given an A score (Blake *et al.*, 2003) for secure allocation concealment. The participants for Coskun *et al.* (2000) were randomized in equal proportions to either day 2/3 or day 5/6 embryo transfer via a sealed envelope on the day of fertilization check. Allocation concealment, again by sealed opaque envelopes, was employed by two trials where randomization took place at the start of the cycle (Livingstone and Bowman, 2001; Van der Auwera *et al.*, 2002). Gardner *et al.* (1998a) gained allocation concealment score B for using a computer-generated allocation on day 8 of the ovarian stimulation cycle, but the method of concealment was unclear. Karaki *et al.* (2002) gained a B score—the 'box containing two types of cards within envelopes' was not explicitly stated to maintain allocation concealment. Another study also performed a 'drawing of lots' on the day of fertilization, and scored B due to the unclear verification of patient allocation (10 unaccounted patients) (Demylle *et al.*, 2000). Rienzi *et al.* (2002) scored B, describing a 'computer generated randomization list' but allocation concealment was not mentioned. The remaining included trials were also allocated a B score for stating that the patients were randomly assigned, or divided with no further details provided (Janny *et al.*, 1993; Motta *et al.*, 1998a,b; Boyarsky *et al.*, 2001; Levitas *et al.*, 2001; Bungum *et al.*, 2002; Levron *et al.*, 2002; Schillaci *et al.*, 2002). The four studies identified as quasi-random, for the use of the weekday of oocyte retrieval or day 2 as the method of allocation, were excluded from the meta-analysis (Scholtes *et al.*, 1996; Gudmundsson *et al.*, 1998; Huisman *et al.*, 2000; Plachot *et al.*, 2000). Quasi-randomization by weekday amounts to inadequate concealment prior to allocation (Blake *et al.*, 2003). It may also introduce a particular form of bias in IVF where patients who respond rapidly or slowly to gonadotrophin stimulation may end up having their oocyte retrievals at predictable times of the week.

Blinding and power analysis

The length of culture and the day of embryo transfer is different for each of the experimental groups, making it impossible to blind which group a patient was in from either the doctor, scientist, nurse or patient. There was no evidence to suggest that the outcome assessor or statistician in any trial was blinded to the assignment status. A power calculation was mentioned in only one trial (Livingstone and Bowman, 2001), although the final results of this trial, in fulfilment of the power calculation to demonstrate a significant reduction in the occurrence of multiple pregnancy, are yet to be reported.

Intention to treat, withdrawals and drop-outs

The 'blastocyst transfer à la carte' policy of Boyarsky *et al.* (2001) was in fact an intention-to-treat (ITT) analysis of the randomized groups where the blastocyst group only proceeded to blastocyst culture if they had ≥2 8-cell embryos on day 3 (11 out of 26; but all 26 were analysed in the 'blastocyst' group). Motta *et al.* (1998a,b) was the only study to clearly include patients where no fertilization took place in the outcome statistics. Although no other trials stated that an ITT analysis was performed, it was possible to express data as an ITT analysis for all trials. Identification of patients failing to have an embryo transfer was not stated or unclear in some trials. Coskun *et al.* (2000) implied that a 100% embryo transfer rate was achieved in both day 2/3 and day 5/6 groups. Although embryos of a lesser stage were transferred in this trial when blastocysts were not available, this transfer rate appears very high. The day 5/6 embryo transfer rate in the remaining studies ranged from 71 to 96%. In one study there was a loss of 10 patients between allocation and embryo transfer that was unaccounted for (Demylle *et al.*, 2000). Whether randomization was performed prior to gonadotrophin stimulation (Livingstone and Bowman, 2001; Van der Auwera *et al.*, 2002), prior to oocyte retrieval (Gardner *et al.*, 1998a), at or after oocyte retrieval (Janny *et al.*, 1993; Motta *et al.*, 1998a,b; Boyarsky *et al.*, 2001; Levitas *et al.*, 2001; Levron *et al.*, 2002), after fertilization check (Coskun *et al.*, 2000; Demylle *et al.*, 2000; Karaki *et al.*, 2002; Rienzi *et al.*, 2002; Schillaci *et al.*, 2002) or on day 2, had an affect on the number of withdrawals in each trial.

Attempts were made to obtain additional information regarding all aspects of randomization, blinding, power analysis and ITT from all trial authors where eligibility of the trial or utility of the data was in doubt.

