

ORIGINAL ARTICLE

In Vitro Fertilization with Single Blastocyst-Stage versus Single Cleavage-Stage Embryos

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ABSTRACT

BACKGROUND

Single-embryo transfer has been recommended to reduce the incidence of multiple gestations when in vitro fertilization is performed in women under 36 years of age. We designed a prospective, randomized, controlled trial to determine whether there were any differences in the rates of pregnancy and delivery between women undergoing transfer of a single cleavage-stage (day 3) embryo and those undergoing transfer of a single blastocyst-stage (day 5) embryo.

METHODS

We studied 351 infertile women under 36 years of age who were randomly assigned to undergo transfer of either a single cleavage-stage embryo (176 patients) or a single blastocyst-stage embryo (175 patients). Multifollicular ovarian stimulation was performed with a gonadotropin-releasing hormone antagonist and recombinant follicle-stimulating hormone.

RESULTS

The study was terminated early after a prespecified interim analysis (which included 50 percent of the planned number of patients) found a higher rate of pregnancy among women undergoing transfer of a single blastocyst-stage embryo ($P=0.02$). The rate of delivery was also significantly higher in this group than in the group undergoing transfer of a single cleavage-stage embryo (32.0 percent vs. 21.6 percent; relative risk, 1.48; 95 percent confidence interval, 1.04 to 2.11). Two multiple births occurred, both of monozygotic twins, both of which were in the group undergoing transfer of a single cleavage-stage embryo.

CONCLUSIONS

These findings support the transfer of a single blastocyst-stage (day 5) embryo in infertile women under 36 years of age.

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ALTHOUGH THE FIRST SUCCESSFUL delivery after in vitro fertilization was achieved after the placement of one embryo,¹ the practice of single-embryo transfer was soon replaced by multifollicular ovarian stimulation followed by the transfer of multiple embryos. Transfer of multiple embryos results in higher pregnancy rates than single-embryo transfer, but multiple gestations are a common and serious complication of this approach.^{2,3} The rate of multiple gestations after in vitro fertilization is 26.4 percent in Europe⁴ and 35.3 percent in the United States,⁵ as compared with 1.1 percent after natural conception.⁶ Adverse outcomes associated with high-order gestations include serious perinatal morbidity and mortality and long-term disabilities.⁶

Single-embryo transfer has been advocated as a strategy to reduce the frequency of multiple births after in vitro fertilization.⁷⁻⁹ The Belgian government has recently instituted a policy whereby the first six in vitro fertilization trials for a couple will be reimbursed by health insurance agencies if elective single-embryo transfer was performed in the first two trials in women under 36 years of age.¹⁰ It has been suggested that extending the embryo culture to day 5 and transferring the embryo at the blastocyst stage would enhance the likelihood of pregnancy as compared with the usual procedure in which the embryo is transferred at the cleavage stage (day 3),^{11,12} but this suggestion remains controversial.¹³

The main disadvantage of transferring the embryo at the cleavage stage rather than at the blastocyst stage is that the morphologic criteria that govern embryo selection on day 3 are highly subjective¹⁴ and less accurately reflect the genetic quality (euploidy status) of embryos than do the criteria used on day 5. For example, among women 36 years of age or older, 59 percent of embryos judged as "top quality" on day 3 were found to be aneuploid, as compared with 35 percent of blastocysts judged as "top quality" on day 5.^{15,16} If these findings also apply to women under 36 years of age (albeit in a lower proportion),¹⁷ then selecting only one cleavage-stage embryo for use (even if it is of top quality) would increase the chances of transferring a chromosomally abnormal embryo. On the other hand, a potential downside to the use of blastocyst-stage embryos is that some embryos are not able to reach the blastocyst stage

in culture.¹⁸ Additional concerns about the use of blastocysts include the possibilities of an increased risk of monozygotic twins, an altered sex ratio of births, and epigenetic effects on the embryo.

We conducted a study to determine whether there were any differences in the rates of pregnancy and delivery between women randomly assigned to undergo transfer of a single cleavage-stage embryo and those assigned to undergo transfer of a single blastocyst-stage embryo.

