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Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis

Evangelos G. Papanikolaou[1], Efstratios M. Kolibianakis, Herman Tournaye, Christos A. Venetis, Human Fatemi, Basil Tarlatzis and Paul Devroey

Centre for Reproductive Medicine, University Hospital, Vrije Univestiteit Brussel (Free University of Brussels), Laarbeeklaaan 101, B-1090 Brussels, Belgium

[1]Correspondence address. Tel: +32-2-4261042/4776607; E-mail: evangelos.papanikolaou@vub.ac.be/drvagpapanikolaou@yahoo.gr

BACKGROUND: Both cleavage-stage and blastocyst-stage embryo transfer policies have advantages and drawbacks. The number of embryos transferred, however, is a crucial parameter that needs to be considered before attempting any comparison. METHODS: An extensive literature search yielded initially 282 studies from which 8 randomized controlled trials met the inclusion criteria: (i) truly randomized design (ii) policy to transfer equal number of embryos in both the cleavage-stage and the blastocyst-stage groups and (iii) published as full text in a peer-review journal. Primary outcome was the live birth rate and secondary outcomes were clinical pregnancy rate, multiple pregnancy rate, cancellation rate and cryopreservation rate. RESULTS: A total of 1654 patients were reviewed. Live birth rate per randomized patient was significantly higher ($n = 6$ studies) in patients who had a blastocyst-stage transfer as compared to patients with cleavage-stage embryo transfer [odds ratio (OR): 1.39, 95% confidence interval (CI): 1.10–1.76; $P = 0.005$]. Clinical pregnancy rate (OR: 1.27, 95% CI: 1.03–1.55; $P = 0.02$) and cancellation rate per patient randomized (OR: 2.21, 95% CI: 1.47–3.32; $P = 0.0001$) were significantly higher in patients with a blastocyst-stage embryo transfer as compared to patients in whom a cleavage-stage embryo transfer was performed. The cryopreservation rate was significantly higher in the cleavage-stage group (OR: 0.28, 95% CI: 0.14–0.55; $P = 0.0002$). CONCLUSIONS: The best available evidence suggests that the probability of live birth after fresh IVF is significantly higher after blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer when equal number of embryos are transferred in the two groups compared.

Keywords: meta-analysis; live birth rate; blastocyst; embryo transfer; cleavage stage

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Introduction

Although the first milestone publication in assisted reproduction technology (ART) reported on the development of human blastocysts in culture (Steptoe *et al*., 1971), subsequently IVF was based on the transfer of embryos at the cleavage stage. During the last decade, the extension of embryo culture to Day 5 has gained popularity in IVF practice (Wilson *et al*., 2002). The main reasons for this new strategy were mainly scientific; first the embryo does not implant before Day 5 and the implantation rates with IVF treatment and Day 2 or 3 embryo transfers were still remaining low (Nyboe Andersen *et al*., 2004); second, the understanding of the different metabolic needs of the embryo during its development and the evolution of new sequential media render possible the culture of embryos *in vitro* until the fifth day with relatively good survival rates (Gardner *et al*., 1998).

The advantages and disadvantages of blastocysts over cleavage-stage embryos are definite (Tsirigotis, 1998). The advantages of blastocysts are the better correlation between morphology and euploidy status, better synchronization with an endometrium already affected by an IVF stimulated cycle and the high implantation potential. On the other hand, blastocysts have certain drawbacks, such as the lack of one day co-culture with endometrial cells (embryo descends into the uterine cavity on Day 4); the increased possibility of some embryos not developing into blastocysts *in vitro* and as a result cancellation of embryo transfer; and the decreased embryo freezing rate combined with technical difficulties in the cryopreservation/thawing process in such expanded embryos.

A recent Cochrane meta-analysis found no evidence of a difference in live birth or pregnancy outcomes between Days 2–3 and 5–6 transfers of embryos (Blake *et al*., 2005).

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Moreover, blastocyst transfer was associated with an increase in failure to transfer any embryos in a cycle and a decrease in embryo freezing rates.

A fundamental error performed in many randomized controlled trial (RCTs) comparing blastocyst transfer over cleavage-stage transfer was that the number of embryos replaced was unequal in the two groups, with more embryos transferred in the cleavage-stage group. Therefore, by allowing by definition (at randomization point) more embryos to be replaced in the Day 3 group, then the relative advantage of blastocysts having better correlation with genetic quality disappears. If we consider that accumulating evidence suggests that in top-quality cleavage-stage embryos up to 60% might be aneuploid, whereas in top-quality blastocysts this percentage might reach 30% (Staessen *et al*., 2004), it is obvious that the fewer embryos we transfer [i.e. single-embryo transfer (SET)] the higher the chance to transfer genetically abnormal embryos when earlier developmental stage embryos are transferred. Thus the transfer of extra embryos in the Day 3 group compared with Day 5 group increases the probability of transferring a euploid embryo and thereby reducing the selection bias in early embryonic developmental stages (cleavage stages).

Since the aforementioned meta-analysis emerged, two more studies have been published increasing by 40% the study population (Papanikolaou *et al*., 2005, 2006). In addition, effort was made to contact authors of published studies to provide further data on live birth rates.

The present meta-analysis attempts to investigate whether live birth rate is influenced by the developmental stage of the embryo, where the policy for the number of embryos replaced was equal in both cleavage-stage and blastocyst-stage groups.

Materials and Methods

Search strategy and identification of studies

A literature search was performed in the following electronic databases: MEDLINE (1966 to July 2007), EMBASE (1980 to July 2007), CENTRAL (The Cochrane Library Issue 1, 2007), the Cochrane Menstrual Disorders and Subfertility Group trial register (searched in 10th July 2007). The free-text search terms 'Day two or Day 2', 'Day three or Day 3', 'cleavage', 'Day five or Day 5', 'Day six or Day 6' and 'blastocyst*' combined with 'embryo* transfer*' were used. Additionally, the citation lists of all relevant publications, review articles and included studies were hand-searched. No language limitations were applied.