Results

All the pre-specified meta-analyses were carried out and the results are presented in the text below; some of the important meta-analyses are shown in Figures 1–3. Not all studies provided data for each of the outcome measures reported.

Primary outcomes

Clinical pregnancy per couple randomized

Eleven RCT, with a combined total of 1107 women, reported pregnancy rate per couple randomized. The meta-analysis (Figure 1a) showed no significant difference in pregnancy rate between day 2/3 and day 5/6 transfer [day 2/3, 39.6% versus day 5/6, 42.0%; Peto odds ratio (OR) 0.91, 95% CI 0.71–1.17].

Subgroup analyses showed no significant benefit of the timing of embryo transfer when trials with transfer of equal numbers of blastocysts and cleavage stage embryos were pooled (Figure 1c) or when trials with transfer of fewer blastocysts than cleavage stage embryos were pooled (Figure 1b). There were also no significant differences in pregnancy rates in any subgroup analysis when the trials were broken down according to good, poor or unselected prognosis.

The results of the meta-analyses were all stable to the inclusion/exclusion of trials with co-interventions—first, assisted hatching, and second, use of different culture media for the two groups.

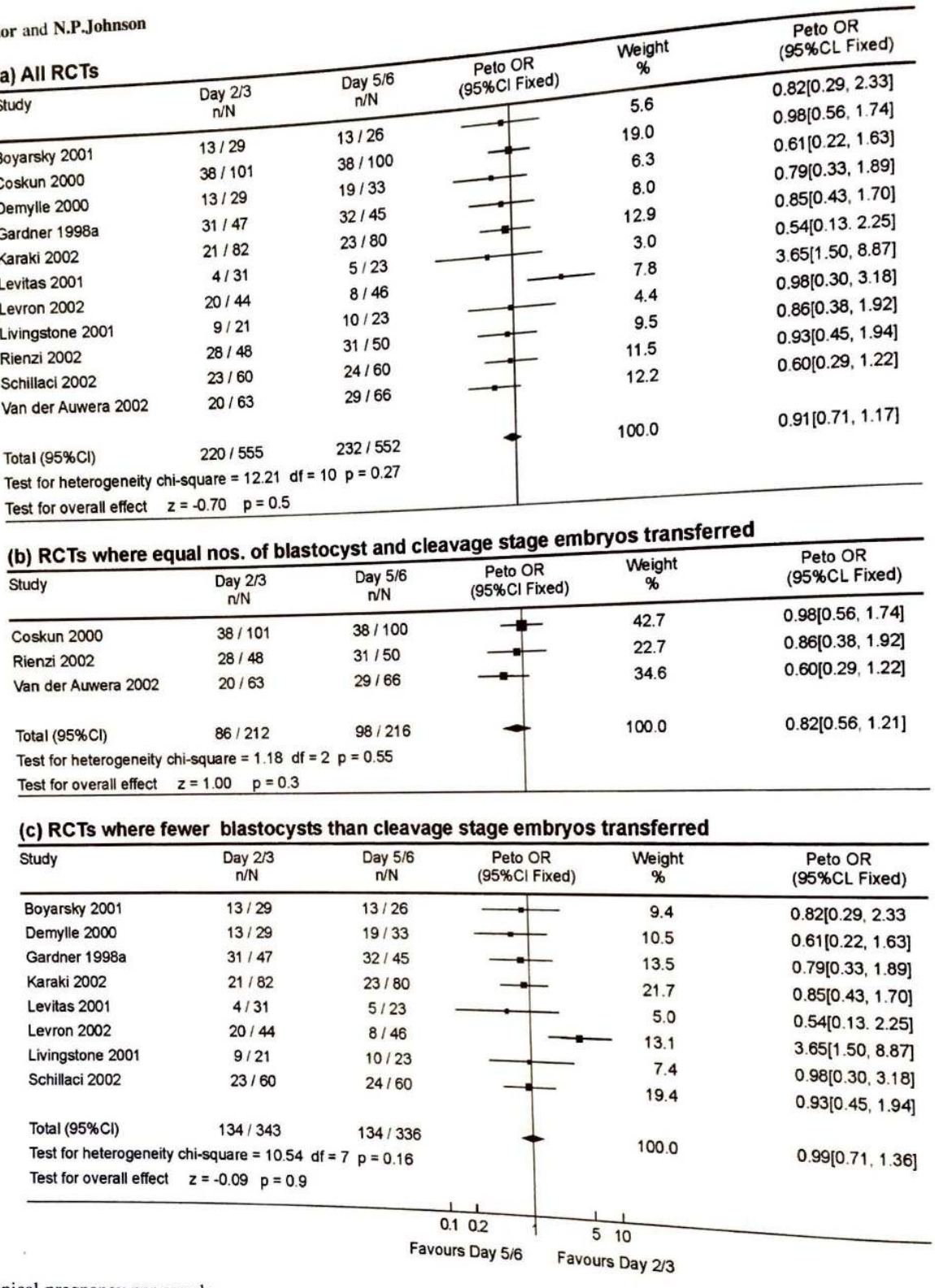

Figure 1. Clinical pregnancy per couple.

There was also no clear trend when pregnancy was expressed per oocyte retrieval or per embryo transfer (Blake *et al.*, 2003).

Live birth per couple randomized

Two RCT (n = 227) reported live birth rates (Rienzi *et al.*, 2002; Van de Auwera *et al.*, 2002). There was no statistically significant difference in live birth per woman randomized between cleavage stage and blastocyst transfer (day 2/3, 36.9% versus day 5/6, 41.4%; Peto OR 0.83, 95% CI 0.48–1.42).

Again for the subgroups, whether only trials with transfer of equal numbers of embryos or fewer blastocysts, or whether only trials with good, poor or unselected prognosis, were analysed, no significant differences were present. The meta-analysis results were stable to sensitivity analyses with inclusion/exclusion of trials with co-interventions.

