ORIGINAL ARTICLE

Elective Single-Embryo Transfer versus Double-Embryo Transfer in in Vitro Fertilization

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ABSTRACT

BACKGROUND
The risks of premature birth and perinatal death are increased after in vitro fertilization. These risks are mainly due to the high incidence of multiple births, which relates to the number of embryos transferred.

METHODS
We performed a randomized, multicenter trial to assess the equivalence of two approaches to in vitro fertilization with respect to the rates of pregnancy that result in at least one live birth and to compare associated rates of multiple gestation. Women less than 36 years of age who had at least two good-quality embryos were randomly assigned either to undergo transfer of a single fresh embryo and, if there was no live birth, subsequent transfer of a single frozen-and-thawed embryo, or to undergo a single transfer of two fresh embryos. Equivalence was defined as a difference of no more than 10 percentage points in the rates of pregnancy resulting in at least one live birth.

RESULTS
Pregnancy resulting in at least one live birth occurred in 142 of 331 women (42.9 percent) in the double-embryo-transfer group as compared with 128 of 330 women (38.8 percent) in the single-embryo-transfer group (difference, 4.1 percentage points; 95 percent confidence interval, −3.4 to 11.6 percentage points); rates of multiple births were 33.1 percent and 0.8 percent, respectively ($P<0.001$). These results do not demonstrate equivalence of the two approaches in rates of live births, but they do indicate that any reduction in the rate of live births with the transfer of single embryos is unlikely to exceed 11.6 percentage points.

CONCLUSIONS
In women under 36 years of age, transferring one fresh embryo and then, if needed, one frozen-and-thawed embryo dramatically reduces the rate of multiple births while achieving a rate of live births that is not substantially lower than the rate that is achievable with a double-embryo transfer.

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With the rapid development of techniques of in vitro fertilization, resulting in higher rates of implantation, a substantial increase in the rate of multiple births has become evident. Several studies have reported less favorable obstetrical outcomes after in vitro fertilization than after spontaneous conception,[1-7] including higher risks of prematurity, low or very low birth weight, and perinatal death. The increased risk of prematurity is primarily due to the greatly increased rate of multiple births. However, even for singletons born after in vitro fertilization, adverse outcomes have been found to be more frequent than among children born after spontaneous conception.[1-7] An increased risk of neurologic sequelae has also been noted, particularly in preterm infants but also in term infants conceived by in vitro fertilization.[8] An increased risk of malformations has been reported in children born after in vitro fertilization, particularly in instances of multiple gestation.[9]

The most important factor influencing the rate of multiple births is the number of embryos transferred. In Sweden, in 1993, there was a voluntary reduction in the number of embryos routinely transferred, from three to two. This resulted in the virtual elimination of the conception of triplets by in vitro fertilization, whereas the pregnancy rate and the live-birth rate remained essentially unaffected at 35 percent and approximately 25 percent per transfer, respectively. The rate of multiple births remained fairly constant at 20 to 25 percent.[10] In a large observational study, a reduction in the number of embryos transferred, from three to two, had little effect on the rates of live births but considerably decreased rates of multiple births; however, multiple births remained frequent.[11]

A strategy involving the transfer of only one embryo would be expected to result mainly in singleton pregnancies but might also lead to a considerable decrease in the overall birth rate. Several studies have been conducted to identify patients suitable for elective single-embryo transfer.[12,13] In a retrospective study,[13] it was found that the only variables predictive of multiple birth were the mother's age and the number of good-quality embryos transferred. From this analysis, cutoff limits were calculated for the woman's age for single-embryo transfer in order to halve the rate of pregnancy with twins in the total population undergoing in vitro fertilization. According to this analysis, the overall birth rate in the total in vitro fertilization population would be reduced from 29 percent to 25 percent after the transfer of the fresh embryo but would be completely restored after an additional transfer of one frozen-and-thawed embryo. These assumptions were used in the design of the present study. Previous randomized trials evaluating single-embryo transfer are few and have included a limited number of patients.[14,15]

We designed this study to test the following hypothesis: among women less than 36 years of age, the rate of pregnancies resulting in at least one live birth in patients who undergo the transfer of a single fresh embryo and, if no live birth results, the subsequent transfer of a frozen-and-thawed embryo, would be equivalent to the rate in patients who undergo the simultaneous transfer of two fresh embryos. We also hypothesized that single-embryo transfers would reduce the rate of multiple gestation.