Selection of studies

Criteria for inclusion/exclusion of studies were established before literature search. A study was considered eligible only if the researchers applied a policy to transfer equal number of embryos in the two groups compared. Moreover, a study was included in the current systematic review, if it followed a prospective, two-arm, parallel design and it was truly randomized. Studies that incorporated pseudo-randomization methods (sequential numbers, date of birth, allocation by week day) were excluded from the main analysis. However, a sensitivity analysis with the inclusion of such studies was carried out to check the robustness of the results obtained.

Studies not published as full manuscripts in peer-reviewed journals were not considered for this review since they cannot be adequately evaluated for their design and quality. Moreover, it has been shown, that although there is a considerable publication deficit in reproductive medicine for RCTs, there is no concomitant publication bias (Evers, 2000).

Identification of studies

The literature search yielded 282 studies, the screening of the titles resulted in 50 publications that could provide information relevant with the question of interest (Fig. 1). The examination of the abstracts reduced the potentially eligible studies to 19, the manuscript of which was retrieved for a more detailed evaluation. By this process, studies were excluded because they (i) did not follow a policy of transferring equal number of embryos in the two groups compared ($n = 6$), (ii) were pseudo-randomized ($n = 1$), (iii) were published as abstracts in meeting proceedings ($n = 2$), (iv) included overlapping data with another eligible study ($n = 1$) and (v) included a co-intervention ($n = 1$) (Table I). The references of all the studies in which the full text was retrieved were hand-searched. However, no additional studies that could provide data to answer the research question were found. Eventually, eight studies were included in the present systematic review and meta-analysis (Table II) (Coskun *et al*., 2000; Rienzi *et al*., 2002; Van der Auwera *et al*., 2002; Bungum *et al*., 2003; Hreeinsson *et al*., 2004; Kolibianakis *et al*., 2004; Papanikolaou *et al*., 2005, 2006).

Data extraction

The following data were recorded from each of the eight eligible studies: demographic (citation data, country, study period, number of patients included, number of cycles performed and age of population), procedural (randomization time point, inclusion criteria at randomization, power analysis,

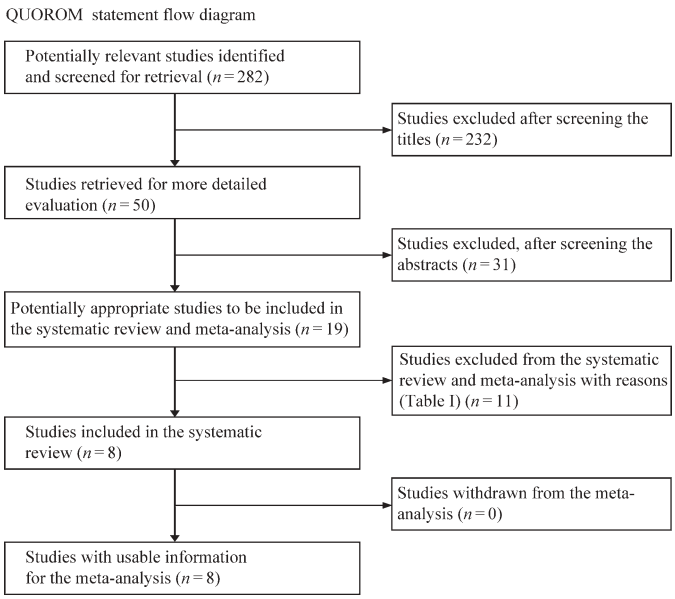

Figure 1: QUOROM statement flow diagram

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Table I. Studies excluded from the systematic review and meta-analysis and reason for exclusion.

Study	Reason for exclusion
Zech *et al*. (2007)	Pseudo-randomized
Minasi *et al*. (2002)	Published as abstract in meeting proceedings
Levron *et al*. (2001)	Published as abstract in meeting proceedings
Scholtes and Zeilmaker (1996)	Did not follow a policy of transferring equal number of embryos in the two groups compared
Karaki *et al*. (2002)	Did not follow a policy of transferring equal number of embryos in the two groups compared
Levron *et al*. (2002)	Did not follow a policy of transferring equal number of embryos in the two groups compared
Emiliani *et al*. (2003)	Did not follow a policy of transferring equal number of embryos in the two groups compared
Pantos *et al*. (2004)	Did not follow a policy of transferring equal number of embryos in the two groups compared
Levitas *et al*. (2004)	Did not follow a policy of transferring equal number of embryos in the two groups compared
Utsunomiya *et al*. (2004)	Included a co-intervention
Kolibianakis *et al*. (2005)	Overlapping data with Kolibianakis *et al*. (2004)

intension to treat analysis or not, blinding regarding allocation arm, type of down-regulation, protocol of ovarian stimulation, type of gonadotrophin administered, time of oocyte retrieval, type of fertilization, number of oocytes retrieved, day of embryo transfer, blastulation rate, number of embryos transferred, culture media used and type of luteal support administered), outcome data (pregnancy achievement, pregnancy loss, multiple pregnancies, cancellation of embryo transfer, number of fetal hearts per transferred embryos, cryopreservation rate and achievement of delivery). Any disagreement was resolved unanimously by discussion.

Outcomes

The main outcome measure chosen for meta-analysis were live birth rate per patient randomized. Secondary outcome measures included clinical pregnancy rate per patient randomized (defined as the detection of fetal heart by ultrasound at 7 weeks of gestation), multiple pregnancy rate per clinical pregnancy, cancellation rate and cryopreservation rate. In case the studies did not report data regarding one or more of the above outcome measures, the authors were contacted and asked to provide the missing information.

Quantitative data synthesis

The dichotomous data results for each of the studies eligible for meta-analysis were expressed as an odds ratio (OR) with 95% confidence intervals (CIs). These results were combined for meta-analysis using the Mantel/Haenszel model, when using the fixed effects method, and the DerSimonian and Laird method, when using the random effects method.

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Table II. Design characteristics of the studies included in the systematic review and meta-analysis.