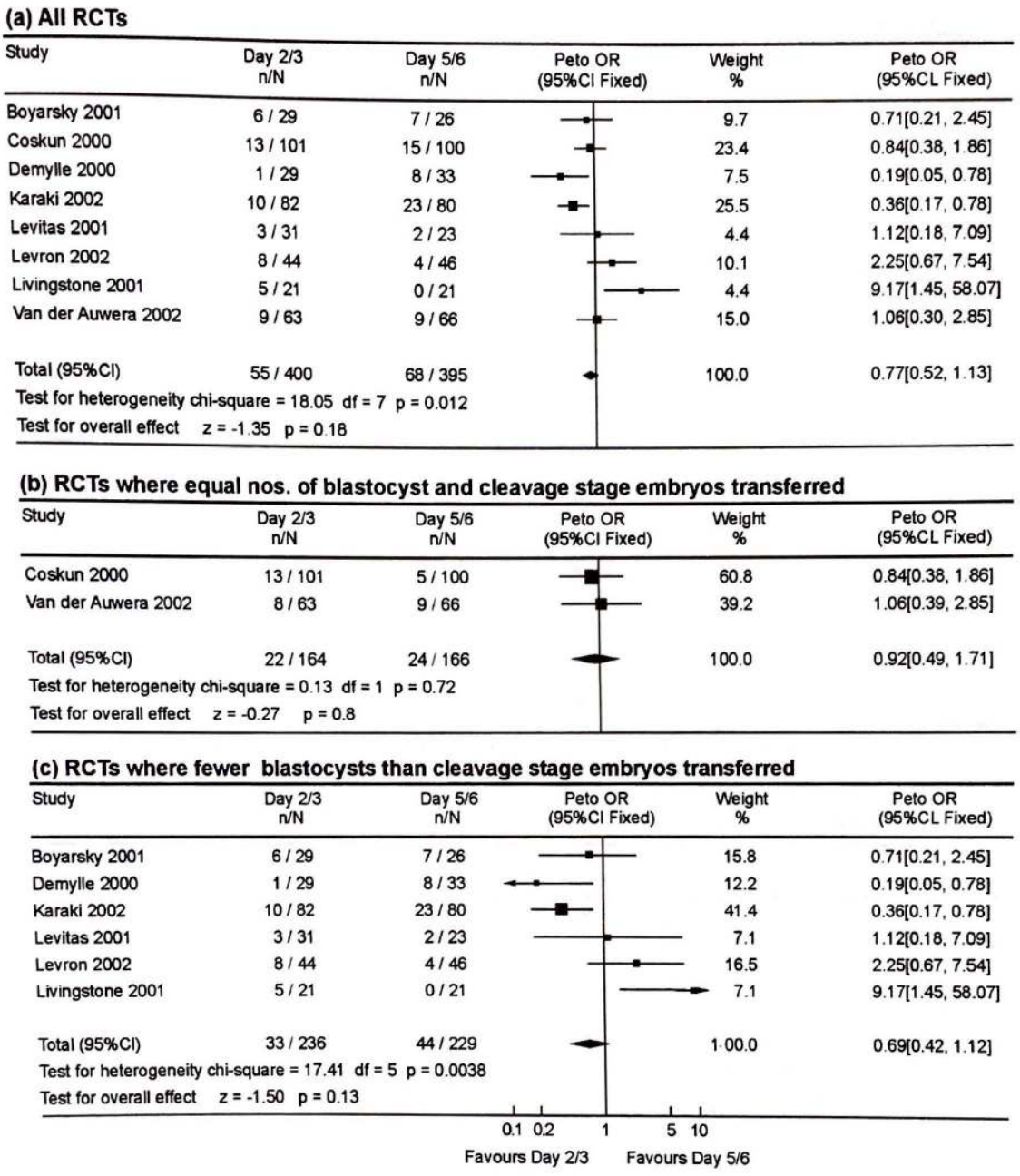

Figure 2. Multiple pregnancies per couple.

Estimation of live birth per oocyte retrieval did not alter the conclusions, although live birth per embryo transfer, based on only one trial (Van der Auwera *et al.*, 2002), was significantly higher in favour of day 5/6 transfer. Interpretation of such results must be cautious—these data do not generate valid estimates of confidence intervals as the unit of analysis (per embryo transfer) is different from the unit of randomization (women or couples).

Multiple pregnancy per couple

Eight RCT ($n = 795$) reported the outcome multiple pregnancy rate per couple. The meta-analysis showed no statistically significant difference in multiple pregnancy per couple between day 2/3 and day 5/6 transfer (Peto OR 0.77, 95% CI 0.52–1.13) (Figure 2a). Ten RCT also reported multiple pregnancy rate per pregnancy. All but two of these trials reported no statistically significant difference in multiple pregnancy rate; Demylle *et al.* (2000) reported a significantly lower rate in the cleavage stage transfer group (Peto OR 0.19; 95% CI 0.04–0.90); Livingstone and Bowman (2001), who had a policy of single blastocyst transfer, had a significantly higher rate of multiple pregnancy in the cleavage stage transfer group than in the blastocyst transfer group, where there were no multiple pregnancies (Peto OR 15.09, 95% CI 2.06–110.48).

Subgroup analyses showed no significant difference in occurrence of multiple pregnancy when equal numbers of embryos (Figure 2b) or when fewer blastocysts than cleavage stage embryos (Figure 2c) were transferred. Subgroup analyses