METHODS

PATIENTS

Women were eligible for randomization if they were less than 36 years of age at the time of the transfer of the fresh embryo, were undergoing their first or second in vitro fertilization cycle, and had at least two embryos of good quality available for transfer or freezing. Good-quality embryos were defined by their morphologic features and cleavage rate and included embryos with less than 20 percent fragmentation and 4 to 6 cells at day 2, 6 to 10 cells at day 3,[16] or expanded blastocysts at day 5 or 6.[17] The original protocol stipulated that the patient had to be less than 35 years of age and have at least three good-quality embryos available, but these criteria were modified in an amendment after the first 215 patients were enrolled, owing to a change in usual clinical practice in Sweden. Eleven clinics, both public and private, participated. The patients were recruited from May 2000 to October 2003.

STUDY DESIGN

The study was approved by the ethics committees of the participating clinics, and all patients provided written informed consent. Each clinic was allowed to follow its own local stimulation protocol. The majority of the women were treated with the use of a stimulation protocol that included down-regulation with a gonadotropin-releasing hormone agonist. Stimulation was performed with recombinant follicle-stimulating hormone (Gonal-F, Serono; Puregon, Organon) or urinary-derived follicle-stimulating hormone (Menopur, Ferring). Oocyte retrieval and fertilization were performed by conventional in vitro fertilization or intracytoplasmic sperm injection by means of standard techniques. Embryo transfer was performed two, three, or five days after oocyte retrieval. Progesterone was administered daily, either intramuscularly or vaginally, from the time of oocyte retrieval until the time of a negative pregnancy test or until two weeks after a positive pregnancy test.

Before the transfer, when the embryos could be evaluated, randomization was performed locally by the embryologist with the use of a computerized randomization program. Randomization was at a ratio of 1:1. Optimal allocation was applied according to Pocock's minimization technique for sequential randomization,[18] with consideration given to the woman's age, the presence or absence of tubal infertility, the number of previous in vitro fertilization cycles involving transfers, the number of previous in vitro fertilization cycles resulting in birth, the day of embryo transfer, and the number of good-quality embryos available.

The study was double-blind; neither the patient nor the physician knew whether one embryo or two embryos had been transferred. The blinding continued until a urine test or a serum pregnancy test was performed — that is, during the implantation phase. Patients in the single-embryo-transfer group who did not conceive in the cycle in which the fresh embryo had been transferred, or who miscarried, subsequently underwent the transfer of a single frozen-and-thawed embryo in a natural or a hormone-stimulated cycle. If the first frozen-and-thawed embryo was not viable, other embryos were thawed, one by one, until a viable embryo could be transferred. If no surviving embryos were available after thawing, no transfer took place.

The primary outcome was the cumulative rate of pregnancy resulting in at least one live birth. Secondary outcomes were the rates of pregnancy, implantation, multiple births (as a percentage of live births), spontaneous abortion, and ectopic pregnancy. A pregnancy was defined as a positive test for human chorionic gonadotropin in urine (>20 IU per liter) or a serum level of human chorionic gonadotropin 2 IU per liter or more two weeks after embryo transfer. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred.

CALCULATION OF SAMPLE SIZE

The study was designed as an equivalence study. Before the start of the study, we calculated sample size on the basis of the primary outcome of the live-birth rate, after applying the following assumptions: if the true rate of live births in the two treatment groups (one fresh-embryo transfer, perhaps followed by one frozen-and-thawed transfer [1+(1)], or one fresh double-embryo transfer [2+0]) is 0.30, then the probability is 0.80 that the upper limit of the 95 percent confidence interval for the difference in the probability of live birth between the groups is lower than 0.10, if 330 patients who can be evaluated are included in each group. The number of patients lost to follow-up was assumed to be zero. Thus, 660 patients were needed.

STATISTICAL ANALYSIS

The primary statistical analysis was carried out according to the intention-to-treat principle and included 661 patients. We also performed a secondary per-protocol analysis, in which 634 patients were analyzed according to the actual treatment given (single-embryo transfer [1+(1)] or double-embryo transfer [2+0]). Patients who did not undergo transfer of a frozen-and-thawed embryo because there were no surviving embryos were still included in both analyses as part of the single-embryo-transfer group. Comparisons of continuous variables were performed with the use of Fisher's nonparametric permutation test, and comparisons of dichotomous variables with the use of Fisher's exact test. For the differences in proportions between groups, 95 percent confidence intervals were calculated. For descriptive statistics, we used means ±SD and range. All tests were two-sided, and the level of significance was 0.05. SPSS software (version 11.5) and SAS software (version 8.2) were used.

The sponsors of this study played no role in the study design, the collection of data, the analysis or interpretation of the data, the writing of the report, or the decision to submit the manuscript for publication.

RESULTS

PATIENTS

A total of 661 patients underwent randomization. Of those, 331 patients were randomly assigned to undergo double-embryo transfer and 330 to undergo elective single-embryo transfer (i.e., there were at least two good-quality embryos to choose from) (Fig. 1). The demographic and clinical characteristics of the patients are shown in Table 1.

