The Influence of Supernumerary Embryos on the Clinical Outcome of IVF Cycles[1]

OSAMA SALHA,[2,3] TUNDE DADA,[2] STEVE LEVETT,[2] VICKI ALLGAR,[2] and VINAY SHARMA[2]

Submitted: August 19, 1999
Accepted: March 6, 2000

Purpose: *To assess the influence of the presence of quality supernumerary embryos on the clinical outcome and risk of multiple conception in patients having their first in vitro fertilization (IVF) cycle.*
Methods: *Retrospective cohort study of 1448 women having their first IVF treatment cycle who received 4004 embryos where at least six embryos were available for transfer treated in an Assisted Conception Unit based in a large teaching hospital.*
Results: *The replacement of three rather than two embryos to women under 35 years who had good-quality supernumerary embryos resulted in a higher twin (12.5 vs. 11.9%) and triplet birth rates (2.1 vs. 0%), without significantly improving the clinical pregnancy (50.5 vs. 45.2%) or total live birth rates (38.9 vs. 35.7%). In the absence of quality spare embryos, these women who had three rather than two embryos replaced had a significantly higher clinical pregnancy rate (39.3 vs. 28.8%;* $P = 0.04$*), total live birth (32.7 vs. 19.4%;* $P = 0.02$*) and singleton birth rate per cycle (20.8 vs. 14.4%;* $P = 0.04$*), without significantly influencing the multiple birth rate. In women over 35 years, the replacement of three instead of two embryos in the presence or absence of quality supernumerary embryos led to a significant improvement in clinical outcome, without being associated with a concurrent increase in the multiple birth rate. Women in both age groups who had either two or three embryos replaced in the presence of quality supernumerary embryos had a notably better clinical outcome compared with their counterparts who had the same number of embryos replaced, but with no quality embryos to spare.*
Conclusions: *The presence of good-quality supernumerary embryos can be used as a reference to determine the optimal number of embryos to transfer and as an indicator of the probability of success of an individual couple in a given cycle. Optimal pregnancy rates and simultaneous reduction of multiple gestation can be achieved with a flexible embryo replacement policy that is based on embryo quality, maternal age, and the presence or absence of surplus quality embryos.*

KEY WORDS: IVF; supernumerary embryos; embryo quality; multiple pregnancy; maternal age.

INTRODUCTION

Assisted reproduction treatment is inevitably associated with an increased risk of multiple conceptions. In fact, nearly 70% of all triplet pregnancies result from ovulation induction or assisted reproductive technologies (ART) (1–3). High-order multiple pregnancies entail additional medical risks (4–8), psychological stress (9), and considerable financial costs for the couple (10). Several authors therefore have suggested the selective replacement of two rather than three embryos (11–16). Nevertheless, it seems that embryo replacement policies have not been significantly influenced by the above-mentioned publications, since the incidence of triplet pregnancies after IVF treatment has remained static at 4–5% (17, 18). The clinicians' as well as the couples' desire to achieve the highest probability of a pregnancy, together with the availability of fetal reduction, are probably the two main responsible factors. The ethical aspects of fetal reduction (9) and the risk of a total pregnancy loss, which varies from 7.7 to 22.9% (19, 20), prevent many couples from accepting selective feticide (5). Therefore, in order to reduce the incidence of multiple pregnancies in IVF cycles, risk factors for such an outcome need to be prospectively identified. The replacement of two instead of three embryos may become more acceptable to couples and clinicians only if it could be conclusively demonstrated that, in certain circumstances, the transfer of three embryos confers no significant advantage.

[1] Presented in part at the British Fertility Society Meeting in Sheffield April 15–17 1998.
[2] Assisted Conception Unit and Medical Statistics Department St. James's University Hospital, Leeds LS9 7TF, England
[3] To whom correspondence should be addressed.

1058-0468/00/0700-0335$18.00/0

Clinical factors influencing the probability of pregnancy after embryo transfer include maternal age (21), cause of infertility, stimulation protocol (22), thickness of the endometrium (23, 24), and embryo morphology (25). Indeed, 14 variables were analyzed by Roseboom *et al.* (26) to obtain a more complete view of the complicated process of achieving a successful pregnancy. All these are cumbersome to apply in routine clinical practice, and it is impossible to combine all these variables to develop one threshold that can be used to predict the outcome. Furthermore, the laboratory variables, expertise of staff, and the techniques applied in each program also would have a significant impact on outcome. Accordingly, the British Fertility Society (BFS) and the American Society of Reproductive Medicine (ASRM) have encouraged centers to review their own data and adapt their own embryo transfer policy. The most important index they were advised to consider was the multiple pregnancy rate in the center. Such a policy may reduce the overall incidence of multiple pregnancy, but the transfer of two or three embryos will always carry an individual risk. Balancing benefit against disadvantage is not easy, since benefit (increased pregnancy rates) is immediately appreciated by both clinicians and patients, whereas the impacts of longer-term consequences are more distant. What is needed is a reliable indicator of a particular couple's risk of multiple pregnancy. This would give comfort that the transfer of two or more embryos would yield good pregnancy rates without the significant risk of high-order multiple pregnancy.