Studies	Journal and study period	Randomization method/blinded	PC, ITT analysis	Randomization time-point	Criteria	Outcome measure	Embryo transfer policy
Coskun *et al*. (2000)	Hum Reprod, not stated	Sealed envelope/No	No-PC ITT analysis	Day 1 post oocyte retrieval	When four (four) 2-PN embryos	Clinical pregnancy rate	Maximum three embryos
Rienzi *et al*. (2002)	Hum Reprod, not stated	Computer-generated randomization list/No blinded	No-PC, ITT analysis	Day 1 post-oocyte retrieval	When eight 2-PN embryos, <38 years, ICSI	Live birth rate	double-embryo transfer—fixed
Van der Auwera *et al*. (2002)	Hum Reprod, not stated	Sealed envelope/No	PC performed but abandoned, no ITT analysis	At initiation of stimulation	<40 years	Live birth rate	Maximum two embryos
Bungum *et al*. (2003)	RBM Online 12/2001-5/ 2002	Sealed envelope/No	No-PC,	Day 3 post-oocyte retrieval	When three or more 8-cell embryos with<20% fragmentation first or second trial	Clinical pregnancy rate	Maximum two embryos
Hreinsson *et al*. (2004)	EJOGRB, not stated	Sealed envelope	Yes-PC, but not achieved	Day prior to HCG	When six follicles were present	Clinical pregnancy rate	Maximum two embryos
Kolibianakis *et al*. (2004)	Hum Reprod	Computer-generated randomization list/No	Yes-PC	Prior to stimulation	<41 years	Ongoing pregnancy rate	Maximum two embryos
Papanikolaou *et al*. (2005)	Hum Reprod	Computer-generated randomization list/No	Yes-PC ITT analysis	Day 3 post-oocyte retrieval	When four embryos >6cell and <10% fragmentation first to third trial	Live birth rate	double-embryo transfer—fixed
Papanikolaou *et al*. (2006)	NEJM 7/ 2003-11/2004	Computer-generated randomization list/No	Yes-PC, ITT analysis terminated earlier	At consultation prior to stimulation	<36 years first or second trial	Live birth rate	SET—fixed

EJOGRB, European Journal of Obsterics and Gynecology and Reproductive Biology;Hum Reprod, Human Reproduction; ITT, intention to treat; NEJM, New England Journal of Medicine; PC, Power calculation; 2-PN, 2 pronuclei.

When the outcome of interest was of a continuous nature, the differences were pooled across the studies, which provided information on this outcome parameter, resulting in a weighted mean difference with 95% CI. The inverse variance method and the DerSimonian and Laird method were used when the fixed or random effects method, respectively, was applied.

All results were combined for meta-analysis with Revman Software (Version 4.2 for Windows, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2003). Study-to-study variation was assessed by using the chi-square statistic (the hypothesis tested was that the studies are all drawn from the same population, i.e. from a population with the same effect size). A fixed effects model was used where no heterogeneity was present, whereas in the presence of significant heterogeneity, a random effects model was applied. A funnel plot analysis and Egger's test were performed in order to detect the presence of publication bias. Statistical significance was set at a P level of 0.05.

Power analysis

It was calculated that a sample size of 2834 subjects (1417 in each group) achieves 80% power to reject the null hypothesis using an α error of 0.05 and a two-tailed Fisher's Exact test, assuming a baseline live birth rate of 30% and a clinically important difference of 5% between the two developmental stages of embryo transfer.

Results

Eight studies fulfilled the inclusion criteria for the systematic review. A total of 1654 patients were reviewed (blastocyst transfer: $n = 815$, cleavage-stage transfer: $n = 839$). A sensitivity analysis with the inclusion of the study by Zech *et al*. (which performed allocation of treatment by using a pseudo-random method) was also performed.

Systematic review

Characteristics of the eligible studies are listed in Tables II and III. All included studies were published between 2000 and 2006 and were performed in single centres. The majority of the studies were published in *Hum Reprod* ($n = 5$). In four studies, randomization of patients was based on a computer-generated randomization list. The time point of randomization varied among the eligible studies from prior to stimulation initiation to Day 3 of embryo culture. Treatment allocation was concealed in four studies. Regarding the policy of embryo transfer that was applied, a maximum of two embryos were transferred in both groups in four studies, in two studies a fixed number of two embryos (double-embryo transfer), in one study a fixed number of one (SET) and in one study a maximum of three embryos were transferred in both groups (Table II). The size of the studies ranged from 98 to 442 patients and the median number of patients included was 154.

For ovarian stimulation, a combination of urinary and recombinant gonadotrophins was used in three studies, in two studies this was achieved with urinary gonadotrophins, while in three studies ovarian stimulation was performed with recombinant gonadotrophins.

To inhibit premature LH surge, the long agonist protocol was used in four studies, in three studies this was performed using both the long agonist and the antagonist protocol in the same study, while in one study a fixed Day 6 antagonist protocol was applied (Table III). Luteal support varied between

Table III. Stimulation and embryological characteristics.

Studies	Ovarian stimulation	Luteal supplementation	Fertilization method	Blastulation rate, %	Centre	Clinical pregnancy definition	Culture media
Coskun *et al*. (2000)	GnRH agonist + hMG	Progesterone i.m	IVF/ICSI	28	Single centre	Fetal heart activity on 7 weeks	Sequential Medicult to Day 3 and then G1/G2 to Day 5
Rienzi *et al*. (2002)	GnRH agonist + rFSH	Not reported	ICSI	44.8	Single centre	Fetal heart activity on 7 weeks	Sequential G1/G2 Vitrolife
Van der Auwera *et al*. (2002)	GnRH agonist + HMG	HCG every 3 days or Progesterone pessaries	IVF/ICSI	44.7	Single centre	Fetal heart activity on 7 weeks	Sequential G1/G2 Vitrolife & Cook IVF
Bungum *et al*. (2003)	GnRH agonist + rFSH	Progesterone vaginal	IVF/ICSI	55.2	Single centre	Fetal heart activity on 7 weeks	Sequential G1/G2 Vitrolife
Hreeinsson *et al*. (2004)	GnRH agonist or GnRH antagonist rFSH and HMG	Not reported	IVF/ICSI	33.0	Single centre	Fetal heart activity on 7 weeks	Sequential Mixture Vitrolife IVF and G1/G2
Kolibianakis *et al*. (2004)	GnRH agonist or GnRH antagonist + rFSH and HMG	Progesterone vaginal	IVF/ICSI	50.7	Single centre	Fetal heart activity on 7 weeks	Sequential G1/G2 Vitrolife
Papanikolaou *et al*. (2005)	GnRH agonist or GnRH antagonist + rFSH and HMG	Progesterone vaginal	IVF/ICSI	51.1	Single centre	Fetal heart activity on 7 weeks	Sequential G1/G2 Vitrolife or Medicult
Papanikolaou *et al*. (2006)	GnRH antagonist + rFSH	Progesterone vaginal	IVF/ICSI	44.6	Single centre	Fetal heart activity on 7 weeks	Sequential G1/G2 Vitrolife or Medicult

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studies. Two studies did not provide details about the type of luteal support used.