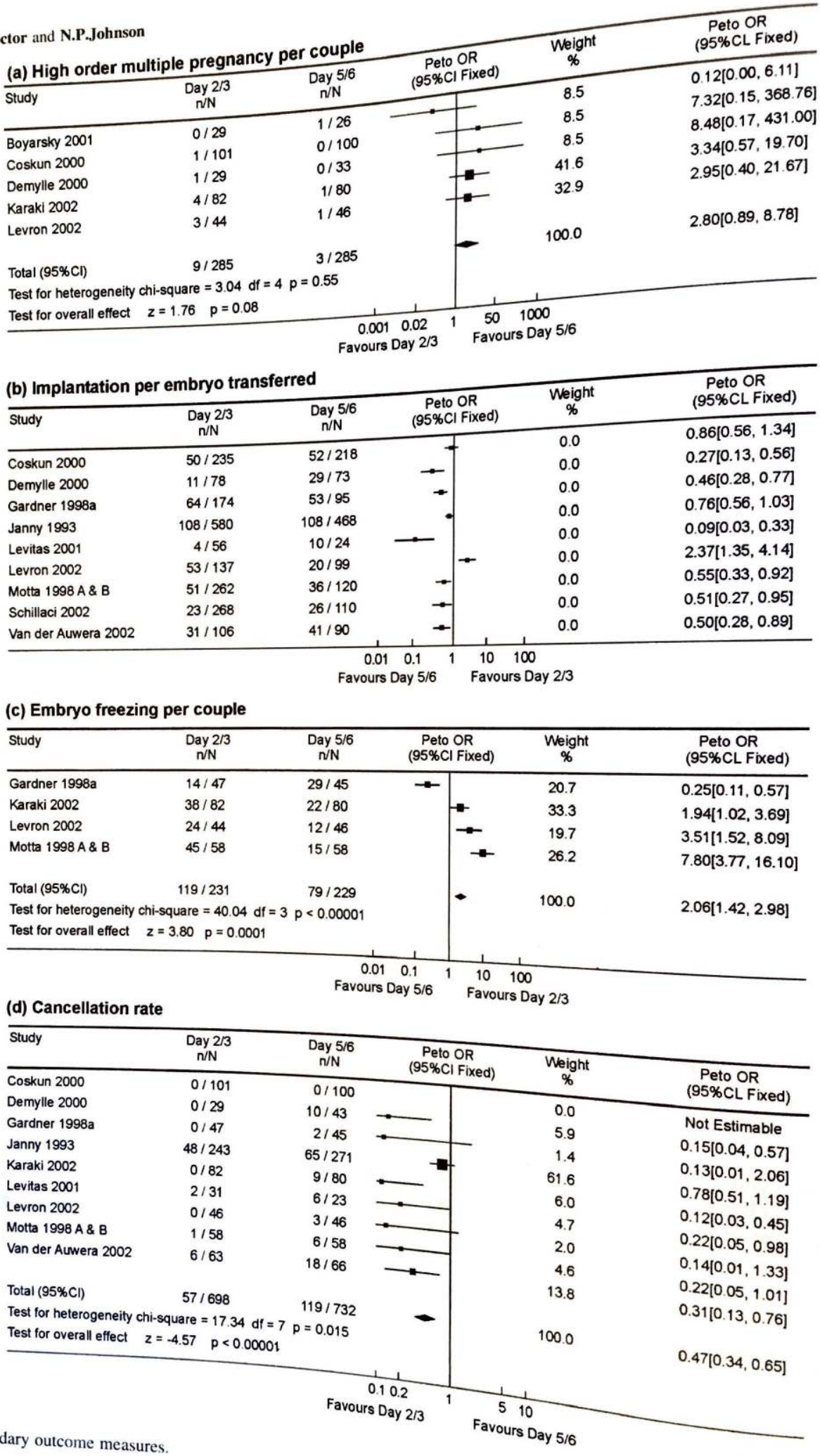

Figure 3. Secondary outcome measures.

Table II. Implications for practice

There is no evidence to suggest a difference in the odds of pregnancy for day 2/3 versus day 5/6 embryo transfer
There is insufficient evidence to suggest a decrease in multiple or high order multiple pregnancy rates following blastocyst transfer, even when a policy of replacement of fewer day 5/6 than day 2/3 embryos is employed
Advantages of blastocyst culture and day 5/6 transfer include:
increased chance of implantation
Maintenance of the chance of pregnancy in the face of transfer of fewer embryos
Advantages of cleavage stage embryo transfer on day 2/3 include:
decreased chance of cancellation between oocyte retrieval and embryo transfer
increased chance of cryopreservation of embryos
There is currently no strong evidence to support the widespread routine use of blastocyst culture in IVF

Table III. Implications for future research. Optimization of extended culture conditions may lead to improved outcomes from blastocyst culture—this must be kept under continual re-evaluation in robust randomized controlled trials (RCT)

Future RCT should have:
adequate power to demonstrate clinically important differences
explicit pre-specified embryo transfer policies for both groups
analysis per woman or per couple randomized, with full disclosure of all study participants, analysed on an intention-to-treat basis, to retain statistical validity
long-term follow-up reports of cumulative live birth rates (including results from frozen embryo cycles)
more complete reporting of secondary outcomes, including miscarriage, ectopic pregnancy, multiple pregnancy (including monozygotic twinning)
Research into improved blastocyst cryopreservation techniques is also required if it is to be considered a reliable and successful replacement for day 2/3 culture
The approach of transfer of a single blastocyst to minimise multiple pregnancies merits wider evaluation (versus double and versus single cleavage stage transfer) in units with a sufficiently high implantation rate

of good, poor or unselected prognosis showed no significant differences.