METHODS

STUDY DESIGN

Between July 2003 and November 2004, 351 women requesting infertility treatment were randomly assigned to undergo transfer of either a single cleavage-stage embryo or a single blastocyst-stage embryo (Fig. 1). The randomization was performed after the final consultation at the outpatient clinic. A computer-generated list was used for randomization; this list was not concealed from the physicians, but it did not explicitly state the treatment strategy, identifying the strategies only as "A" or "B." A patient could enter the study only once. The study was approved by our institutional review board, and all the patients gave written informed consent. The embryo transfers were performed without ultrasound guidance by clinicians and embryologists who were blinded only with respect to the patient's participation in the study.

The patients included in the study were women under 36 years of age who were undergoing a first or second trial of in vitro fertilization or intracytoplasmic sperm injection, whose serum follicle-stimulating hormone level on day 3 of the menstrual cycle was 12 IU per liter or less, and who were undergoing transfer of one embryo. The use of preimplantation genetic diagnosis was an exclusion criterion. The primary outcomes were the rate of ongoing pregnancy at 12 weeks of gestation or more and the rate of delivery.

MULTIFOLLICULAR OVARIAN STIMULATION

The protocol for use of the gonadotropin-releasing hormone antagonist and recombinant follicle-stimulating hormone has been described elsewhere.¹⁹ In brief, injections of recombinant follicle-stimulating hormone, follitropin beta (Puregon, NV Organon) were initiated at a dose of 100 to 250 IU on day 2 of the menstrual cycle (day 1 of stimula-

tion) and subcutaneous administration of the antagonist ganirelix (Orgalutran, NV Organon) was started at a dose of 0.25 mg on day 6 of stimulation. Final oocyte maturation was induced by the administration of 10,000 IU of human chorionic gonadotropin (Pregnyl, NV Organon), when at least three follicles 17 mm in diameter were visualized on ultrasonography. The luteal phase was supported by 600 mg of vaginally administered micronized natural progesterone (Utrogestan, Besins International) per day. To assess the treatment outcome, serum human chorionic gonadotropin was measured 14 and 17 days after oocyte retrieval. An increase in serum human chorionic gonadotropin levels above 20 IU per liter indicated pregnancy. Clinical pregnancy was defined by the observation of fetal cardiac activity on ultrasonography after seven weeks of gestation. An ongoing pregnancy was defined by the observation of a fetal heartbeat on ultrasonography after 12 weeks of gestation.

EMBRYO CULTURE, EVALUATION, AND SELECTION FOR TRANSFER

Sperm preparation, in vitro fertilization and intracytoplasmic sperm injection procedures, and embryo culture were carried out as described by Van Landuyt et al.²⁰ Embryo quality was assessed daily until the moment of transfer or (for supernumerary embryos) freezing, as described by Papanikolaou et al.²¹ In brief, on the morning of day 3, the embryos were removed from cleavage medium and placed in blastocyst medium (sequential medium). The embryo quality on day 3 was assessed on the basis of the number of blastomeres (cells), the rate of fragmentation (the volume of the embryo with anucleate fragments), multinucleation of the blastomeres, and early compaction (junction of cells). Each embryo received a score of 1 (top quality), 2 (good quality), 3 (fair quality), or 4 (poor quality). Embryos with a score of 4 were not transferred. Supernumerary embryos were frozen on day 3.

The embryo quality on day 5 was assessed according to the criteria of Gardner and Schoolcraft.²² This scoring system is based on the formation and degree of expansion (from early blastocyst [BL1] to fully expanded blastocyst [BL6]) of the blastocoele, the development of the inner cell mass, and the development of the trophoctoderm. The combined score ranged from 1 to 4, with