Fertilization methods included ICSI ($n = 1$) and IVF/ICSI ($n = 7$). Regarding embryo culture, sequential media were used in all studies. The type of media used, however, varied among studies (Table III).

Meta-analysis

Primary outcome

Live birth rate

The probability of live birth was significantly higher in patients who had a blastocyst-stage compared with those with cleavage-stage embryo transfer (OR: 1.39, 95% CI: 1.10–1.76; $P = 0.005$; heterogeneity: $P = 0.30$; fixed effects model) (Fig. 2). Similarly, the rate difference (RD) was 7% in favour of the blastocyst-stage transfer group (RD: +7% (95% CI: +2 to +12%; $P = 0.005$; heterogeneity: $P = 0.30$; fixed effects model) (Fig. 3). A funnel plot of the included studies is shown in Supplementary Fig. S1. No publication bias was detected in the studies analysed (Egger's test: $P = 0.93$). Six studies offered data for this outcome measure.

Secondary outcomes

Clinical pregnancy rate

The probability of clinical pregnancy was significantly higher in patients with a blastocyst-stage embryo transfer compared with patients in which a cleavage-stage embryo transfer was performed (OR: 1.27, 95% CI: 1.03–1.55; $P = 0.02$; heterogeneity: $P = 0.22$; fixed effects model) (Fig. 4). Eight studies offered data for this outcome measure. A sensitivity analysis with the inclusion of the study by Zech *et al*. (which performed allocation of treatment by using a pseudo-random method) did not materially change the results obtained (OR: 1.29, 95% CI: 1.07–1.56; $P = 0.008$; heterogeneity: $P = 0.28$; fixed effects model) (Fig. 4).

Multiple pregnancy rate

The multiple pregnancy rate was not significantly different between patients in which a blastocyst-stage embryo transfer was performed compared with patients which had a cleavage-stage embryo transfer (OR: 0.86, 95% CI: 0.58–1.29; $P = 0.46$; heterogeneity: $P = 0.57$; fixed effects model) (Fig. 5). Seven studies offered data for this outcome measure.

Cancellation rate

The cancellation rate was significantly higher in patients randomized to have a blastocyst-stage embryo transfer compared with patients randomized to undergo a cleavage-stage embryo transfer (OR: 2.21, 95% CI: 1.47–3.32; $P = 0.0001$; heterogeneity: $P = 0.45$; fixed effects model) (Fig. 6). Eight studies offered data for this outcome measure.

Cryopreservation rate

The cryopreservation rate was significantly lower in the group of patients who had a blastocyst-stage embryo transfer compared with those who had a cleavage-stage embryo transfer (OR: 0.28, 95% CI: 0.14–0.55; $P = 0.0002$; heterogeneity: $P < 0.00001$; random effects model) (Fig. 7). Seven studies offered data for this outcome measure.

Discussion

The current meta-analysis suggests that, when an equal number of embryos are transferred in a fresh IVF cycle, the probability of both live birth and clinical pregnancy is significantly higher when performing the embryo transfer at the blastocyst stage than the cleavage stage. Especially for the live birth rate, this has been estimated in 81% of the total sample size (after vigorous contact with authors of the published studies). The above finding, however, needs further elucidation for it to be the basis of guidelines for use in every day clinical practice.

One of the larger studies included which used SET (Papanikolaou *et al*., 2006) showed that the implantation potential of an *in vitro* cultured blastocyst is higher compared with an *in vitro* cleavage-stage embryo. The current meta-analysis provides the clinical confirmation of the above finding, when an equal number and up to two embryo transfer policy is followed. This seems rational, as through embryonic development there is a natural selection, which does not allow most of the

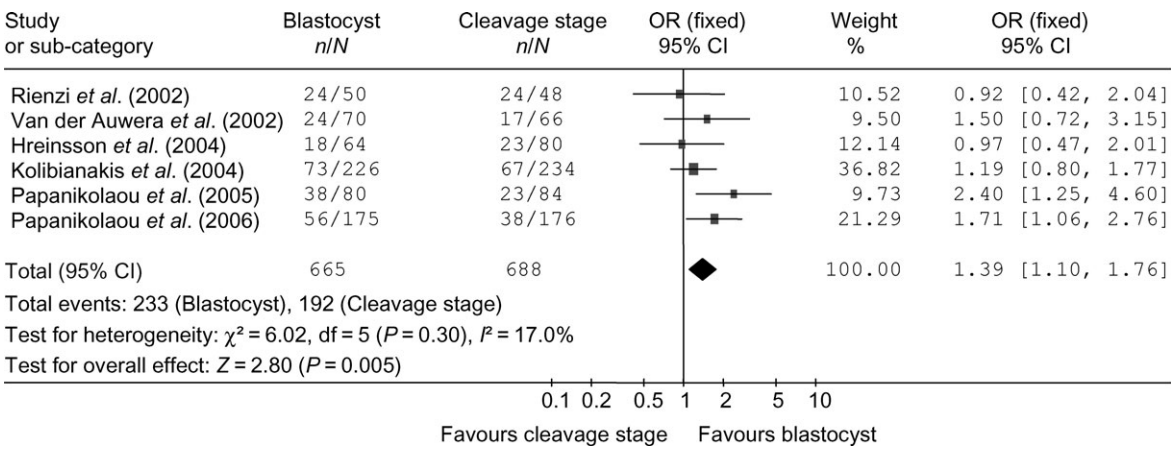

Figure 2: Live birth rate per randomized couple[a]

[a]Additional data were obtained after authors replied to letter sent (Hreinsson *et al*., 2004, Kolibianakis *et al*., 2004).