In this study we analyzed the records of patients in their first IVF cycle with a view to establishing accurate prognostic information regarding the optimum number of embryos required to achieve the maximum probability of a pregnancy without significantly increasing the multiple pregnancy rate. This was done by comparing the clinical and multiple pregnancy rates per cycle in patients who had good-quality spare embryos with those who had none to spare.

MATERIALS AND METHODS

Patients

We studied all couples in their first cycle of IVF treatment reaching oocyte recovery and embryo transfer at the Assisted Conception Unit at St. James's University Hospital, Leeds, during a 7-year period from 1991 to 1998. Only patients who had at least six embryos available for transfer were included in the study. Of the 2032 patients involved, 1448 couples fulfilled the inclusion criteria and were included in the analysis.

IVF Procedure

The protocol for pituitary down-regulation and ovarian stimulation as well as the techniques for cycle monitoring, oocyte retrieval, and embryo transfer have been described previously (27). Briefly, pituitary down-regulation was achieved using either Nafarelin intranasal spray 100 μg 8 hr (Searle, High Wycombe, UK) or subcutaneous Buserelin acetate 0.5 mg daily (Hoechst, Hounslow, Middlesex, UK) commencing on the first day of the menstrual cycle. Twenty-one days later, pituitary desensitization was assessed using transvaginal ultrasonography (Kretz Technik Combison 310, Crawley, Sussex, UK), confirming that there were no ovarian follicles > 5 mm in diameter and the endometrial thickness was < 5 mm. Thereafter, desensitization was maintained and multiple follicular stimulation was achieved using human menopausal gonadotropin (hMG) (Pergonal; Serono Laboratories, Welwyn Garden City, Herts., UK) or follicle-stimulating hormone (FSH) (Metrodin HP; Serono Laboratories, Herts, UK; or Orgafol; Organon Laboratories, Cambridge, UK). Tracking of follicular growth was performed using transvaginal ultrasonography from day 6 of gonadotrophin stimulation. The mean maximal follicular diameter was calculated from four measurements of the leading follicle(s), in sagittal and transverse plane. In each plane, the follicle was measured along its longitudinal axis and at 90° to this axis. When the leading follicle(s) had a mean diameter of $\geq$ 18 mm, human chorionic gonadotropin (hCG) (Profasi; Serono Laboratories, UK; or Pregnyl; Organon Laboratories, Cambridge, UK) was administered at a dose of 5000 IU subcutaneously or intramuscularly. Transvaginal oocyte retrieval under ultrasound guidance was performed 35 to 37 hr later using a double lumen oocyte harvesting needle (Rocket Medical; Watford, UK). Once identified, the oocyte–cumulus complex was placed in 1 ml of Earle's balanced salt solution containing 0.03 M sodium pyruvate, 15% human serum albumin, penicillin, and gentamycin in a Nunclon 4-well dish, incubated at 37 °C, and gassed with 5% CO_2. All normal-appearing oocytes were inseminated with 100,000 sperm/ml, 40 to 42 hr after hCG administration. Oocytes were examined for fertilization (presence of two pronuclei) at 16 to 20 hr after insemination.

Embryo Grading

Embryo transfers were performed approximately 48 to 52 hr post-egg collection when the majority of embryos were at the 2- or 4-cell stage. The numbers of cell divisions and embryo morphology at the time of embryo transfer were regarded as a reflection of embryo development up to that point. Embryo morphology was evaluated according to the number of blastomeres, the symmetry of the blastomeres, and percentage of extracellular fragmentation: grade 1 embryos had even-sized blastomeres with minimal or no fragmentation; grade 2 embryos had uneven-sized blastomeres with up to 20% fragmentation; grade 3 embryos had highly irregular blastomeres with significant fragmentation; and grade 4 embryos had barely defined blastomeres with grainy cytoplasm.

The number of embryos transferred ranged from two to three, depending on the patients' age, the availability and quality of the embryos. After a comprehensive counseling of the advantages and disadvantages of replacing two versus three embryos, the final decision on the number of embryos to be replaced was made by the couple. Couples who had more than three supernumerary good-quality (grade 1 and 2) embryos were counseled regarding freeze-storage of these embryos for later use. Lower-grade embryos (grades 3 and 4) not deemed suitable for cryopreservation were observed in culture for a further 48 hr before being discarded.

