Defining women who are prone to have twins in in vitro fertilization—a necessary step towards single embryo transfer

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Purpose: The aim of the present study was to identify subsets of women undergoing two-embryo transfer in in vitro fertilization (IVF) who are prone to give birth to twins.

Methods: During 1990 to 2002 4580 day 2 or day 3 two-embryo transfers were conducted and these constituted the study population.

Results: By selecting combinations of factors, some subsets of patients where single-embryo transfer might be conducted would reduce the estimated twin rate by 60%, but requiring single-embryo transfer in only approximately one third of the patients. Examples of such selected groups were patients less than 33 years of age with two top quality embryos, patients less than 39 years of age with two top quality embryos and conducting their first IVF cycle, and patients less than 39 years of age with two top quality embryos and two optimal cleavage cells.

Conclusion: By combining factors, subsets of patients with both a high birth and twin rate that could be recommended single embryo transfer were identified.

KEY WORDS: Age; cryo preservation; embryo quality; in vitro fertilization; male infertility; twins.

INTRODUCTION

Multiple pregnancies, whether twins or more, are increasingly considered an adverse effect (1) or even failure in in vitro fertilization (IVF), and the delivery of a single, healthy child should be regarded as the optimal outcome of the embryo transfer (2). Despite this, multiple births still account for 25–45% of IVF births (3,4). ESHRE recently concluded that a 10% multiple birth ratio could be a reasonable goal in IVF (5), which would be acceptable also in certain clinics (6).

Reduction of embryos transferred to a maximum of two eliminates the risk for triplets, which often have disastrous consequences for the children. But also a twin birth substantially increases the risk for both the mother and the children regardless if the pregnancy is a result of natural conception or IVF (7–11). These include preeclampsia, prematurity, small for gestational age, and perinatal mortality and morbidity. A two-fold risk of preclampsia and obstetric severe hemorrhages, and a three-fold increase in perinatal mortality has been estimated (11). An increased risk for anomalies, neurological sequelae including cerebral palsy, has been found in children born after IVF and the increase was interpreted as mainly due to the increased frequency of twins (9). In addition, health-care costs for delivery and the newborn have been estimated to increase by 5.4 times (12). Having twins also pose a social and psychological burden for the parents.

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Decreasing twin rates in in vitro fertilization (IVF) to the rates of natural conceptions is at present not realistic if good overall pregnancy rates in IVF should persist. This requires single embryo transfer in all patients undergoing IVF. The pregnancy rates in an IVF population are higher with multiple embryo transfer than with single embryo transfer even in well-designed studies (5). Thus, there is a necessity to define groups of women who are prone to have twins and which have high pregnancy rates, for the selection of women for single embryo transfer, without hampering the pregnancy rates excessively (6).

The aim of this study was to define groups of patients that are prone to have twins with two-embryo transfer and to estimate twin ratio if these groups of patients were selected to have single embryo transfer. Pregnancy rates with two-embryo transfer were included in the analyses.

MATERIAL AND METHODS

Controlled ovarian hyperstimulation and oocyte retrieval was made according to a standard "long" gonadotropin-releasing hormone agonist protocol, or in a few cases a standard antagonist protocol. For ovarian stimulation a recombinant follicle stimulating hormone (FSH), highly purified FSH or human menopausal gonadotropin was used depending on the time period of the study, as has been described previously (13).

Embryos were cultured for two days, except at oocyte retrieval on Fridays, when three-day culture was performed before embryo transfer (20% of the IVF cycles). As pregnancy rate varied only insignificantly, with 3% increased pregnancy rate with two-day transfer, the IVF cycles were analysed together.

Embryo quality was graded from 1 to 4, where one was the top-quality embryo. A top-quality day-2 embryo was characterized by absence of fragmentation, equally sized and symmetric blastomeres, no intercellular spatia and an irregular zona pellucida. Grade 2 included at least two equally sized blastomeres and less than 25% fragmentation and sometimes with a thick zona pellucida, while grade 3 had blastomeres of different sizes and no more than 50% fragmentation. Grade 4 had more than 50% fragmentation.

Initial analysis proved that 4-cell cleavage stage at two-day transfer and 8-cell cleavage stage at 3 day transfer was optimal for IVF outcome

During recent years assisted partial hatching was performed with Tyrone's solution when there was

a thick zona pellucida, as judged by the embryologist. Intracytoplasmic sperm injection (ICSI) was performed since 1994 and in 47.2% of the cycles. Spare good quality embryos were frozen in freezing media from Medicult^R.

At ET, luteal support was given with intramuscular progesterone 50 mg daily or with micronized progesterone in vaginal gel 800 mg daily, until the result of the pregnancy test, on average 18 days after ET. A clinical pregnancy was defined as fetal heartbeats on ultrasonography. Birth rates were calculated per embryo transfer.