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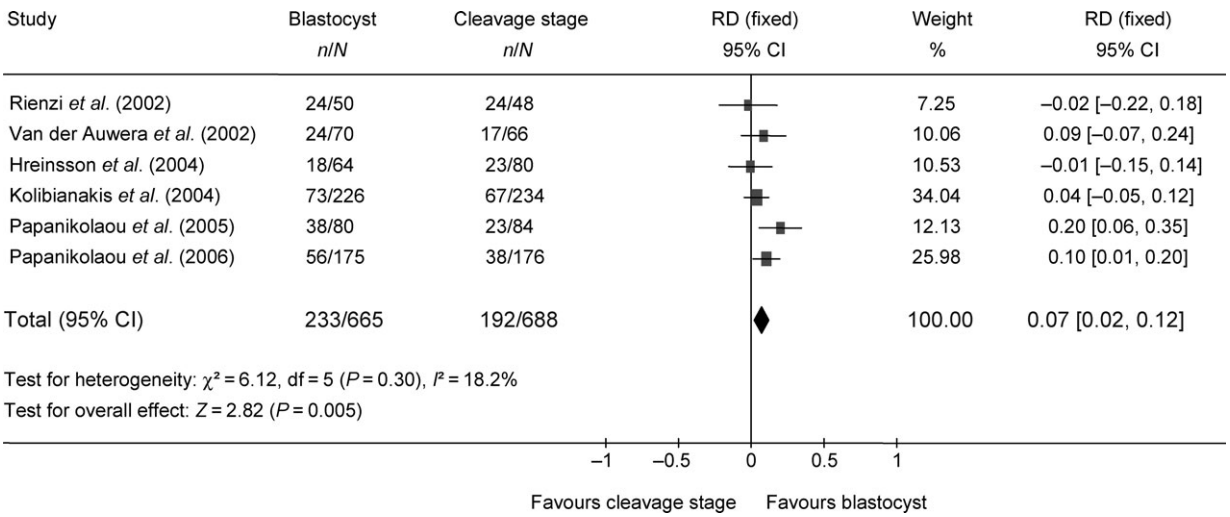

Figure 3: Rate difference regarding live birth rate

chromosomally abnormal embryos to reach higher developmental stages and ultimately to implant. However, *in vitro* culture conditions cannot substitute for endometrial conditions during the natural cycle *in vivo*, and on the other hand the *in vivo*-stimulated endometrial environment can not be compared with endometrium in a natural cycle (Bourgain and Devroey, 2003).

Therefore, choosing at which stage to transfer an embryo is a dilemma; if the blastocyst stage is chosen there are higher chances to transfer a genetically healthy embryo along with better synchronization with a stimulated endometrium. There is a risk, however, that some normal embryos might not reach that stage just because were cultured outside their natural environment. When opting for cleavage-stage embryo transfer, we do not further expose the embryos to *in vitro* culture and therefore a larger pool of embryos is available to select from. Nevertheless, we acknowledge the increased hazard of transferring an aneuploid embryo (though judged morphologically of top quality) and to prematurely exposing an embryo to an altered endometrial environment.

Onto this dilemma, the number of embryos to be transferred is a crucial variable, with a definite impact on the final outcome, which is the establishment and continuation of a pregnancy. By transferring more than two embryos (three or four) at the cleavage stage, the chances of transferring chromosomally normal embryos are increased and that might relatively balance out the higher implantation potential of a blastocyst. By transferring more than two blastocysts, the