INTENTION-TO-TREAT ANALYSIS

The outcomes of the two approaches to in vitro fertilization, on the basis of the intention-to-treat analysis, are shown in Table 2. In the single-embryo-transfer group, 128 women had a pregnancy resulting in at least one live birth (38.8 percent; 95 percent confidence interval, 33.5 to 44.3 percent), as compared with 142 women in the double-embryo transfer group (42.9 percent; 95 percent confidence interval, 37.5 to 48.4 percent). The absolute difference

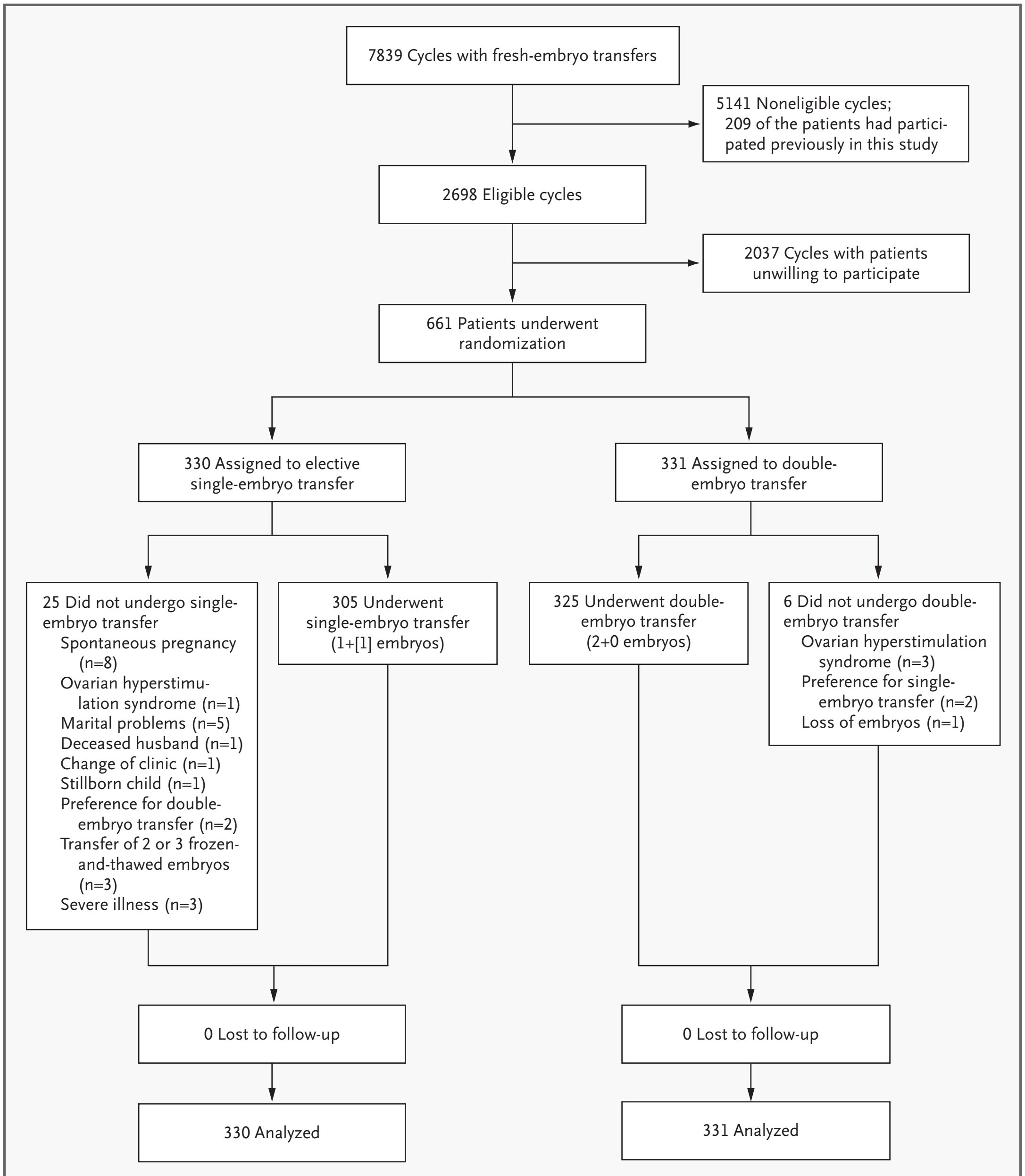

Figure 1. Flow Chart Showing the Enrollment and Status of Cycles in the Study.
Among patients who received embryo transfers, 1+[1] denotes one fresh-embryo transfer, followed, if necessary, by one frozen-and-thawed embryo; and 2+0 denotes one transfer of two fresh embryos.