During 1990–2002 6528 day 2 and day 3 embryo transfers were conducted. As a rule two embryos were transferred ($n = 4580$, 70.2%), which constituted the study group. In the beginning of the study period three-embryo transfers were more often performed, giving a total of 1094 (16.8%) of ET. Single embryo transfer ($n = 836$, 12.8%) was in general only performed when only one embryo was available. These embryos were on average of more poor quality than those where two-embryo transfer was performed.

Birth and twin rates were analyzed for 33 variables: cause of infertility; tubal factor, endometriosis, hormonal, other, idiopathic and male, IVF/ICSI, number of retrieved oocytes, number of fertilized oocytes, embryo quality of each of the two embryos, cell cleavage state of each of the two embryos, IVF cycle rank, treatment with acetyl salicylic acid, sperm quality, sperm preparation method, sperm leucocytes, doctor responsible for controlled ovarian hyperstimulation, oocyte retrieval and embryo transfer, biomedical staff, body mass index, previous pregnancy with husband or other, previous childbirth with husband or other, previous induced or spontaneous abortions, previous extrauterine pregnancy and smoking habits.

The results were calculated on the JMP 3.1 statistical program (SAS Institute). Logistic regression (log likelihood test) were used when adjustment for possible confounding factors was made, and for estimation of odds ratios (OR) and 95% confidence intervals (95% CI).

Estimated twin ratio was the actual proportion of remaining twin pregnancies in the whole study population when groups of women that were selected for single embryo transfer were expected to have singleton pregnancies. A less than 1% expected monozygotic twin ratio in single embryo transfer was not considered.

RESULTS

The study included 4580 two embryo transfers. These represented 2190 patients. Birth rate and twinning varied insignificantly by IVF cycle rank. More than four IVF cycles were rarely conducted. Birth rates at the first IVF cycle, as compared to the fourth, were 27.1% versus 22.9%. The corresponding rates for twinning were 26.1% vs. 27.3%. All IVF cycles were therefore analyzed as one group. Average age was 33.0 years (*SD* 4.3) with an average IVF cycle rank of 2.0. Only 11.3% of the IVF cycles were rank four or more. The main indications for IVF were tubal factor (34.7%), male factor (24.4%) and idiopathic infertility (27.6%). Mean implantation rate was 16.2%, average birth rate per embryo transfer was 26.2% and twin rate 24.4%. There were four triplets included in twin rates.

Factors that were associated with twin rate were age, infertility other than caused by the male, embryo cleavage stage, embryo quality, number of fertilized, but not retrieved, oocytes, and ability to freeze at least one good quality embryo. In multifactorial analyses where cleavage stage was adjusted for, number of fertilized oocytes and ability to freeze embryos were no longer significantly associated with a duplex pregnancy, in contrast to cleavage state. These two variables were therefore not included in the final analyses.

No single infertility cause other than male was associated with twin rate and other indications for IVF

were therefore grouped as a single factor. With male infertility there was a significantly lower twin ratio, than in the remaining study population. Tubal factor infertility had non-significantly lower birth rates (24.6% vs. 27.1%, $p = 0.07$) but no higher rates of twin pregnancies (25.6% vs. 23.8%, $p = 0.50$) than the rest of the study population.

There was no significant difference in birth and twin rate according to rank of IVF cycle, neither when all ranks was compared, nor when the first to third IVF cycle was compared with four or more. Since it is our experience that IVF cycle rank is important for the clinician to decide whether to transfer one or two embryos and as the actual twin ratio was higher, although not significantly, in the first IVF cycle, as compared with the subsequent, this factor was included in the final analyses (Table I).

The other factors that were associated with an increased twin rate were dichotomized after different models had been evaluated and cut-off levels were chosen to give the best predictive value for differences in pregnancy and twin rate. Thus, for age cut-off values for 32 years and 38 years were chosen. A high quality embryo was defined as grade 1 or 2 and was compared to grade 3 and 4 embryos. Four-cell embryos on day two and eight-cell embryos on day 3 were found optimal and was compared with other cleavage stages. Fertilized oocytes were divided into four or less versus five or more and ability to freeze at least one good quality embryo was compared to no frozen embryos.

Table I. Variables Associated with an Increased or Decreased Twin Pregnancy Ratio with Two-Embryo Transfer (Average Birth Ratio 26.2%, Twin Ratio 24.4%)

	Per cent of all patients (study group)	Birth rate in the study group (%)	Twin rate (%)		Odds ratio	95% CI
			Study group	Comparison group		
Age ≤ 32 years vs. ≥ 33 years	50.4	29.6	27.9	19.8	1.57	1.19–2.07
Age ≤ 38 years vs. ≥ 39 years	91.4	27.6	25.0	7.3	4.22	1.51–17.52
Other causes vs. male infertility	75.6	26.4	26.5	17.5	1.70	1.22–2.40
One IVF cycle vs. ≥ 2 IVF cycles	47.8	27.1	26.1	22.7	1.20	0.92–1.57
No 4/8 cleavage cells vs. two 4/8 cleavage cells	16.1	14.8	19.0	28.7	0.58	0.33–0.98
Only one 4/8 cleavage cell vs. two 4/8 cleavage cells	35.5	23.2	18.3	28.7	0.56	0.40–0.77
Two 4/8 cleavage cells vs. all others	48.3	32.8	28.7	18.4	1.78	1.33–2.41
No high quality embryo vs. two high quality embryos	8.8	10.4	11.4	25.6	0.38	0.11–0.96
One high quality embryo vs. two high quality embryos	15.6	20.2	20.0	25.6	0.73	0.45–1.40
Two high quality embryo vs. all others	75.5	29.7	25.6	18.6	1.51	1.00–2.31
Ability to freeze at least one high quality embryo	32.5	33.0	27.4	22.3	1.31	1.00–1.71
≥ 5 fertilized oocytes	50.7	30.7	26.7	20.9	1.38	1.05–1.82