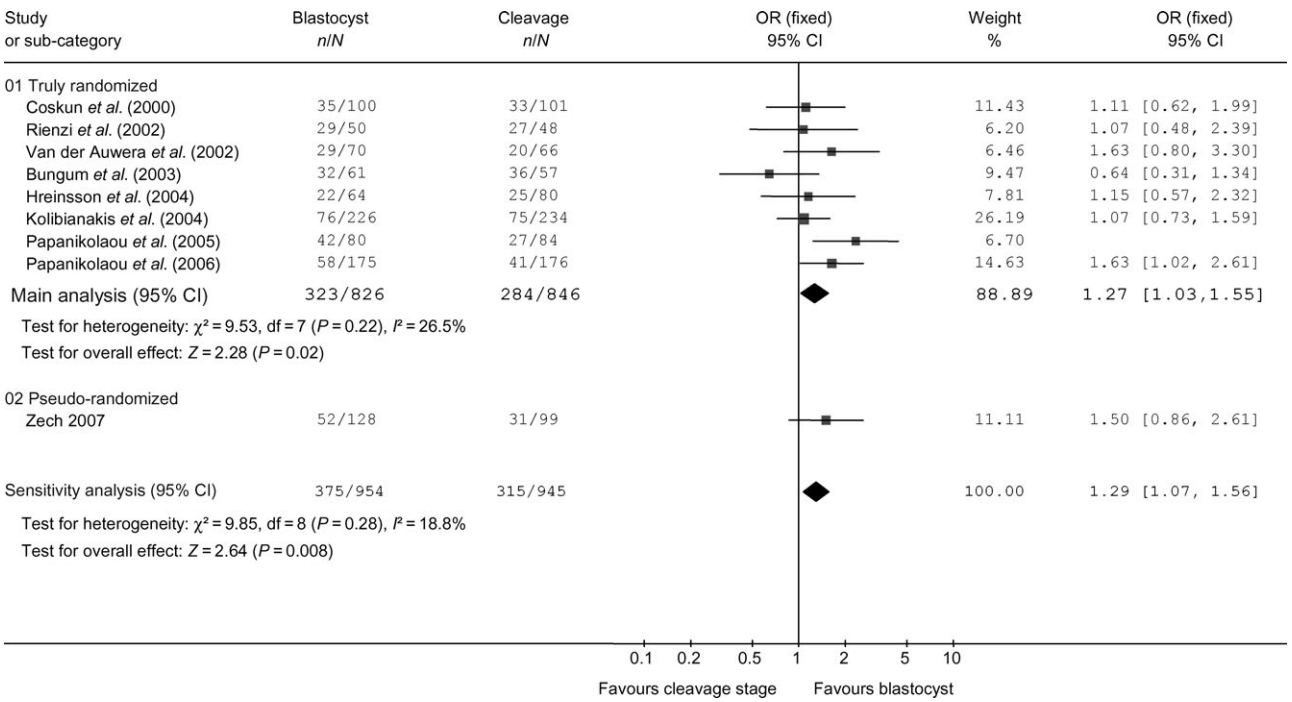

Figure 4: Clinical pregnancy rate per randomized couple

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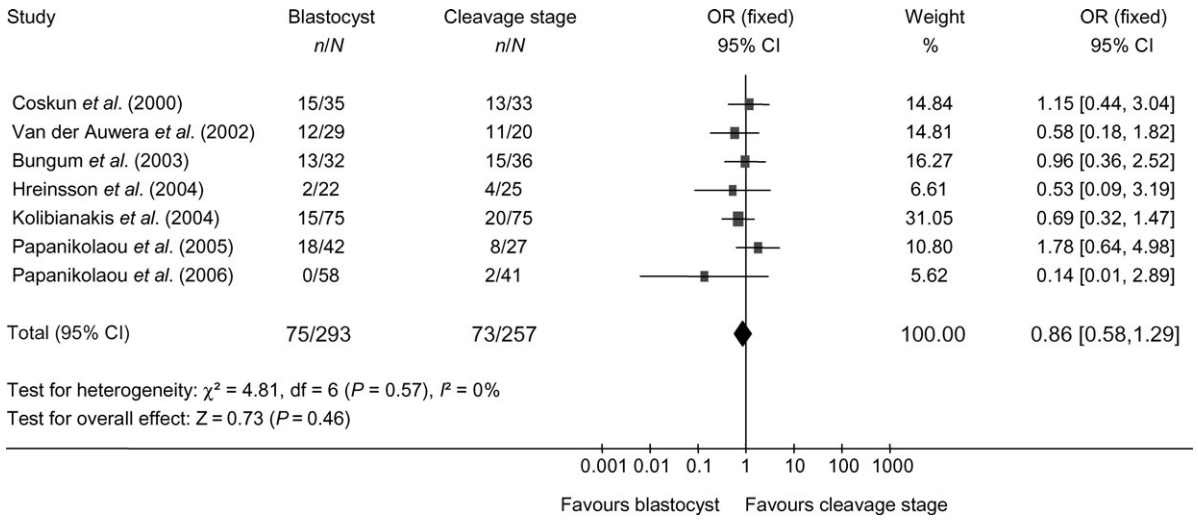

Figure 5: Multiple pregnancy rate per clinical pregnancy

most likely probability that might increase is the likelihood for multiple gestations.

On the other hand, the surplus number of embryos to be transferred cannot counteract the endometrial impact on the pregnancy outcome. In previous work by our group (endometrium biopsies were taken in fresh IVF cycles with Day 3 embryo transfer), endometrial advancement was found to be present on the day of oocyte retrieval and this was negatively associated with the achievement of pregnancy (Kolibianakis *et al*., 2002). Moreover, it has been shown (Papanikolaou *et al*., 2007) that progesterone rise in the late follicular phase (day of HCG administration) has a detrimental impact on the implantation potential of Day 3 embryos, whereas Day 5 embryo performance seems minimally affected. The above findings suggest that, in cases of extreme advancement of luteal endometrium, a transferred cleavage-stage embryo has the disadvantage of interacting with out of phase factors that might hinder its development to the blastocyst stage and thus its implantation (Barnes, 2000). Alternatively, blastocysts have the advantage of interacting with a less out of phase endometrium (studies show that endometrium, at least histologically, recovers within the implantation window), resulting in a better interaction with the implantation molecular milieu.

Although the current meta-analysis suggests the clinical superiority of blastocyst-stage transfer policy, when an equal number and up to two embryos are transferred, there are still some issues to be analysed.

The first issue is the higher probability of embryo transfer cancellation in the blastocyst group (Fig. 6). The risk of not performing embryo transfer, though devastating for the patient undergoing IVF treatment, has also other important parameters. If the blastulation rate of an embryology laboratory exceeds 50% of the fertilized ova (considered as a satisfactory cut-off), then the probability of a 2-pronuclei embryo not reaching the blastocyst stage due to *in vitro* culture conditions decreases significantly. That clinically signifies that the likehood of a euploid embryo to undergo cleavage arrest and not to be transferred due to extra-uterine culture is relatively low. Therefore, the cancellation of embryo transfer in certain cycles with a blastocyst policy might be regarded as avoidance of a redundant embryo transfer offering the patient false hopes for an expected pregnancy.