Table 1. Characteristics of the Patients.

Characteristic	Elective Single-Embryo Transfer (N=330)	Double-Embryo Transfer (N=331)	P Value
Age at fresh-embryo transfer — yr			
Mean ±SD	30.9±3.0	30.8±3.0	0.70
Range	22.6–35.9	21.6–35.9	
Body-mass index*			
Mean ±SD	24.5±4.6	24.5±4.4	0.84
Range	17.2–49.2	16.9–40.6	
Duration of infertility — yr			
Mean ±SD	3.6±1.7	3.8±3.9	0.29
Range	0–12	1–12	
Cause of infertility — no. (%)†			
Tubal factor	70 (21.2)	60 (18.1)	0.33
Endometriosis	58 (17.6)	38 (11.5)	0.03
Hormonal factor	64 (19.4)	80 (24.2)	0.16
Male factor	164 (49.7)	155 (46.8)	0.48
Other, including unknown	55 (16.7)	71 (21.5)	0.14
History of previous pregnancies — no. (%)	90 (27.3)	105 (31.7)	0.23
Live birth	27 (8.2)	36 (10.9)	0.29
Miscarriage	36 (10.9)	57 (17.2)	0.03
Ectopic pregnancy	8 (2.4)	15 (4.5)	0.20
Termination of pregnancy	35 (10.6)	20 (6.0)	0.04
History of previous treatment with in vitro fertilization — no. (%)	75 (22.7)	70 (21.1)	0.64
Pregnancy	9 (2.7)	16 (4.8)	
Live birth	2 (0.6)	4 (1.2)	

* The body-mass index is the weight in kilograms divided by the square of the height in meters.
† More than one diagnosis per couple was possible; 513 couples had one, 140 had two, and 8 had three diagnoses.

between the groups was 4.1 percent, and the 95 percent confidence interval for the difference was −3.4 to 11.6 percent. These results do not demonstrate equivalence of the two approaches with respect to the rates of live births according to prespecified criteria, but they do indicate that the plausible rate of live births for the group undergoing elective single-embryo transfers ranges from 11.6 percent lower to 3.4 percent greater than that in the double-embryo-transfer group.

In the cycle in which a single fresh embryo was transferred, the rate of live births was significantly lower than in the double-embryo-transfer group (27.6 percent vs. 42.9 percent, $P<0.001$). In the subsequent cycle, involving transfer of a frozen-and-thawed embryo, the rate of live births was 29 of 177 (16.4 percent). Thirty-eight patients did not receive a thawed-embryo transfer because they had no surviving embryos after thawing; they were included in the single-embryo-transfer group in both the intention-to-treat and the per-protocol analyses. The rate of multiple births was 47 of 142 (46 pairs of twins and 1 set of triplets) in the double-embryo-transfer group, as compared with 1 of 128 (1 pair of twins) in the single-embryo-transfer group (33.1 percent vs. 0.8 percent, $P<0.001$). The results in the single-embryo-transfer group were calculated by combining the results of 120 live births after conception by in vitro fertilization and 8 births after spontaneous pregnancies (Table 2). Since there was a shift during the trial toward less strict inclusion criteria for randomization, a subgroup analysis was performed that compared patients who underwent randomization before and after this change. No significant difference in the cumulative rate of live births was noted between the first period and the second period (83 of 215 [38.6 percent] vs. 187 of 446 [41.9 percent]).

PER-PROTOCOL ANALYSIS

The outcomes of treatment based on the per-protocol analysis are shown in Table 3. According to the per-protocol analysis, the rate of live births after a fresh-embryo transfer and the cumulative rate of live births were 91 of 307 (29.6 percent) and 119 of 307 (38.8 percent), respectively, in the single-embryo-transfer group. In the double-embryo-transfer group, the rate of live births was 142 of 327 (43.4 percent). The rate of multiple births as a percentage of live births was 33.1 percent in the double-embryo-transfer group and 0.8 percent in the single-embryo-transfer group.

DISCUSSION

In this large, randomized study, transferring one fresh embryo, followed if necessary by the transfer of one frozen-and-thawed embryo, did not result in a substantially lower rate of pregnancy resulting in at least one live birth than did transferring two fresh embryos on a single occasion. Equivalence in the rates of live births between the two methods of transfer was not confirmed according to our prespecified criteria, but the 95 percent confidence interval for the difference demonstrates that the plausible rate would not be more than 11.6 percentage points lower for single-embryo transfers than for double-embryo transfers. At the same time, the use of single-embryo transfers resulted in a marked reduction in the rate of multiple gestations. These data provide support for the use of single-embryo transfers in certain patients undergoing in vitro fertilization.