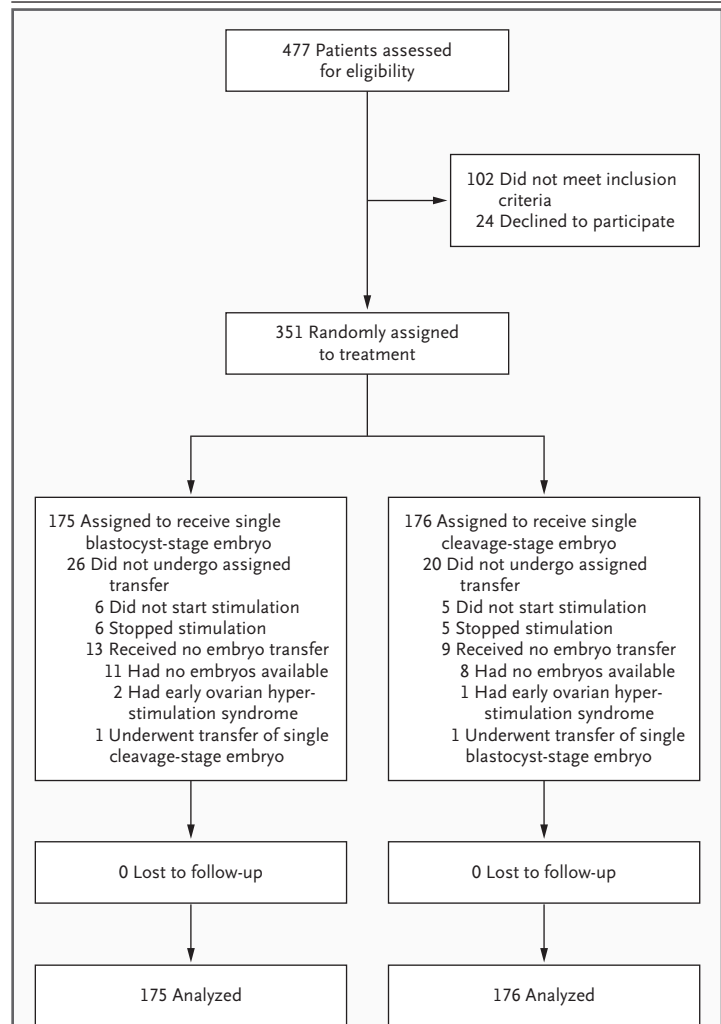


Figure 1. Flow Diagram of the Phases of the Study.

Stimulation was stopped in six patients in the blastocyst-stage group because of a surge in luteinizing hormone, an endometrial polyp, a poor ovarian response, a high risk of the ovarian hyperstimulation syndrome, the development of a cyst, and the injection of human chorionic gonadotropin instead of gonadotropin (one patient each). Stimulation was stopped in five patients in the cleavage-stage group because of hospitalization for abdominal pain in one patient, a surge in luteinizing hormone in one patient, a poor ovarian response in two patients, and the involvement of a relative of one patient in an automobile accident.

higher scores indicating poorer quality; embryos with a score of 4 (poor quality) were not transferred. Supernumerary embryos were frozen on day 5 or 6.

STATISTICAL ANALYSIS

Analysis was performed according to the intention to treat. For descriptive statistics, we used means

±SD. Normally distributed continuous variables were analyzed by the t-test for independent samples, and non-normally distributed metric variables by the Mann–Whitney test. The chi-square test was used to analyze nominal variables. For the differences in pregnancy rates between the groups, relative risks and 95 percent confidence intervals were calculated. The relative risk was defined as the ratio of the pregnancy rate in the intervention (blastocyst-stage) group to the pregnancy rate in the control (embryo-stage) group; the relative risk was calculated with Review Manager software (RevMan version 4.2.2, Cochrane Collaboration). All tests were two-sided, and a P value of less than 0.05 was considered to indicate statistical significance.

Using group sequential methods, we calculated that the enrollment of 351 patients in each group would give the study a statistical power of 80 percent to detect an absolute difference of 10 percent in the rate of ongoing pregnancy between the groups (given rates of 20 and 30 percent) at an alpha level of 0.05 with the use of a two-sided z-test. These results assume that two sequential tests are performed with the use of the Pocock spending function to determine the test boundaries and that the first interim analysis (time one) yields a P value of 0.03. It was prespecified that the study would be stopped if the first interim analysis identified a significant difference ($P=0.03$) in pregnancy rates between groups. At the first interim analysis (after the enrollment of 50 percent of the projected 702 patients), the pregnancy rate in the blastocyst-stage group was greater than that in the cleavage-stage group at an alpha level of 0.02, and therefore, the study was terminated.

RESULTS

A total of 351 patients were randomly assigned to undergo transfer of either a single cleavage-stage embryo (176 patients) or a single blastocyst-stage embryo (175 patients). The proportion of patients undergoing their first in vitro fertilization or intracytoplasmic sperm insertion trial under the new legislation was 92.6 percent in the cleavage-stage group and 90.9 percent in the blastocyst-stage group. There were no significant differences between the groups in demographic, cycle, or embryologic characteristics. However, significantly more embryos were cryopreserved in the cleavage-stage group than in the blastocyst-stage group

(4.2 vs. 2.2, $P=0.001$). The percentage of patients with a top-quality embryo for transfer was similar in the two groups (70.0 percent in the blastocyst-stage group and 65.0 percent in the cleavage-stage group) (Table 1).