The Unit's policy is to freeze-store only grade 1 or grade 2 embryos when there are at least three such embryos supernumerary. Thus, patients who had spare grade 1 or grade 2 embryos would have had either grade 1 or grade 2 embryos replaced. Accordingly, there were no specific criteria to differentiate between embryos selected for transfer and those to be cryopreseved, since all embryos were of similar grade.

Luteal support was provided with either hCG (Pregnyl or Profasi) 2500 IU given on the day of embryo transfer and 72 hours later, or if $>$ 15 follicles were aspirated, with progesterone (100 mg by intramuscular injections once daily; Gestone, Ferring, Middlesex, UK). Pregnancy tests were performed 14 days post-embryo transfer, using a commercial urinary kit (ICON II, hCG, San Diego, CA).

Definitions

Since all patients included in the study were those who had their first IVF treatment cycle, the number of cycles was equivalent to the number of patients. A clinical pregnancy was defined as any pregnancy confirmed by a transvaginal ultrasound scan performed at 6 to 7 weeks gestation demonstrating a gestational sac containing a fetal pole with an audible–visible fetal heart. The implantation rate was calculated by dividing the number of gestational sacs seen at the initial pregnancy scan by the total number of embryos transferred multiplied by 100. The clinical pregnancy rate per cycle (CPR/cycle) was calculated by dividing the number of clinical pregnancies by the total number of treatment cycles multiplied by 100, while the live birth rate per cycle was calculated by dividing the number of live births by the total number of cycles multiplied by 100.

Statistical Analysis

The data were analyzed using χ^2 test, where a P value < 0.05 was considered to be statistically significant.

RESULTS

Between January 1991 and January 1998, a total of 4004 embryos were transferred to 1448 patients in their first IVF treatment cycle who met the inclusion criteria (Table I). A total of 565 clinical pregnancies were achieved (overall CPR/cycle, 39.0%) of which 34 were ectopic pregnancies (ectopic pregnancy rate/cycle, 2.3%) and 82 ended in a first trimester miscarriage (miscarriage rate/cycle, 5.7%). Of the remaining 449 live births (live birth/cycle, 31.0%) 307 were singletons (singleton pregnancy rate/cycle, 21.2%), 121 were twins (twinning rate/cycle, 8.4%) and 21 were triplets (triplet rate/cycle, 1.5%). The rate of first trimester pregnancy loss as well as the outcome of continuing pregnancies is illustrated in Table II. The data

Table I. Summary of the Two Age Groups

	Maternal age $\leq$ 35 years	Maternal age $>$35 years	Total
Number of cycles	1126	322	1448
Number embryos transferred	3097	907	4004
Number embryos implanted	673	155	828
Embryo implantation rate	21.7%	17.1%	20.7%
Clinical pregnancies	468	97	565
Clinical pregnancy rate/cycle	41.6%	30.1%	39.0%
Live births	370	79	449
Live birth rate/cycle	32.8%	24.5%	31.0%

Table II. Summary of Pregnancy Outcome of the Two Age Groups

	Maternal age ≤ 35 years	Maternal age >35 years	Total
Clinical pregnancies	468	97	565
Ectopics (% of conceptions)	32 (6.8%)	2 (2.1%)	34 (6.0%)
1st Trimester miscarriage (% of conceptions)	66 (14.1%)	16 (16.5%)	82 (14.5%)
Continuing pregnancies	370	79	449
Singleton pregnancy (% of continuing pregnancies)	236 (63.7%)	71 (89.9%)	307 (68.4%)
Twin pregnancy (% of continuing pregnancies)	114 (30.8%)	7 (8.9%)	121 (26.9%)
Triplet pregnancy (% of continuing pregnancies)	20 (5.4%)	1 (1.3%)	21 (4.7%)

were further analyzed according to the number of embryos transferred and the availability of good-quality spare embryos.

Clinical Outcome for Women ≤ 35 Years (n = 1126)

Clinical Outcome When Two or Three Embryos Were Replaced. In the presence of three grade 1 or grade 2 spare embryos, the replacement of three rather than two embryos was not associated with a significant improvement in any of the clinical parameters analyzed (Table III). The twin pregnancy rate/cycle in patients receiving three embryos was 12.5% compared to 11.9% when two embryos were transferred, while patients who had three embryos replaced had a 2.9% chance of having triplets. Thus, the replacement of three instead of two embryos in this age group only served to increase the multiple pregnancy rate without significantly improving the clinical (50.5 vs. 45.2%) or the live birth rates (38.9 vs. 35.7%).