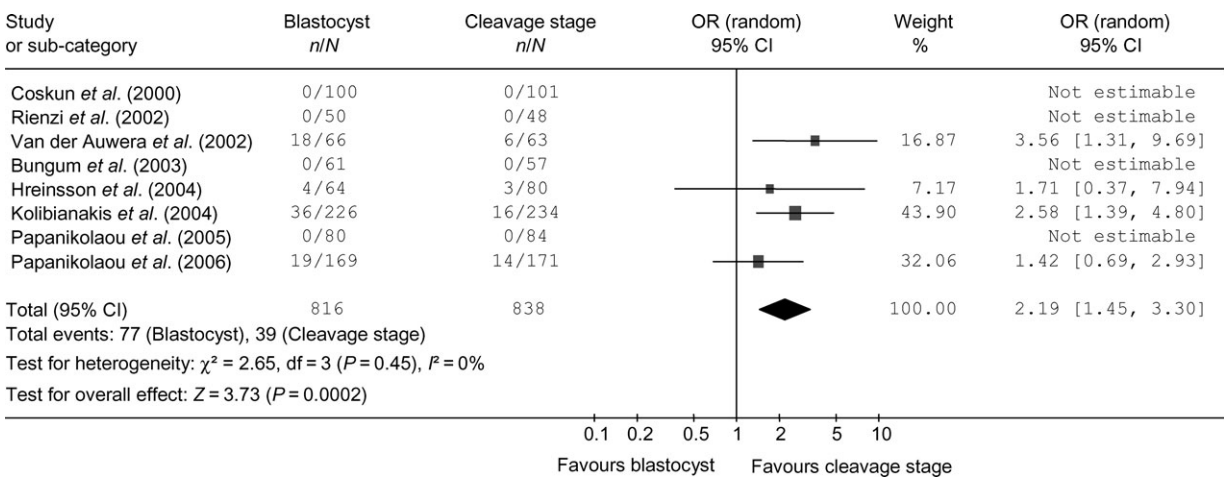

Figure 6: Embryo cancellation rate per patient reaching oocyte retrieval

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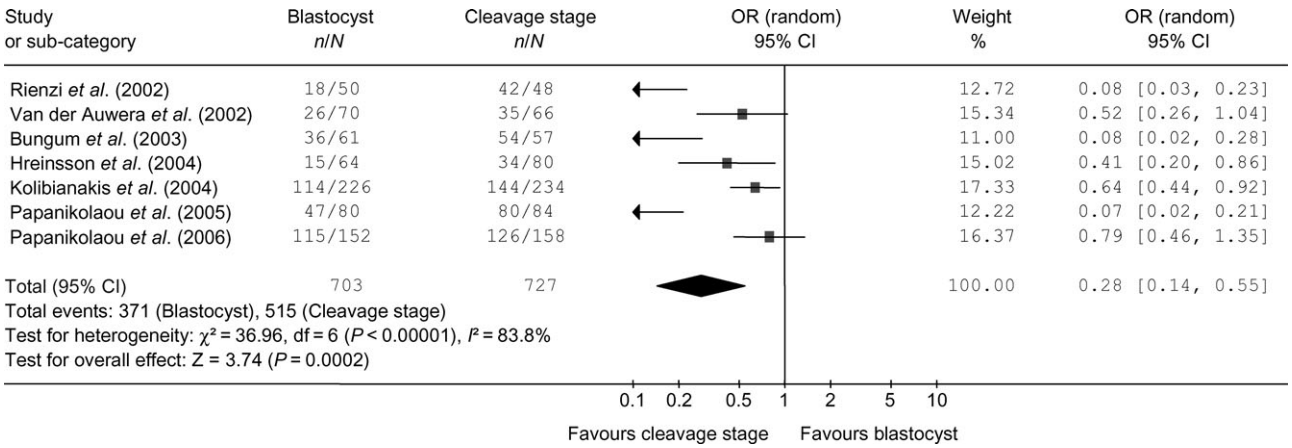

Figure 7: Cryopreservation rate per couple

The second issue arising in this debate is the cryopreservation rate of each policy. Certainly, there are more surplus embryos available for cryopreservation when a cleavage-stage embryo transfer is performed (Fig. 7). Considering the proven universal efficacy of cryopreservation for cleavage-stage embryos (Thurin *et al*., 2004), whereas the results with cryopreserved blastocysts vary significantly among studies (Van den Abbeel *et al*., 2005; Medved *et al*., 2006) the cumulative delivery rate should be the optimal outcome for comparison in the future. However, the inclusion of thawed cycles in the current meta-analysis and in any might follow is impossible due to the study design of the included studies. These RCTs were not designed for a cumulative pregnancy analysis (with the exception of the study by Rienzi *et al*.) and as a result, non-pregnant women having cryopreserved embryos were not instructed to perform a frozen–thawed embryo transfer cycle before they attempt a new fresh trial.

In cleavage-stage transfer policy, however, the odds of cryopreserving euploid embryos that will be successfully thawed and transferred in a subsequent cycle is relatively high. Thus, on a cumulative basis, in the cleavage-stage transfer policy even half of the offsprings could arise from embryos exposed to a three-fold stress (*in vitro* culture, cryopreservation and thawing) with anything this might imply, in terms of congenital abnormalities (Belva *et al*., 2007), imprinting disorders plus the increased cost for additional trials.

A third issue and relative weakness of the current meta-analysis is that the huge variety of ART used among laboratories worldwide cannot be clearly addressed. Principally, the blastocyst formation rate (individual laboratories report from <20 up to $>70\%$) may reflect the quality of the laboratory, the media used, stimulation protocol, embryo transfer technique, freezing-protocol etc. The improvement of culture media and of other laboratory parameters (e.g. number of incubators, gas mixture) may have played an important role in the area under the learning curve all these years covered by the present meta-analysis. However, we are not aware of a more appropriate method, currently available, for reviewing, combining and drawing conclusions from the available evidence regarding the research question of interest.

The aim of reproductive practitioners remains the improvement of IVF efficacy in terms of birth rates. The embryo transfer of up to two blastocysts appears able to translate the proven higher implantation potential of a blastocyst into increased pregnancy rates. However, the optimal outcome in current IVF practice should be the delivery of singleton infants. To this end, single-blastocyst transfer emerges as highly efficient method with the additional support of governmental funding.

Supplementary material

Supplementary material is available at *Hum Reprod* Journal online.

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References

Barnes FL. The effects of the early uterine environment on the subsequent development of embryo and fetus. *Theriogenology* 2000;**53**:649–658.

Belva F, Henriet S, Van den Abbeel E, Haentjens P, Camus M, Devroey P, Van der Elst J, Liebaers I, Bonduelle M. *Neonatal outcome of 937 children born after transfer of cryopresrved embryos obtained by conventional IVF and ICSI between 1986 and 2006*. ESHRE, Lyon, 2007, Abstract book P-300.

Blake D, Proctor M, Johnson N, Olive D. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. The Cochrane Library, Copyright 2005, The Cochrane Collaboration, Vol. (4), 2005.

Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. *Hum Reprod Update* 2003;**9**:515–522.

Bungum M, Bungum L, Humaidan P, Yding Andersen C. Day 3 versus day 5 embryo transfer: a prospective randomized study. *Reprod BioMed Online* 2003; April 7:98–104.

Coskun S, Hollanders J, Al-Hassan S, Al-Sufyan H, Al-Mayman H, Jaroudi K. Day 5 versus day 3 embryo transfer: a controlled randomized trial. *Hum Reprod* 2000;**15**:1947–1952.

Emiliani S, Delbaere A, Vannin A, Biramane J, Verdoodt M, Englert Y, Devreker F. Similar delivery rates in a selected group of patients, for day 2 and day 5 embryos both cultured in sequential medium: a randomized study. *Hum Reprod* 2003;**18**:2145–2150.