Single-embryo transfers seem suitable for many patients in clinical practice. In the present study, 37.1 percent of 7839 consecutive cycles (2698 eligible cycles plus 209 cycles in patients who had previously participated in this study) at the participating in vitro fertilization clinics met the inclusion criteria (Fig. 1). This is probably a representative estimate for many in vitro fertilization clinics. The percentage of cycles suitable for single-embryo transfer and the findings regarding the rates of live births and multiple gestation are consistent with those of our previous retrospective study.[13] Studies of elective single-embryo transfer that have been performed in Belgium and Finland have shown satisfactory results in the group of patients who undergo single-embryo transfers, with rates of clinical pregnancy of 30 to 40 percent per transfer.[14,15,19-24]

Data comparing single-embryo transfer and double-embryo transfer are limited. In a randomized trial comparing the two types of fresh-embryo-transfer cycles among 53 women in Belgium,[14] the rate of ongoing pregnancy was significantly higher in the double-embryo-transfer group than in the single-embryo-transfer group (74.0 percent vs. 38.5 percent). In a similar trial in Finland[15] involving 144 patients, the clinical pregnancy rate was 32.4 percent in the group that underwent single-embryo transfer and 47.1 percent in the group that underwent double-embryo transfer. In contrast, the present study included the possibility of receiving two single-embryo transfers, but only one at a time — if the fresh-embryo transfer did not result in a live birth, one cycle involving the transfer of a frozen-and-thawed embryo was performed. Unlike the report from Finland,[15] our study showed a significantly lower rate of pregnancy resulting in at least one live birth after a single fresh-embryo transfer than with the transfer of two fresh embryos at one time. Thus, this report emphasizes the importance of performing additional cycles with frozen-and-thawed embryos.

It is well known that the risk of an adverse outcome after in vitro fertilization is attributable in large part to the greatly increased rate of multiple births.[1-5,7] The rates of multiple gestation after in vitro fertilization in Europe and the United States are 26.4 percent and 35.4 percent, respectively,[25,26] underscoring the magnitude of the problem. However, higher risks of preterm delivery and low birth weight have also been observed for singletons conceived by in vitro fertilization and have been attributed at least in part to parental characteristics associated with infertility, such as high maternal age and nulliparity; these, of course, would not be influenced by the use of single-embryo transfer.[1-7] Nonetheless, a substantial reduction in multiple births after in vitro fertilization would dramatically decrease the risks associated with prematurity and low birth weight for children born after in vitro fertilization.

A possible disincentive to the use of single-embryo transfers is the concern that the chance of pregnancy will be reduced. This concern was noted among the reasons for nonparticipation in our study during the initial year. Similar attitudes were noted in other countries during the late 1990s.[27] In Sweden, however, a combination of media coverage and

Table 2. Outcomes According to Treatment Group in the Intention-to-Treat Analysis.*

Variable	Elective Single-Embryo Transfer (N=330)	Double-Embryo Transfer (N=331)	P Value	95% CI for the Difference in Percentage between Groups
Treatment of patients — no. (%)†				
With IVF	178 (53.9)	189 (57.1)	0.43	
With ICSI	137 (41.5)	121 (36.6)	0.20	
With mixed IVF and ICSI‡	15 (4.5)	21 (6.3)	0.39	
Aspirated oocytes — no.				
Mean ±SD	13.0±5.7	12.6±5.4	0.37	
Range	2 to 31	3 to 44		
Fertilized oocytes — no.				
Mean ±SD	8.5±3.8	8.3±3.7	0.47	
Range	2 to 21	2 to 31		
Available good-quality embryos — no.				
Mean ±SD	4.6±2.4	4.6±2.4	0.98	
Range	2 to 16	2 to 17		
Day of transfer — no. (%)§				
Day 2	305 (92.4)	306 (92.4)		
Day 3	16 (4.8)	16 (4.8)		
Day 5	8 (2.4)	8 (2.4)		
Patients who received assigned treatment in the fresh-embryo cycle — no.	327	325		
Patients who did not receive assigned treatment in the fresh-embryo cycle — no.¶	3	6		
Patients who did not receive assigned treatment in the frozen-and-thawed cycle — no.‖	60	—		
Pregnancies — no. (%)				
Fresh-embryo cycle	111 (33.6)	174 (52.6)	<0.001	11.4 to 26.5
Thawed-embryo cycle**	43 (24.3)	—		
Cumulative, fresh-embryo and thawed-embryo cycles††	158 (47.9)	174 (52.6)	0.24	−2.9 to 12.3
95% CI for the estimated cumulative rate — %	42.5 to 53.4	47.1 to 58.0		
Ectopic pregnancies — no.				
Fresh-embryo cycle	0	1		
Thawed-embryo cycle	1	—		
Spontaneous abortions at ≤12 weeks of gestation — no. (%)				
Fresh-embryo cycle	17 (15.3)	27 (15.5)		
Thawed-embryo cycle	13 (30.2)	—		
Spontaneous abortions at >12 weeks of gestation — no.				
Fresh-embryo cycle‡‡	2	3		
Thawed-embryo cycle	0	—		

new legislation, which stated that single-embryo transfer should be the routine procedure and that two embryos could be transferred only in selected cases, has led to a general implementation of single-embryo transfer and a change in attitude among patients.