Secondary outcomes

High order multiple pregnancy

Five RCT (n = 570) reported high order multiple pregnancy rate per couple randomized (Figure 3a) and showed no statistically significant difference between day 2/3 and day 5/6 transfer (Peto OR 2.80, 95% CI 0.89–8.78). Six RCT reported rate per pregnancy, with a pooled odds ratio significantly increased for the day 2/3 transfer group (Peto OR 3.83, 95% CI 1.58–9.27).

One sextuplet pregnancy identified in a quasi-randomized trial (Plachot *et al.*, 2000) was the result of three IVF implantations and three natural conception implantations as the couple had intercourse on the day of oocyte retrieval and only three embryos were transferred.

Monozygotic twinning

No trials reported data on monozygotic twinning rates.

Implantation per embryos transferred.

Raw reported data for implantation rate per embryo transferred were either reported or able to be calculated in nine RCT (Figure 1b). Of these trials, six showed a statistically significant increase in implantation rate for day 5/6 transfer (Gardner *et al.*, 1998a; Motta *et al.*, 1998a,b; Demylle *et al.*, 2000; Levitas et al., 2001; Schillaci *et al.*, 2002; Van der Auwera *et al.*, 2002), two showed no significant difference and one showed a significant increase in implantation rate for day 2/3 transfer (Levron *et al.*, 2002).

Miscarriage

The one RCT in the meta-analysis to assess miscarriage per couple randomized (Coskun *et al.*, 2000) showed no statistically significant difference between day 2/3 and day 5/6 transfer (Peto OR 1.66, 95% CI 0.41–6.81). A similar result was obtained when miscarriage was expressed per pregnancy.

Ectopic pregnancy

No trials reported ectopic pregnancy data.

Embryo freezing rate

Four RCT (n = 460) provided data on the number of couples with embryos available for cryopreservation (Figure 3c). There was a significant increase in the number of couples with embryos freeze-stored in the day 2/3 versus day 5/6 group (Peto OR 2.06, 95% CI 1.42–2.98). However, significant heterogeneity was detected (χ^2 = 40.04, df = 3, P < 0.00001). Removal of the RCT with the unusually low day 2/3 freezing rate (Gardner *et al.*, 1998a) eliminated heterogeneity.

Embryo transfer rate

Nine RCT provided data that enabled a meta-analysis of embryo transfer rate that was inversely expressed as 'cancellation rate' (defined as the number of cycles failing to result in an embryo transfer divided by the number of cycles having an oocyte retrieval) (Figure 3d). There was a significantly lower cancellation rate in the day 2/3 group (8.2%) compared with day 5/6 (16.3%) (Peto OR 0.47, 95% CI 0.34–0.65).

Embryo utilization rate

One RCT and one quasi-randomized trial provided adequate information for the utilization rate (total number of embryos transferred and cryopreserved divided by the total number of pronuclear embryos) to be calculated for each group. The RCT (Van der Auwera *et al.*, 2002) showed no significant difference between day 2/3 and day 5/6 transfer (Peto OR 1.08, 95% CI 0.81–1.45); the quasi-randomized trial (Huisman *et al.*, 2000) had a utilization rate of 67.1% for day 2/3 and 54.8% for day 5/

6, a significant difference in favour of day 2/3 transfer (Peto OR 1.68, 95% CI 1.53–1.84).

Discussion

This systematic review of randomized trials has found no evidence to support an improvement in pregnancy or live birth rates from a policy of blastocyst versus cleavage stage embryo transfer for couples entering an IVF programme. The implications for practice are summarized in Table II. Regrettably the fact that so few trials have reported live birth as an outcome is a serious indictment of research in this field, and the implications for further research are summarized in Table III. The improved implantation rate of blastocysts appears to be counteracted by the increased likelihood of cancellation between oocyte retrieval and embryo transfer (and thus failure to achieve an embryo transfer) in couples for whom blastocyst transfer is planned.