On the other hand, when there were no supernumerary grade 1 or grade 2 embryos available, young women who had three embryos replaced (n = 529) had a significantly higher clinical pregnancy (39.3 vs. 28.8%; P = 0.04), live birth (32.7 vs. 19.4%; P = 0.01), and singleton birth rate per cycle (20.8 vs. 14.4%; P 0.3) compared to those who had two embryos replaced (n = 160) (Table III). Furthermore, the twin (9.8 vs. 5%) and triplet (2.1 vs. 0%) birth rates were similar in the two groups. Thus, in the absence of quality surplus embryos, the replacement of three embryos was associated with a significant improvement in the clinical outcome.

Clinical Outcome When Two Embryos Were Replaced in the Presence or Absence of Quality

Table III. Outcome of IVF Cycles Where Maternal Age Is ≤ 35 Years (n = 1126 Patients)[a]

Embryos transferred	Quality embryos available Two embryos (n = 126 cycles)	Quality embryos not available Two embryos (n = 160 cycles)	P value	Quality embryos available Three embryos (n = 311 cycles)	Quality embryos not available Three embryos (n = 529 cycles)	P value
Number embryos transferred	252	320		933	1587	
Number embryos implanted	78	57		228	310	
Embryo implantation rate	30.9%	17.8%	0.01	24.4%	19.5%	NS[b]
Clinical pregnancies	57	46		157	208	
Clinical pregnancy rate/cycle	45.2%	28.8%*	0.03	50.5%	39.3%*	NS
Live births	45	31		121	173	
Live birth rate/cycle	35.7%	19.4%**	0.01	38.9%	32.7%**	NS
Singletons	30	23		73	110	
Singleton birth rate/cycle	23.8%	14.4%***	0.02	23.5%	20.8%***	NS
Twins	15	8		39	52	
Twin birth rate/cycle	11.9%	5%	NS	12.5%	9.8%	NS
Triplets	0	0		9	11	
Triplet birth rate/cycle	0%	0%		2.9%	2.1%	NS

[a] Significantly higher ($P < 0.05$): *Clinical pregnancy rates/cycle; **Live birth rate/cycle; ***Singleton birth rate/cycle when three embryos are replaced compared to two in the absence of good quality spare embryos.
[b] NS, Not significant.

Spare Embryos. Women aged 35 years or less who received two embryos in the presence of at least three grade 1 or grade 2 spare embryos (n = 126) had a significantly higher embryo implantation (30.9 vs. 17.8%; P 0.01), clinical pregnancy (45.2 vs. 28.8%; P 0.03), live birth (35.7 vs. 19.4%; P 0.01), and singleton birth rates (23.8 vs. 14.4%; P 0.02) compared to those who had less than three quality embryos to spare (n = 160) (Table III). Although the twin birth rate per cycle was higher in the former group than that in the latter (11.9% vs. 5.0%), the numbers in each group were too small to demonstrate a significant difference. Hence, the presence of quality spare embryos was associated with a significant improvement in clinical outcome.

Clinical Outcome When Three Embryos Were Replaced in the Presence or Absence of Quality Spare Embryos. Although the replacement of three embryos to women under the age of 35 years in the presence of at least three grade 1 or grade 2 spare embryos (n = 311) resulted in a better clinical outcome compared to those who had less than three quality spare embryos available (n = 529) (Table III), the difference was not statistically significant.

Clinical Outcome of Women Over 35 Years (n = 322 Patients)

Clinical Outcome When Two versus Three Embryos Were Replaced. In women over 35 years old with more than three grade 1 or grade 2 spare embryos, the replacement of three rather than two embryos was associated with a significantly higher embryo implantation (27.8 vs. 15.0%), clinical pregnancy (37.7 vs. 20.0%), and live birth rate per cycle (30.6 vs. 10.0%) (Table IV). Furthermore, in this age group the replacement of three rather than two grade 1 or grade 2 embryos did not result in a concurrent increase in either the twin or triplet birth rates.

Clinical Outcome When Two Embryos Were Replaced in the Presence or Absence of Quality Spare Embryos. In the presence of at least three spare grade 1 or 2 embryos, 10-women aged 35 years or over out of the 322 studied (3.1%) selectively requested to have two embryos replaced. When no spare quality embryos were available, 49 women elected to have two rather than three embryos replaced. This decision was partly influenced by the poor quality of the remaining embryos and partly by the couple's desire to reduce their risk of multiple conception. As illustrated in Table IV, when good-quality spare embryos were available, the replacement of two embryos resulted in a better implantation (15.0 vs. 12.2%), clinical pregnancy (20.0 vs. 18.4%), and singleton birth rates per cycle (20.0 vs. 12.2%).