The acceptability of single-embryo transfer for both patients and clinicians will relate in part to how

Table 2. (Continued.)

Variable	Elective Single-Embryo Transfer (N=330)	Double-Embryo Transfer (N=331)	P Value	95% CI for the Difference in Percentage between Groups
Stillborn infants ≥28 weeks of gestation — no.				
Fresh-embryo cycle	1	1		
Thawed-embryo cycle	0	—		
Live births — no. (%)				
Fresh-embryo cycle	91 (27.6)	142 (42.9)	<0.001	8.0 to 22.6
Thawed-embryo cycle**	29 (16.4)	—		
Cumulative, fresh-embryo and thawed-embryo cycles§§	128 (38.8)	142 (42.9)	0.30	–03.4 to 11.6
95% CI for the estimated cumulative rate — %	33.5 to 44.3	37.5 to 48.4		
Multiple births — no. (%)¶¶	1 (0.8)	47 (33.1)	<0.001	

* IVF denotes in vitro fertilization, ICSI intracytoplasmic sperm injection, and CI confidence interval. A dash denotes not applicable.
† Because of rounding, percentages may not total 100.
‡ Mixed IVF and ICSI denotes cycles in which 50 percent of the oocytes were fertilized by IVF and 50 percent by ICSI.
§ In the single-embryo-transfer group, one patient canceled the transfer owing to ovarian hyperstimulation syndrome. In the double-embryo-transfer group, one patient's embryos were lost during transfer.
¶ In the single-embryo-transfer group, one patient canceled the transfer because of ovarian hyperstimulation syndrome, and two patients requested a double-embryo transfer. In the double-embryo-transfer group, one patient's embryos were lost during transfer, three patients had only one embryo transferred because of ovarian hyperstimulation syndrome, and two patients requested a single-embryo transfer.
‖ Of the patients who did not receive treatment in the frozen-and-thawed cycle, 38 had no surviving embryos after freezing and thawing, 8 had a spontaneous pregnancy between the fresh and the frozen-and-thawed cycle, 5 had marital problems, 3 had severe illnesses, 1 patient's husband died, 1 changed clinics, 1 continued with a new fresh-embryo cycle after a fresh single-embryo transfer resulted in a stillborn child, and 3 received two or three embryos in the frozen-and-thawed cycle.
** The percentage was calculated on the basis of 177 thawed-embryo cycles.
†† In the single-embryo-transfer group, four patients had two pregnancies each, one from a cycle with a fresh embryo and one from a cycle with a frozen-and-thawed embryo. This group also includes eight pregnancies that occurred after spontaneous conception between the cycle with the fresh embryo and the cycle with the frozen-and-thawed embryo.
‡‡ One patient in the single-embryo-transfer group underwent termination of pregnancy owing to fetal acrania and was included in the miscarriage group in the study.
§§ The single-embryo-transfer group includes eight live births that occurred after spontaneous conception between the cycle with the fresh embryo and the cycle with the frozen-and-thawed embryo.
¶¶ The double-embryo-transfer group includes one multiple birth in which one fetus died in utero at 24 weeks of gestation.

the cost of in vitro fertilization is covered. Single-embryo transfer is likely to be more readily accepted when there is insurance coverage for in vitro fertilization. If individual patients pay for in vitro fertilization, however, they might prefer that more embryos be transferred, in an effort to maximize their chances of having a live-born child. However, in Sweden, the introduction of single-embryo transfer during the past two years has been well accepted at both private and public in vitro fertilization clinics. Other disadvantages of single-embryo transfer, such as inconvenience and the stress of an additional cycle (although, in this case, a cycle not requiring ovarian stimulation and the induction of ovulation), must be balanced against the much higher risk of multiple gestation with double-embryo transfer.

It is possible that psychological factors, such as anxiety, may affect rates of implantation.[28] Because the patients in our study were randomly assigned to therapy, and the patients and physicians were blinded to the number of fresh embryos transferred, expectations and psychological factors would be expected to be similar in the two groups.