Six patients starting stimulation in the blastocyst-stage group and five patients starting stimulation in the cleavage-stage group did not undergo oocyte retrieval (Fig. 1). In the blastocyst-stage group, 13 of 169 patients (7.7 percent) did not undergo transfer because of lack of embryos (11 patients) or occurrence of early ovarian hyperstimulation syndrome (2 patients). Of the 11 women in this group in whom embryo transfer was canceled, only 1 would have received an embryo transfer if she had been randomly assigned to the other group. In the cleavage-stage group, embryo transfer was not performed in 9 of 171 patients (5.3 percent), eight of whom lacked embryos on day 3 and one of whom had early ovarian hyperstimulation syndrome.

The pregnancy outcomes of the two groups are shown in Table 2. In both groups, a relatively high percentage of patients had an initial positive human chorionic gonadotropin test (41.7 percent in the blastocyst-stage group and 33.5 percent in the cleavage-stage group, $P=0.11$). As compared with the cleavage-stage group, the blastocyst-stage group had a significantly higher rate of both ongoing pregnancy (33.1 percent vs. 21.6 percent; relative risk, 1.54; 95 percent confidence interval, 1.08 to 2.18) and delivery (32.0 percent vs. 21.6 percent; relative risk, 1.48; 95 percent confidence interval, 1.04 to 2.11). This difference may be partially attributable to a higher rate of pregnancy loss in the first trimester in the cleavage-stage group than in the blastocyst-stage group, although this difference was not significant (33.9 percent vs. 19.2 percent; 95 percent confidence interval, 0.31 to 1.02). In the blastocyst-stage group, one patient underwent elective termination of pregnancy for anencephaly of the embryo and one patient had a second-trimester miscarriage. The overall rate of multiple births was 2.1 percent (2 of 94 deliveries). Both multiple pregnancies occurred in the cleavage-stage group and consisted of monozygotic twins.

DISCUSSION

The results of this randomized, controlled trial suggest that among women under 36 years of age

Table 1. Patient Demographic, Cycle, and Embryologic Characteristics.*

Characteristic	Single Blastocyst-Stage Embryo Transferred (N=175)	Single Cleavage-Stage Embryo Transferred (N=176)	P Value
Age — yr	30.4±3.6	30.5±3.2	0.84
Duration of infertility — yr	3.5±2.1	3.7±2.2	0.75
Cause of infertility — no. (%)			0.68
Male	93 (53.1)	103 (58.5)	
Female	45 (25.7)	40 (22.7)	
Both male and female	15 (8.6)	16 (9.1)	
Idiopathic	22 (12.6)	17 (9.7)	
Duration of stimulation — days	9.6±2.4	9.6±2.6	0.93
Total gonadotropin dose — IU	1704±574	1699±515	0.94
Fertilization procedure — no./total no. (%)			0.73
In vitro fertilization	54/163 (33.1)	56/166 (33.7)	
Intracytoplasmic sperm insertion	103/163 (63.2)	107/166 (64.5)	
Both procedures†	6/163 (3.7)	3/166 (1.8)	
No. of oocytes retrieved (cumulus–oocyte complexes)	12.5±7.1	13.9±8.1	0.13
No. of fertilized oocytes (2 pronuclei)	7.5±4.7	8.0±5.4	0.23
No. of embryos cryopreserved	2.2±2.7	4.2±4.1	0.001
≥1 cryopreserved embryo —no./total no. (%)	115/152 (75.7)	126/158 (79.7)	0.38
Quality of transferred embryos‡			0.10
Top — %	70.0	65.0	
Good — %	26.7	33.7	
Fair — %	3.3	1.3	
Mean§	1.34±0.5	1.36±0.4	0.67

* Plus–minus values are means ±SD.

† Half the retrieved oocytes were fertilized by conventional in vitro fertilization and half by intracytoplasmic sperm insertion.

‡ A top-quality cleavage-stage embryo had at least eight cells with no more than 10 percent fragmentation, blastomeres of regular size, and no multinucleation. A top-quality blastocyst-stage embryo was at least in the expanded-blastocyst stage (BL3) and had a type A inner cell mass (in which cells are tightly packed, with many cells present) and a trophoblast that was either type A (with many cells forming a cohesive epithelium) or type B (with few cells forming a loose epithelium).