Clinical Outcome When Three Embryos Were Replaced in the Presence or Absence of Quality Spare Embryos. The replacement of three grade 1 or grade 2 embryos in the presence of at least three grade 1 or grade 2 spare embryos to women over 35 years (n = 85) led to a significant improvement in the embryo implantation (27.8 vs. 12.9%; P 0.03), clinical pregnancy (37.7 vs. 30.3%; P 0.04), live birth (30.6 vs. 24.7%; P 0.04), and singleton birth rate/cycle (29.4 vs. 21.3%; P 0.03) in comparison with those who had less than three quality spare embryos available (n = 178) (Table IV). This improvement did not extend, however, to increase the twin or triplet birth rate per cycle (0% vs. 0.6%).

DISCUSSION

The ultimate goal of IVF reatment is to maximize pregnancy rates while minimizing multiple gestation rates. However, as IVF treatment becomes more widely applied, there is a justifiably growing concern about the resulting greater percentage of multiple pregnancies (3,15,28–31). The problem exists because infertility specialists still practice an imprecise and risky strategy of multiple embryo replacements to maintain acceptable pregnancy rates. In view of the increased prevalence of maternal and neonatal complications and higher costs associated with multiple gestations, we have attempted in this study to identify patients who are at risk by controlling for two primary variables: the number of embryos transferred and maternal age.

In the U.K., there is a legal restriction that limits the number of embryos transferred to a maximum of three. Within these legal restrictions, a recent postal survey (32) showed that the couple's final decision on the number of embryos to be replaced was based on information obtained from a clinician. Since informed consent depends on the level and depth of the information given, in the absence of accurate information, the transfer of too many or too few embryos may disadvantage some couples.

Of the many factors judged to influence the pregnancy rate after IVF-ET, the number of oocytes collected and fertilized and the number of good-quality embryos transferred appear to be the most important prognostic factors (22,33). For embryo grading several criteria have been adopted, including developmental

Table IV. Outcome of IVF Cycles Where Maternal Age Is > 35 years (n = 322 Patients)

Embryos transferred	Quality embryos available Two embryos (n = 10 cycles)*	Quality embryos not available Two embryos (n = 49 cycles)**	P value	Quality embryos available Three embryos (n = 85 cycles)*	Quality embryos not available Three embryos (n = 178 cycles)**	P value
No. embryos transferred	20	98		255	534	
No. embryos implanted	3	12		71	69	
Embryo implantation rate	15.0%[a]	12.2%	NS	27.8%[a]	12.9%	0.03
Clinical pregnancies	2	9		32	54	
Clinical pregnancy rate/cycle	20.0%[b]	18.4%[e]	NS	37.7%[b]	30.3%[e]	0.04
Live births	2	7		26	44	
Live birth rate/cycle	20.0%[c]	14.3%[f]	NS	30.6%[c]	24.7%[f]	0.04
Singletons	2	6		25	38	
Singleton birth rate/cycle	20.0%[d]	12.2%[g]	NS	29.4%[d]	21.3%[g]	0.03
Twins	0	1		1	5	
Twin birth rate/cycle	0%	2.0%	NS	1.2%	2.8%	NS[b]
Triplets	0	0		0	1	
Triplet birth rate/cycle	0	0		0	0.6%	NS

* Superscripts a–d, statistically higher ($P < 0.05$): a, Implantation rates; b, Clinical pregnancy rates/cycle; c, Live birth rates/cycle; d, Singleton birth rates/cycle when three embryos are replaced compared to two in the presence of good quality spare embryos.
** Superscripts e–g, significantly higher ($P < 0.05$): e, Clinical pregnancy rate/cycle; f, Live birth rate/cycle; g, Singleton birth rate/cycle when three embryos are replaced, compared to two, in the absence of good quality spare embryos.
[b] NS, not significant.

speed (34), symmetry and uniformity of the blastomeres in size and shape (33), presence of granularity of ooplasm and anucleate fragmentation (35), and some other biochemical methods such as pyruvate-glucose uptake (36), oxygen consumption (37), and production of platelet-activating factor, macrophage-inhibiting factor, leukotrienes, and histamine-releasing factors. Unfortunately all these factors cannot be applied in routine clinical practice. Hence, simpler, readily available, and yet reasonably reliable methods to assess the couples' chances of conception in a given cycle are needed.

The practice of grading embryos, with the assignment of a numerical or a letter designation based on criteria such as degree of fragmentation and uniformity of blastomeres, has become standard in IVF laboratories. While all IVF programmes occasionally observe normal pregnancies after the transfer of "poor"-grade embryos, most embryos that exhibit significant fragmentation or obvious abnormalities in cytokinesis do not develop and indeed have been shown be chromosomally abnormal (38). On the other hand, higher implantation and pregnancy rates are expected to result from the transfer of good-quality embryos (39). These findings indicate that morphological observations alone can be of some predictive value in assessing the relative developmental potential of embryos, especially if evaluations are made during the 2- to 4-cell stage (40). Embryo grading, although not entirely reliable, gives the clinician an approximation of an embryo's chance of implantation, and hence could be used as a prognostic indicator when determining the number of embryos to be transferred. Although the relationship between the grading of an individual embryo and its ability to give rise to a pregnancy has been described (41), most treatments result in the transfer of several embryos, and hence the impact this has on overall pregnancy rates is difficult to establish.