There is also insufficient evidence to support a reduction in the multiple pregnancy or high order multiple pregnancy rate with blastocyst transfer, even when only trials with a policy of transferring fewer blastocysts than cleavage stage embryos are considered. Although the common theme was to replace fewer blastocysts than cleavage stage embryos, the different policies of the absolute number of replaced embryos at each stage in different trials reflects genuine differences of opinion in current clinical practice. The only trial with a policy of transferring fewer blastocysts to show a significant reduction in multiple pregnancies (Livingstone and Bowman, 2001) used single blastocyst transfer versus transfer of two cleavage stage embryos. It is possible that, in order to see a genuine reduction in the multiple pregnancy rate, it is necessary to move to a single embryo transfer policy. Although this approach is gaining popularity, particularly in Europe (Gerris and Van Royen, 2000; De Sutter *et al.*, 2003; Tiitinen *et al.*, 2003), internationally many institutions remain far from this policy. A further important question is whether extended culture and blastocyst transfer are essential prerequisites for single embryo transfer, given the impressive results reported in some series of single cleavage stage embryo transfer (Martikainen *et al.*, 2001).

Most RCTs found a significant increase in implantation rates for blastocyst versus cleavage stage embryo transfer. The most plausible explanations for this are either an improved selectability at the blastocyst stage or the opportunity to replace embryos into a more synchronized uterine environment compared to day 2/3 transfer. Conversely one trial found a significant decrease in implantation rates associated with blastocyst transfer, which adversely affected clinical pregnancy rate per couple in that trial (Levron *et al.*, 2002), emphasizing the exacting nature of culture conditions where extended culture is employed. This was highlighted by the original meta-analysis which showed that the enhanced implantation potential of blastocysts was more pronounced when sequential culture media (compared to single media) were used (Blake *et al.*, 2003). The fact that the experimental and control groups were often not grown in the same culture media does introduce a confounding factor that makes comparisons difficult, although the meta-analysis results and conclusions were stable to these sensitivity analyses. In reality, many of the trials using sequential media either used completely different media for the two transfer groups, for example Ham's F-10 versus G1/G2, or they used a combination of media brands (both manufacturers and in-house made). Ultimately the most clinically relevant study would be to compare these two approaches to culture and embryo transfer, using the best available technique-specific media for each respective stage of embryo.

One patient selection policy that has recently grown in popularity is allowing only those who have 2, 3 or more high quality 8-cell embryos on day 3 to continue on with blastocyst culture for day 5/6 embryo transfer (Racowsky *et al.*, 2000). This so called 'à la carte' approach to blastocyst culture was compared with a control group of women randomized for day 2/3 embryo transfer in the study carried out by Boyarsky *et al.* (2001). The fact that the data for the day 5/6 group in this study include a subgroup of women who did not receive blastocyst culture is in essence an 'intention to treat' and therefore appropriate to include in the meta-analysis (Vail and Gardener, 2003). Nevertheless, a sensitivity analysis revealed that exclusion of this study results in no significant alteration to any of the meta-analysis outcomes.

This meta-analysis demonstrates well the importance of expressing pregnancy and live birth per woman randomized rather than per oocyte retrieval or, particularly, per embryo transfer. With an increased implantation potential for blastocysts, but an increased cancellation rate between oocyte retrieval and embryo transfer for blastocyst culture, it would be reasonable to expect a higher pregnancy rate per embryo transfer in the day 5/6 group. It was thus surprising that a higher pregnancy rate per embryo transfer in the day 5/6 group was not observed. One possible explanation was the widely variable policy for minimal quality of embryos for transfer that may have existed amongst the trials—some accepted transfer of developmentally delayed embryos on day 5/6, whilst other trials were more selective and refused to transfer embryos that were anything less than a late morula or early blastocyst. Blastocyst formation rates may also influence the pregnancy rate per embryo transfer for each trial. They ranged from 28% (Coskun *et al.*, 2000) where the pregnancy rate per embryo transfer for day 5/6 was 38%, to 46.5% (Gardner *et al.*, 1998a) which had a corresponding 74.4% pregnancy rate. Both trials used sequential media and had identical numbers of embryos transferred (2.2), which highlights the issue that there are many other factors that play a part in pregnancy rates such as exact media constituents, culture conditions, number and quality of retrieved oocytes and patient population.

Pre-selection of good prognosis couples would be expected to maximize the chance of each woman having viable embryos for transfer on day 5, taking into account the 50–60% embryo attrition rate commonly experienced with blastocyst culture. On the other hand, selection of couples with multiple IVF failures for blastocyst transfer might be expected to eliminate endometrial asynchrony as a cause for their previous lack of success. However, subgroup analysis has provided no evidence that selection of couples based on good or poor prognosis for

IVF altered the results—there was no evidence of benefit of day 5/6 transfer even in good prognosis couples selected on the basis of an expectation to do well with blastocyst culture.