§ Scores could range from 1 (top quality) to 4 (poor quality).

who are undergoing a first or second cycle of in vitro fertilization or intracytoplasmic sperm injection, transfer of a single blastocyst-stage embryo significantly increases the probability of pregnancy and birth of a child as compared with transfer of a single cleavage-stage embryo. Furthermore, the low rate of multiple births in the study group as a whole provides more evidence of the ability of single-embryo transfer to markedly reduce this complication of in vitro fertilization procedures.

Despite the well-recognized risks associated with multiple gestations, many patients still want to have multiple embryos transferred to enhance

the probability of pregnancy.²³ Increasing awareness of the costs and risks of multiple births has led the medical community to reduce the number of embryos transferred. For single-embryo transfer to be accepted by patients, doctors, and payors, however, it must be associated with acceptable pregnancy rates.

One approach to optimizing pregnancy rates after single-embryo transfer is to follow it with transfer of a frozen–thawed embryo in cases in which the initial transfer is not successful. In a previous randomized trial, this strategy achieved a rate of live births that was not substantially

Table 2. Rates of Pregnancy and Delivery after the Transfer of Single Blastocyst-Stage and Cleavage-Stage Embryos.

Variable	Single Blastocyst-Stage Embryo Transferred (N = 175)	Single Cleavage-Stage Embryo Transferred (N = 176)	Relative Risk (95% CI)*	P Value
	% (no.)			
Rate/patient randomly assigned to treatment				
Pregnancy†	41.7 (73)	33.5 (59)	1.23 (0.95–1.63)	0.11
Clinical pregnancy	33.1 (58)	23.3 (41)	1.42 (1.01–2.00)	0.04
Ongoing pregnancy	33.1 (58)	21.6 (38)	1.54 (1.08–2.18)	0.02
Pregnancy loss‡				
Ectopic pregnancy	1.4 (1)	1.7 (1)		
1st Trimester	19.2 (14)	33.9 (20)	0.57 (0.31–1.02)	0.07
2nd Trimester	2.7 (2)§	0		
Delivery	32.0 (56)	21.6 (38)	1.48 (1.04–2.11)	0.03
Multiple births	0	5 (2)	0.14 (0.01–2.77)	0.16
Rate/patient starting stimulation¶				
Pregnancy	43.2 (73)	34.5 (59)	1.25 (0.96–1.64)	0.10
Clinical pregnancy	34.3 (58)	24.0 (41)	1.43 (1.02–2.01)	0.04
Ongoing pregnancy	34.3 (58)	22.2 (38)	1.54 (1.09–2.19)	0.01
Delivery	33.1 (56)	22.2 (38)	1.49 (1.05–2.12)	0.03
Rate/embryo transfer 				
Pregnancy	48.7 (73)	37.6 (59)	1.30 (1.00–1.68)	0.05
Clinical pregnancy	38.7 (58)	26.1 (41)	1.48 (1.06–2.06)	0.02
Implantation**	38.7 (58)	27.4 (43)	1.41 (1.02–1.95)	0.04
Ongoing pregnancy	38.7 (58)	24.2 (38)	1.60 (1.13–2.25)	0.007
Delivery	37.3 (56)	24.2 (38)	1.54 (1.09–2.18)	0.01

* CI denotes confidence interval.

† Pregnancy was defined by a positive human chorionic gonadotropin test.

‡ The percentages are based on 73 initial pregnancies in the blastocyst-stage group and 59 initial pregnancies in the cleavage-stage group.

§ One patient underwent elective termination of pregnancy at 15 weeks for anencephaly of the embryo, and one patient had a second-trimester miscarriage at 13 weeks.

¶ The percentages are based on 169 patients in the blastocyst-stage group and 171 patients in the cleavage-stage group.

|| The percentages are based on 150 patients in the blastocyst-stage group and 157 patients in the cleavage-stage group.