Evers JL. Publication bias in reproductive research. *Hum Reprod* 2000;**15**:2063–2066.

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Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil Steril* 1998;**69**:84–88.

Hreeinsson J, Rosenlund B, Fridstrom M, Ek I, Levkov L, Sjoblom P, Hovatta O. Embryo transfer is equally effective at cleavage stage and blastocyst stage: a randomized prospective study. *Eur J Obstet Gynecol Reprod Biol* 2004;**117**:194–200.

Karaki RZ, Samarraie SS, Younis NA, Lahloub TM, Ibrahim MH. Blastocyst culture and transfer: a step toward improved in vitro fertilization outcome. *Fertil Steril* 2002;**77**:114–118.

Kolibianakis E, Bourgain C, Albano C, Osmanagaoglu K, Smitz J, Van Steirteghem A, Devroey P. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. *Fertil Steril* 2002;**78**:1025–1029.

Kolibianakis EM, Zikopoulos K, Verpoest W *et al.* Should we advise patients undergoing IVF to start a cycle leading to a day 3 or a day 5 transfer? *Hum Reprod* 2004;**19**:2550–2554.

Kolibianakis EM, Zikopoulos K, Verpoest W *et al.* Day 5 embryo transfer did not increase the pregnancy rate over day 3 transfer for unselected couples in ART cycles. *Evid Based Obstet Gynecol* 2005;**7**:147–148.

Levitas E, Lunenfeld E, Har-Vardi I, Albotiano S, Sonin Y, Hackmon-Ram R, Potashnik G. Blastocyst-stage embryo transfer in patients who failed to conceive in three or more day 2–3 embryo transfer cycles: a prospective, randomized study. *Fertil Steril* 2004;**81**:567–571.

Levron J, Bider D, Shulman A, Rabinovichi Y, Seidman D, Dor J. A randomized prospective study on blastocyst versus day 2–3 embryo transfer. *Fertil Steril* 2001;**76**:S4.

Levron J, Shulman A, Bider D, Seidman D, Levin T, Dor J. A prospective randomized study comparing day 3 with blastocyst-stage embryo transfer. *Fertil Steril* 2002;**77**:1300–1301.

Medved R, Virant-Klun I, Meden-Vrtovec H, Tomazevic H. Outcome of frozen-thawed blastocysts derived from gonadotropin releasing hormone agonist or antagonist cycles. *J Assist Reprod Genet* 2006;**23**:275–279.

Minasi MG, Rienzi L, Ubaldi F, Iacobelli M, Ferrero S, Greco E. Day 3 versus day 5 fresh and cryopreserved embryo transfers: controlled comparison of cumulative pregnancy rates. *Hum Reprod* 2002;**17**:12.

Nyboe-Andersen A, Gianaroli L, Nygren KG. Assisted reproductive technology in Europe, 2000: results generated from European registers by ESHRE. *Hum Reprod* 2004;**19**:490–503.

Pantos K, Makrakis E, Stavrou D, Karantzis P, Vaxevanoglou T, Tzigounis V. Comparison of embryo transfer on day 2, day 3, and day 6: a prospective randomized study. *Fertil Steril* 2004;**81**:454–455.

Papanikolaou EG, D'haeseleer E, Verheyen G, Van de Velde H, Camus M, Van Steirteghem A, Devroey P, Tournaye H. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day-3 of embryo culture. A randomized prospective study. *Hum Reprod* 2005;**20**:3198–3203.

Papanikolaou EG, Camus M, Kolibianakis EM, Van Lunduyt L, Van Steirteghem A, Devroey P. Transfer of a single blastocyst-stage embryo as compared with a single cleavage-stage embryo for in vitro fertilization. *N Engl J Med* 2006;**354**:1139–1146.

Papanikolaou EG, Kolibianakis E, Pozzobon C, Tank P, Tournaye H, Bourgain C, Van Steirteghem A, Devroey P. Progesterone rise on the day of HCG administration impairs pregnancy outcome in day-3 single embryo transfer, while has no effect on day-5 single blastocyst transfer. *Fertil Steril* 2007 (Epub ahead of print).

Rienzi l, Ubaldi F, Iacobelli M, Ferrero S, Minasi MG, Martinez F *et al.* Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. *Hum Reprod* 2002;**17**:1852–1855.

Scholtes MC, Zeilmaker GH. A prospective, randomized study of embryo transfer results after 3 or 5 days of embryo culture in in vitro fertilization. *Fertil Steril* 1996;**65**:1245–1248.

Staessen C, Platteau P, Van Assche E, Van Steirteghem A, Devroey P. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004;**19**:2849–2858.

Steptoe PC, Edwards RG, Purdy JM. Human blastocysts grown in culture. *Nature* 1971;**229**:132–133.

Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, Bergh C. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. *N Engl J Med* 2004;**351**:2392–2402.

Tsirigotis M. Blastocyst stage transfer: pitfalls and benefits—too soon to abandon current practice? *Hum Reprod* 1998;**13**:3285–3289.

Utsunomiya T, Ito H, Nagaki M, Sato J. A prospective, randomized study: day 3 versus hatching blastocyst stage. *Hum Reprod* 2004;**19**:1598–1603.

Van den Abbeel E, Camus M, Verheyen G, Van Waesberghe L, Devroey P, Van Steirteghem A. Slow controlled-rate freezing of sequentially cultured human blastocysts: an evaluation of two freezing strategies. *Hum Reprod* 2005;**20**:2939–2945.

Van der Auwera I, Debrock S, Spiessens C, Afschrift H, Bakelants E, Meuleman C *et al.* A prospective randomized study: day 2 versus day 5 embryo transfer. *Hum Reprod* 2002;**17**:1507–1512.

Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, Lyles R. Integration of blastocyst transfer for all patients. *Fertil Steril* 2002;**77**:693–696.

Zech NH, Lejeune B, Puissant F, Vanderzwalmen S, Zech H, Vanderzwalmen P. Prospective evaluation of the optimal time for selecting a single embryo for transfer: day 3 versus day 5. *Fertil Steril* 2007;**88**:244–246.

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