While higher pregnancy rates are achieved with the replacement of a higher number of embryos (42), Staessen *et al.* (12) have shown that the overall pregnancy rate was not significantly different when only two embryos were replaced in cycles with a "good IVF prognosis." They defined a good prognosis by the following criteria: first IVF attempt, age less than 37 years, and good embryo development. Tasdemir *et al.* (14) reported that the transfer of two embryos did not affect the pregnancy rate as long as one good quality embryo was available. Other authors also have encouraged the replacement of two embryos reporting pregnancy rates similar to those after triple transfer (11, 16). Nonetheless, in the majority of cases, the issue remains contentious, since no prognostic indicators have been identified to objectively assess the ideal number of embryos needed to achieve an acceptable pregnancy rate without increasing the risk of multiple pregnancy.

Although the outcome of previous IVF attempts is considered to be a determinant of the number of embryos to be transferred, most women who achieve pregnancy do so within the first three attempts with very similar live birth rates (17). Subsequent live birth

rates remain fairly stable until the eighth attempt, after which it drops significantly (25, 43). However, only 1% of couples continue treatment for so long. Thus, the outcome of previous treatments may be relevant to IVF outcome but only to a minor degree. By evaluating patients in their first IVF cycle, we attempted to offer prognostic information applicable to all patients.

The risks of multiple gestations after IVF treatment has been examined by a number of authors reporting on individual unit's practice (12, 14, 15, 44). However, some have included the results of fresh as well as frozen cycles (12, 44), while others have analyzed IVF cycles where up to four embryos were replaced (15, 44). In comparison to the above-mentioned studies, this is the largest comprehensive analysis assessing the chances of conception and the risks of multiple pregnancy for an individual couple in their first IVF attempt where a maximum of three embryos were replaced per cycle.

The predictive value of good-quality supernumerary embryos in achieving a pregnancy after embryo transfer was assessed in this study by comparing the clinical outcome of IVF cycles when the same number of embryos were replaced in the presence or absence of supernumerary quality embryos. Women in both age groups who had either two or three embryos replaced in the presence of quality supernumerary embryos had a significantly better clinical outcome compared with their counterparts who had the same number of embryos replaced, but with no quality embryos to spare. This is in agreement with other investigators (26, 39, 45) and suggests that the presence of quality spare embryos is a good prognostic indicator of the likely outcome in a particular treatment cycle. Our results also are consistent with the findings of Templeton and Morris (29) who analyzed treatment outcomes using the comprehensive data produced by HFEA to define clinical factors that are predictive of pregnancy and multiple gestation after IVF treatment. They concluded that when more than four embryos are available for transfer, the replacement of two embryos will not decrease the couple's chances of conception, but will only reduce their risk of multiple births.

In this analysis the replacement of three as opposed to two embryos to women under 35 years who had more than three good-grade supernumerary embryos only served to increase the multiple pregnancy rate without improving the clinical pregnancy or live birth rates. Furthermore, in this age group, the replacement of three embryos resulted in higher twin and triplet birth rates (12.5 and 2.9%, respectively) compared with those obtained when two embryos were transferred (11.9 and 0%, respectively); this difference, however, did not reach statistical significance. On the other hand, in the absence of quality spare embryos, these women had a significantly better clinical outcome when three embryos rather than two were replaced without significantly influencing the multiple pregnancy rate. In women over 35 years, the picture was quite different. The replacement of three instead of two embryos in the presence or absence of quality supernumerary embryos resulted in a significantly improved clinical outcome without being associated with an increase in the multiple pregnancy rate.

We, therefore, propose an individualized embryo transfer policy. Young women with quality spare embryos could have only two embryos replaced in the knowledge that such a policy will not be detrimental to their chances of conception, whereas in the absence of quality spare embryos, which reflects a lack of choice among embryos available for transfer, the replacement of three embryos is justifiable since it improves their likelihood of conception without increasing their risk of multiple pregnancy. In older women, however, the replacement of three embryos seems necessary to obtain acceptable clinical pregnancy rates (21).