**The implantation rate is the number of gestational sacs with a fetal heartbeat divided by the number of embryos transferred.

lower than the rate after transfer of two fresh cleavage-stage (day 2) embryos (38.8 percent vs. 42.9 percent) and was associated with a dramatic reduction in the rate of multiple births (0.8 percent vs. 33.1 percent).²⁴ However, these results were obtained among women under 36 years of age who had at least two good embryos available for transfer and thus had a good prognosis, and the plausible difference in rates of live births between the two protocols (on the basis of the 95 percent confidence interval) was as high as 11.6

percent. For some couples, this difference may not be acceptable, particularly given the observation that the stress associated with in vitro fertilization leads many couples to abandon treatment without proceeding to a second trial, even if they can afford it.²⁵ Increasing the pregnancy rate in the cycle in which fresh embryos are used would be expected to increase the acceptability of single-embryo transfer.²⁶

In our study of women under 36 years of age who were undergoing a first or second attempt at

in vitro fertilization, transfer of a single blastocyst-stage embryo resulted in a higher pregnancy rate than transfer of a single cleavage-stage embryo. Moreover, the rate of live births after transfer of a single fresh blastocyst-stage embryo was similar to that after transfer of two fresh cleavage-stage (day 2) embryos in the Scandinavian study.²⁴ Smaller studies^{27,28} have found rates of ongoing pregnancy of up to 60 percent after elective transfer of a single blastocyst. Similarly, analysis of a population-based sample of 50,819 in vitro fertilization procedures in the United States in 2001 revealed that transfer of a single blastocyst-stage embryo resulted in a live-birth rate ranging from 31.6 to 39.5 percent among women 20 through 37 years of age.²⁹

The success of single-blastocyst transfer will require effective methods of cryopreservation of blastocysts. Data are needed to compare the rates of live births (including both births resulting from transfer of fresh embryos and those resulting from transfer of frozen-thawed embryos) after transfer of cleavage-stage and blastocyst-stage embryos. We did not include in the initial design a plan for the subsequent transfer of frozen embryos in patients who did not conceive. However, we have recently reported a live-birth rate of 11 percent after transfer of frozen-thawed blastocyst-stage embryos (94 of 857 cycles),³⁰ whereas the live-birth rate after transfer of freeze-thawed cleavage-stage embryos is approximately 15 percent.

A concern in randomly assigning patients to treatment at the time of consultation, before embryologic data were available, was that it might lead to a high rate of cancellation of transfers of blastocyst-stage embryos. However, among this population of women under 36 years of age who were undergoing a first or second trial of in vitro fertilization, cancellation of transfer on day 5 was rare. Prospective studies are needed to determine whether blastocyst transfer is appropriate in older women or in women with repeated implantation failures. Recent data,^{21,31} however, suggest that the number of embryos with two pronuclei (day 1) or cleavage-stage embryos may serve as a predictor of the success of blastocyst transfer.

An increased risk of monozygotic twins has previously been reported with blastocyst transfer in retrospective studies.^{32,33} There were no cases

of monozygotic twins after single-blastocyst transfer in our study, but the study was not powered to assess the risk of monozygotic twins or other potential risks associated with extension of embryo culture to day 5, such as birth defects and long-term health outcomes. The two second-trimester pregnancy losses in the blastocyst-transfer group, including the elective termination owing to anencephaly, may be a cause of concern, although the rates of such complications did not differ significantly between the groups. Larger studies are required to address these issues.

The higher rates of ongoing pregnancy and delivery after transfer of a single blastocyst-stage embryo than of a single cleavage-stage embryo are probably due to a better selection process.²¹ Although some genetically abnormal embryos are able to reach the blastocyst stage, the risk of aneuploidy in a top-quality blastocyst is lower than that in a top-quality cleavage-stage embryo.^{15,16} A recent meta-analysis comparing transfer of cleavage-stage and blastocyst-stage embryos did not show a difference in favor of the latter embryos.¹³ However, this analysis included studies involving transfer of more cleavage-stage embryos than blastocyst-stage embryos, and the transfer of more cleavage-stage embryos could have compensated for the potentially lower quality of any individual cleavage-stage embryo relative to that of a blastocyst-stage embryo. We have recently shown that in appropriately selected patients (with more than three good embryos on day 3), transfer of two blastocysts results in a significantly higher delivery rate than transfer of two cleavage-stage embryos, but with the drawback that almost half of the initial pregnancies involved twins.²¹

In conclusion, our findings demonstrate that in women under 36 years of age who are undergoing their first or second trial of in vitro fertilization, transfer of a single blastocyst-stage embryo significantly increases the likelihood of pregnancy and delivery as compared with transfer of a single cleavage-stage embryo. More work is needed to determine whether this approach is appropriate for other groups of women.

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