Eight patients conceived spontaneously between the cycles with the fresh and the thawed embryos. These patients were included in the intention-to-treat analysis as pregnant but were not included in the per-protocol analysis, since the pregnancy was

Table 3. Outcome According to Treatment Group in the Per-Protocol Analysis.*

Variable	Elective Single-Embryo Transfer (N=307)	Double-Embryo Transfer (N=327)	P Value	95% CI for the Difference in Percentage between Groups
Treatment of patients — no. (%)				
With IVF	163 (53.1)	188 (57.5)	0.30	
With ICSI	130 (42.3)	119 (36.4)	0.13	
With mixed IVF and ICSI†	14 (4.6)	20 (6.1)	0.38	
Aspirated oocytes — no.				
Mean ±SD	12.9±5.2	12.5±5.0	0.27	
Range	2 to 44	3 to 30		
Fertilized oocytes — no.				
Mean ±SD	8.4±3.9	8.2±3.6	0.43	
Range	2 to 31	2 to 23		
Available good-quality embryos — no.				
Mean ±SD	4.5±2.3	4.6±2.4	0.63	
Range	2 to 16	2 to 17		
Day of transfer — no. (%)				
Day 2	284 (92.5)	305 (93.0)		
Day 3	14 (4.6)	16 (4.9)		
Day 5	9 (2.9)	6 (1.8)		
Implantation rate, fresh-embryo cycle — no. (%)‡	100 (32.6)	220 (33.6)		
Pregnancies — no. (%)				
Fresh-embryo cycle	107 (34.9)	173 (52.9)	<0.001	10.3 to 25.8
Thawed-embryo cycle§	42 (23.9)	—		
Cumulative, fresh-embryo and thawed-embryo cycles¶‖	145 (47.2)	173 (52.9)	0.18	−2.1 to 13.5
95% CI for the estimates, cumulative rate — %	41.5 to 53.0	47.4 to 58.5		
Ectopic pregnancies — no.				
Fresh-embryo cycle	0	1		
Thawed-embryo cycle	1	—		
Spontaneous abortions at ≤12 weeks of gestation — no. (%)				
Fresh-embryo cycle	14 (13.1)	26 (15.0)		
Thawed-embryo cycle	13 (31.0)	—		
Spontaneous abortions at >12 weeks of gestation — no.				
Fresh-embryo cycle**	2	3		
Thawed-embryo cycle	0	—		

not a direct result of the treatment given. Thirty-eight patients received no thawed embryos, since no embryos survived. This outcome was expected with this approach, and those 38 patients were included in the single-embryo-transfer group in both the intention-to-treat and the per-protocol analyses.

Results of cycles with frozen-and-thawed embryos vary considerably among clinics. The approach based on cumulative single-embryo transfer requires a well-functioning freezing program, and clinics contemplating recommending single-embryo transfers to their patients must focus on the freezing procedure in order to improve their cumulative rate of pregnancies resulting in live births.[29]

Table 3. (Continued.)

Variable	Elective Single-Embryo Transfer (N=307)	Double-Embryo Transfer (N=327)	P Value	95% CI for the Difference in Percentage between Groups
Stillborn infants ≥28 weeks of gestation — no.				
Fresh-embryo cycle	0	1		
Thawed-embryo cycle	0	—		
Live births — no. (%)				
Fresh-embryo cycle	91 (29.6)	142 (43.4)	0.001	6.3 to 21.3
Thawed-embryo cycle§	28 (15.9)	—		
Cumulative, fresh-embryo and thawed-embryo cycles‖	119 (38.8)	142 (43.4)	0.26	−3.0 to 12.3
95% CI for the estimated cumulative rate — %	33.3 to 44.5	38.0 to 49.1		
Multiple births — no. (%)††	1 (0.8)	47 (33.1)		

* IVF denotes in vitro fertilization, ICSI intracytoplasmic sperm injection, and CI confidence interval. A dash denotes not applicable.
† Mixed IVF and ICSI indicates cycles in which 50 percent of the oocytes were fertilized by IVF and 50 percent by ICSI.
‡ Percentages were calculated on the basis of 307 embryos in the single-embryo-transfer group, and 654 in the double-embryo-transfer group.
§ The percentage was calculated on the basis of 176 thawed-embryo cycles.
¶ In the single-embryo-transfer group, four patients had two pregnancies each, one from a cycle with a fresh embryo and one from a cycle with a frozen-and-thawed embryo.
‖ The single-embryo-transfer group does not include eight live births that occurred after spontaneous conception between the cycle with the fresh embryo and the cycle with the frozen-and-thawed embryo.
** One patient in the single-embryo-transfer group underwent a legal abortion owing to fetal acrania and was included in the miscarriage group in the study.
†† The double-embryo-transfer group includes one multiple birth in which one fetus died in utero at 24 weeks of gestation.