In conclusion, the quality of surplus embryos is an important prognostic factor in identifying couples at risk of multiple pregnancy. This study illustrates that the age of the female partner and the presence of supernumerary quality embryos can be used as a reference to determine the optimum number of embryos to transfer and as an indicator of the probability of success of an individual couple in a given cycle. This information is useful when counseling couples to assist them in reaching an informed and evidence-based decision regarding the number of embryos to be replaced in the knowledge that their decision would not be detrimental to their chances of conceiving nor would it lead to unnecessary risk of high-order multiple pregnancy.

REFERENCES

1. Levene MI, Wild J, Steer P: Higher order births and the modern management of infertility in Britain. Br J Obstet Gynaecol 1992;99:607–613
2. Tuppin P, Blondel B, Kaminiski M: Trends in multiple deliveries and infertility treatments in France. Br J Obstet Gynaecol 1993;100:383–385
3. Callahan TL, Hall JE, Ettner SL, Christiansen CL, Greene MF, Crowley WF: The economic impact of multiple-gestation pregnancies and the contribution of assisted-reproduction techniques to their incidence. N Engl J Med 1994;331:244–249

4. Seoud MA, Toner JP, Kruithoff C, Muasher SJ: Outcome of twin, triplet and quadruplet in vitro fertilisation pregnancies: the Norfolk experience. Fertil Steril 1992;57:825–834
5. Lipitz S, Reichman B, Uval J, Shalev J, Archiron R, Barkai G: A prospective comparison of the outcome of triplet pregnancies managed expectantly or by mutifetal reduction to twins. Am J Obstet Gynecol 1994;170:874–879
6. Sassoon DA, Castro LC, Davis JL, Hobel CJ: Perinatal outcome in triplets versus twin gestation. Obstet Gynecol 1990;75:817–820
7. Yokoyama Y, Shimizu T, Hayakawa K: Incidence of handicaps in multiple births and associated factors. Acta Genet Med Gemellol 1995;44:81–91
8. Yokoyama Y, Shimizu T, Hayakawa K: Prevalence of cerebral palsy in twins, triplets and quadruplets. Int J Epidemiol 1995;24:943–948
9. Vauthier-Brouzes D, Lefébvre G: Selective reduction in multifetal pregnancies: Technical and psychological aspects. Fertil Steril 1992;57:1012–1016
10. Goldfarb JM, Austin C, Lisbona H, Peskin B, Clapp M: Cost-effectiveness of in-vitro fertilisation. Obstet Gynecol 1996;87:18–21
11. Waterstone J, Parsons J, Bolton V: Elective transfer of two embryos. Lancet 1991;I:975–
12. Staessen C, Janssenswillen C, Van Den Abbeel E, Devroey P, Van Steirteghem AC: Avoidance of triplet pregnancies by elective transfer of two good quality embryos. Hum Reprod 1993;8:1650–1653
13. Staessen C, Nagy ZPM, Lui J: One year's experience with elective transfer of two good quality embryos in the human in-vitro fertilisation and intracytoplasmic sperm injection programmes. Hum Reprod 1995;10:3305–3312
14. Tasdemir M, Tasdemir I, Kodama H, Fukuda J, Tanaka T: Two instead of three embryo transfer in in-vitro fertilisation. Hum Reprod 1995;10:2155–2158
15. Roest J, Van Heusden AM, Verhoeff A, Mous HVH, Zeilmaker GH: A triplet pregnancy after in-vitro fertilisation is a procedure-related complication that should be prevented by replacement of two embryos only. Fertil Steril 1997;67:290–295
16. Matson PL, Browne J, Deakin R, Bellinge B: The transfer of two embryos instead of three to reduce the rate of multiple pregnancy: a retrospective analysis. J Assist Reprod Genet 1999;16:1–5
17. Human Fertilisation and Embryology Authority Sixth Annual Report 1997. HFEA, London, UK
18. Society for Assisted Reproductive Technology (SART), American Society for Reproductive Medicine (ASRM). Assisted reproduction technology in the United States and Canada: 1993 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 1995;64:13–21
19. Bollen N, Camus M, Tournaye H, Wisanto, A, Van Steirteghem AC, Devroey, P: Embryo reduction in triplet pregnancies after assisted procreation: a comparative study. Fertil Steril 1993;60:504–509
20. Berkowitz RL, Lynch L, Stone J, Alvarez M: The current status of multifetal pregnancy reduction. Am J Obstet Gynecol 1996;174:1265–1272
21. Widra EA, Gindoff PR, Smotrich DB, Stillman RJ: Achieving multiple-order embryo transfer identifies women over 40 years of age with improved in-vitro fertilisation outcome. Fertil Steril 1996;65:103–108