There were few data for miscarriage and the finding of no significant difference based on one RCT must be interpreted cautiously. Theoretically the rate of miscarriage might be expected to be lowest with the transfer of highly selected embryos into a synchronous uterine environment. There were no data at all for ectopic pregnancy. It has been suggested that extended culture may create alterations in the zona pellucida that place the embryo at risk of abnormal hatching resulting in monozygotic twinning (De Felici and Siracusa, 1982; Cohen *et al.*, 1990). Indeed a multi-centre retrospective analysis of blastocyst transfers has reported an increased frequency of monozygotic twinning (Behr *et al.*, 2000). Unfortunately, none of the included trials in this systematic review reported on the presence or absence of monozygotic twinning.

Data for overall embryo utilization (the proportion of all embryos which were either transferred or cryopreserved) were available for only one RCT and one quasi-randomized study. The RCT showed no significant difference in embryo utilization (Van der Auwera *et al.* 2002). The large size of the quasi-randomized study by Huisman *et al.* (2000) does, however, strengthen our confidence in its result, showing a significantly higher utilization in the day 2/3 transfer group. The number of high quality excess embryos available for freezing after transfer of fresh embryos primarily influences this factor. Four included RCT did, however, report on the number of couples who had embryos cryopreserved in each group. Overall the rate of embryo freezing was significantly higher for the day 2/3 group (51.5%) than the day 5/6 group (34.1%). This result is not unexpected owing to the reduced number of morphologically normal embryos remaining after extended selective culture and day 5/6 transfer.

The number of embryos frozen is an important consideration when assessing the effectiveness of a treatment because it offers couples an additional opportunity to achieve a pregnancy. When considering an alteration in embryo transfer from day 2/3 to day 5/6, the benefits of higher implantation rates with the disadvantages of fewer cryopreserved embryos must be weighed up. Yet another consideration is the issue of time—it has been suggested that a policy of day 5/6 transfer may result in pregnancy sooner and from fewer embryo transfer cycles than day 2/3 transfer (Blake *et al.*, 2003). Freezing protocols for early cleavage and blastocyst stage embryos are fundamentally different and the effectiveness of the latter has yet to be widely accepted, particularly in embryos that have been cultured in sequential media. None of the included trials fully reported data on pregnancies following transfer of the frozen embryos. Such reporting is also unlikely to be forthcoming in the future because of the long time span particularly between a woman's pregnant cycle and a subsequent frozen embryo cycle. Ultimately the crucial statistic is the proportion of couples to achieve a (preferably singleton) live birth from a single IVF stimulation cycle, taking into account transfer of both fresh and frozen embryos resulting from that cycle (the 'total cryo-augmented live birth rate'). Such survival analysis data are rarely reported in trials and often take many years to accumulate.

Advocates of blastocyst culture have suggested that patients may prefer to be informed on day 5 if their embryos had low viability with no embryo transfer, rather than continue and be given a chance of pregnancy (albeit small). However, there has been little research into the emotional status of women given such choices (Borg *et al.*, 2000). Such confidence in the culture conditions during extended culture may need to be treated with caution for two reasons. Firstly, what is the certainty of an embryo's viability based on its morphology on day 5? Indeed there are widespread reports of pregnancies from developmentally delayed morulas on day 5, although this is also true for poor morphology in cleavage-retarded embryos on day 2/3. The evidence of higher implantation rates of blastocysts, particularly with sequential media, suggests that either selection criteria or viability *per se* are improved by extending culture. Secondly, if blastocyst culture is used strictly to select out the most viable embryos, there is the possibility that the slow-cleaving embryo on day 3 may have a higher chance of pregnancy if replaced into the uterus early than if subjected to extended culture (Racowsky *et al.*, 2000). Adaptability of an embryo to survive extended culture may come at the price of viability.

Cost comparisons of treatment have not been investigated in this review but are also important. From the laboratory's perspective, the cost of setting up for blastocyst culture may be substantial. An additional incubator is often required due to the extra 2–3 days that the embryos remain in culture. The extra media costs, on the other hand, are negligible. Blastocyst culture is moderately more labour intensive, however, and laboratory staff may be required to perform more weekend work, particularly if embryos from two different stages of development need 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. Ultimately the cost of the treatment mode must be weighed against the odds of a healthy take-home baby.

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