Single-embryo transfer may be particularly useful in cycles with frozen-and-thawed embryos, since the embryos can be frozen and thawed one by one without further losses, whereas the use of single-embryo transfer in cycles with fresh embryos (with freezing of surplus embryos) will result in some loss of embryo quality due to the freezing-and-thawing procedure itself.

In conclusion, this study shows that a single fresh-embryo transfer, followed (if there was no live birth) by the transfer of one frozen-and-thawed embryo, results in a marked reduction in the rate of multiple gestations but not in a substantial reduction in the rate of pregnancy resulting in one or more live births. Our results strongly support the introduction of elective single-embryo transfer as an effective method for women less than 36 years of age to reduce the high risk of multiple births associated with a transfer of two embryos.

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APPENDIX

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references

1. Bergh T, Ericsson A, Hillensjö T, Nygren K-G, Wennerholm U-B. Deliveries and children born after in-vitro fertilisation in Sweden 1982-1995: a retrospective cohort study. Lancet 1999;354:1579-85.
2. Gissler M, Malin Silverio M, Hemminki E. In-vitro fertilization pregnancies and perinatal health in Finland 1991-1993. Hum Reprod 1995;10:1856-61.
3. Westergaard HB, Johansen AM, Erb K, Andersen AN. Danish national in-vitro fertilization registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. Hum Reprod 1999;14:1896-902.
4. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med 2002;346:731-7.
5. Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ 2004;328:261-5.
6. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol 2004;103:551-63.
7. Wennerholm UB, Bergh C. Outcome of IVF pregnancies. Fetal Matern Med Rev 2004;15:27-57.
8. Strömberg B, Dahlquist G, Ericson A, Finnström O, Köster M, Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. Lancet 2002;359:461-5.
9. Ericson A, Källen B. Congenital malformations in infants born after IVF: a population-based study. Hum Reprod 2001;16:504-9.
10. The National Board of Health and Welfare. Official statistics of Sweden. (Accessed November 3, 2004, at http://www.sos.se.)
11. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after in vitro fertilization. N Engl J Med 1998;339:573-7.
12. Coetsier T, Dhont M. Avoiding multiple pregnancies in in-vitro fertilization: who's afraid of single embryo transfer? Hum Reprod 1998;13:2663-4.
13. Strandell A, Bergh C, Lundin K. Selection of patients suitable for one-embryo transfer reduces the rate of multiple births by half without impairment of overall birth rates. Hum Reprod 2000;15:2520-5.
14. Gerris J, De Neuborg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M. Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. Hum Reprod 1999;14:2581-7.
15. Martikainen H, Tiitinen A, Tomás C, et al. One versus two embryo transfer after IVF and ICSI: a randomized study. Hum Reprod 2001;16:1900-3.
16. Steer CV, Mills CL, Tan SL, Campbell S, Edwards RG. The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. Hum Reprod 1992;7:117-9.
17. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Hum Reprod 1998;13:3434-40.
18. Pocock SJ. Clinical trials: a practical approach. Chichester, England: John Wiley, 1983.
19. Van Royen E, Mangelschots K, De Neubourg D, et al. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999;14:2345-9.
20. Gerris J, De Neubourg D, Mangelschots K, et al. Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum Reprod 2002;17:2626-31.
21. De Sutter P, Van der Elst J, Coetsier T, Dhont M. Single embryo transfer and multiple pregnancy rate reduction in IVF/ICSI: a 5-year appraisal. Reprod Biomed Online 2003;6:464-9.
22. Vilska S, Tiitinen A, Hydén-Granskog C, Hovatta O. Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. Hum Reprod 1999;14:2392-5.
23. Tiitinen A, Unkila-Kallio L, Halttunen M, Hydén-Granskog C. Impact of elective single embryo transfer on the twin pregnancy rate. Hum Reprod 2003;18:1449-53.
24. Söderström-Anttila V, Vilska S, Mäkinen S, Foudila T, Suikkari A-M. Elective single embryo transfer yields good delivery rates in oocyte donation. Hum Reprod 2003;18:1858-63.
25. 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.
26. Assisted reproductive technology in the United States: 2000 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 2004;81:1207-20.
27. Pinborg A, Loft A, Schmidt L, Andersen AN. Attitudes of IVF/ICSI-twin mothers towards twins and single embryo transfer. Hum Reprod 2003;18:621-7.
28. Smeenk JMJ, Verhaak CM, Eugster A, van Minnen A, Zielhuis GA, Braat DDM. The effect of anxiety and depression on the outcome of in-vitro fertilization. Hum Reprod 2001;16:1420-3.
29. Tiitinen A, Halttunen M, Härkki P, Vuoristo P, Hydén-Granskog C. Elective single embryo transfer: the value of cryopreservation. Hum Reprod 2001;16:1140-4.