22. Wood C, McMaster R, Rennie G, Trounson A, Leeton J: Factors influencing pregnancy rates following in-vitro fertilisation and embryo transfer. Fertil Steril 1985;43:245–250
23. Check JH, Nowroozi K, De Cooman S, Thomas K: Influence of endometrial thickness and echo patterns on pregnancy rates during in-vitro fertilisation. Fertil Steril 1991;56:1173–1175
24. Paulson RJ, Sauer MV, Lobo RA: Embryo implantation after human in vitro fertilisation: Importance of endometrial receptivity. Fertil Steril 1990;53:870–874
25. Copperman AB, Selick CE, Grunfeld L, Sandler B, Bustillo M: Cumulative number and morphological score of embryos resulting in success: Realistic expectations from in-vitro fertilisation-embryo transfer. Fertil Steril 1995;64:88–92
26. Roseboom TJ, Vermeiden JW, Schoute E, Lens JW, Schats R: The probability of pregnancy after embryo transfer is affected by the age of the patient, cause of infertility, number of embryos transferred and the average morphology score, as revealed by multiple logistic regression analysis. Hum Reprod 1995;10:3035–3041
27. Salha O, Nugent D, Dada T, Kaufmann S, Levett S, Jenner L, Lui S, Sharma V. The relationship between follicular fluid aspirate volume and oocyte maturity in in-vitro fertilisation cycles. Hum Reprod 1998;13:1901–1906
28. Collins JA, Bustillo M, Visscher RD, Lawrence LD: An estimate of the cost of in-vitro fertilisation in the United States in 1995. Fertil Steril 1995;64:538–545
29. Templeton A, Morris JK: Reducing the risk of multiple births by transfer of two embryos after in-vitro fertilisation. N Engl J Med 1998;339:573–577
30. Bronson R: How should the number of embryos transferred to the uterus following in-vitro fertilisation be determined to avoid the risk of multiple gestation? Hum Reprod 1997;12:1605–1607
31. Faber K: IVF in the US: Multiple gestation, economic competition, and the necessity of excess. Hum Reprod 1997;12:1614–1616
32. Murdoch A: Triplets and embryo transfer policy. Hum Reprod 1997;12(Suppl 2):88–92
33. Hill AG, Freeman M, Bastias MC, Rogers BC, Herbert CM, Osteen KG, Wentz AC: The influence of oocyte maturity and embryo quality on pregnancy rate in a program for in-vitro fertilisation-embryo transfer. Fertil Steril 1989;52:801–806
34. Claman P, Armant RD, Seibel MM, Wang TN, Oskowitz SP, Taymor ML: The impact of embryo quality and quantity on implantation and establishment of viable pregnancies. J Vitro Fertil Embryo Transfer 1987;4:218–222
35. Puissant F, Rysselberge MV, Barlow P, Deweze J, Leroy F: Embryo scoring as a prognostic tool in IVF treatment. Hum Reprod 1987;2:705–708
36. Leese HJ, Hoope MAK, Edwards RG, Smith AMJ: Uptake of pyruvate by early human embryos determined by a non-invasive technique. Hum Reprod 1986;1:181–
37. Magnuson C, Hillenjo T, Homberger L, Nilson L: Oxygen consumption by human oocyte and balstocysts grown in vitro. Hum Reprod 1986;1:183–
38. Munné S, Alikani M, Tomkin G, Grifo J, Cohen J: Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil Steril 1995;64:382–391
39. Hu Y, Maxson W, Hoffman D, Ory S, Eager S, Dupre J, Lu C: Maximising pregnancy rates and limiting higher-order multiple conceptions by determining the optimal number of

embryos to transfer based on quality. Fertil Steril 1998;69:650–657
40. Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A: Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Hum Reprod 1998;13:182–187
41. Shulman A, Ben-Nun I, Ghetler Y, Kaneti H, Shilon M, Beyth, Y: Relationship between embryo morphology and implantation rate after in vitro fertilisation treatment in conception cycles. Fertil Steril 1993;60:123–126
42. Medical Research International, Society for Assisted Reproductive Technology (SART), The American Fertility Society. In-vitro fertilisation-embryo transfer (IVF-ET) in the United States: 1990 results from the IVF-ET Registry. Fertil Steril 1992;57:15–24
43. Seibel, MM, Ranoux C, Kearnan M: In-vitro fertilisation: how much is enough. N Engl J Med 1989;321:1052–1053
44. Svendsen T, Jones D, Butler L, Muasher S: The incidence of multiple gestations after in vitro fertilisation is dependent on the number of embryos transferred and maternal age. Fertil Steril 1996;65:561–565
45. Van Os H C, Alberda ATh, Janssen-Caspers HAB, Leerentveld RA, Scholtes MCW, Zeilmaker GH: The influence of the interval between in-vitro fertilisation and embryo transfer and some other variables on treatment outcome. Fertil Steril 1989;51:360–362