Table II. Estimated Total Twin Ratio in an IVF Population by Performing Single Embryo Transfer in Selected Groups of Patients and Two-Embryo Transfer in the Remaining Patients (Average Birth Ratio 26.2% Twin Ratio 24.4%)

Single embryo transfer	Percentage of all patients	Birth rate (%)	Percentage of all twins	Estimated twin Frequency ^a (%)
Age ≤32 years	50.4	29.6	65.1	8.5
Age ≤38 years	91.4	27.6	99.0	0.25
Other causes of infertility than male	75.6	26.4	82.8	4.2
First IVF cycle	47.8	27.1	53.1	11.4
Two 4/8 cleavage cells	48.3	32.8	69.9	7.4
Two high quality embryos	75.5	29.7	88.3	2.9
Ability to freeze at least one high quality embryo vs. no frozen embryo	32.5	33.0	45.9	13.2
≥5 fertilized oocytes vs. <5 fertilized oocytes	50.7	30.7	65.3	8.5

^aEstimated total pregnancy rate in the whole population if single embryo transfer is performed in the particular group of patients. Expected monozygotic twins are not included.

Age, embryo quality, cleavage state, infertility cause and IVF cycle rank were thus included in the unilateral analyses (Table I). High twin rates, in general accompanied with high birth rates were confirmed in the observed groups of patients. There was a decreased twin rate with age more than 38 years, male infertility, no 4/8 cleavage cells and no high quality embryos. Multifactorial analyses confirmed embryo quality (adjusted odds ratio 1.38), 4/8 cell stage (adjusted odds ratio 1.72), and other infertility cause than male (adjusted odds ratio 1.89) as well as age, to be independently correlated with a high twin ratio and with similar odds ratios as in unilateral analyses, although embryo quality did not entirely reach statistical significance (95% confidence interval: 0.91–2.15).

The estimated twin ratio when single factors are chosen as determinants for single embryo transfer is given in Table II. It is in the nature of things that the larger the group selected for single embryo transfer, the higher decrease in twin ration. By selecting age less than 33 years or the presence of 4/8 cell embryos, both groups with a high birth rate, it would be possible to achieve twin rates of less than 10%, but will require single embryo transfer for half of the population.

In Table III factors associated with a high twin rate as well as birth rate are combined. An estimated twin rates at or below 10%, a high pregnancy rate and a relatively low frequency of elective single embryo transfer is observed when groups chosen for single embryo transfer are those who are less than 33 years of age in combination with two top quality embryos, or with other than male indication. If all women below 39 years of age with two top-quality embryos and two 4/8 cleavage cells would have single embryo

transfer the estimated twin rate would be below 10% and with a reasonable proportion of single embryo transfers.

DISCUSSION

This study raises two major questions: Which patients should be selected for single embryo transfer in order to diminish the overall twin ratio, and how would this affect overall pregnancy ratio? To answer the first question it is obvious that the ultimate action would be to select all patients for single embryo transfer. The challenge is to find a balance where as small group as possible gives as large decrease of twins as possible.

The aim of this study was not to give a definite answer of this problem, but to give alternatives for an IVF single embryo transfer program. This study has defined a number of groups of patients that might be selected for single embryo transfer. Performing single embryo transfer in the 20.5% of our patients that are less than 33 years of age and have two top quality and 4/8 cleavage cells would reduce the frequency of twins by 44% (overall ratio 13.7%). If the age limit would be less than 39 years the twin ratio would decrease by 63% (overall ratio 9.1%) by performing single embryo transfer in 38.1% of the patients. Finally, performing single embryo transfer in the 37.7% of patients who are below 33 years of age and have two top quality embryos would reduce the frequency of twins by 57% (overall ratio 10.5%).

It is evident, according to our results, that age, cleavage rate, and embryo quality are the most important predictors both for a high birth and for a high twin rate. A high age is an established factor for a negative IVF outcome (14,15) and must be part of a

Table III. Estimated Total Twin Ratio an IVF Population by Performing Single Embryo Transfer in Combinations of Selected Groups of Patients and Two-Eembryo Transfer in the Remaining Patients (Average Birth Ratio 26.2%, Twin Ratio 24.4%)

Single embryo	Transfer	Percentage of all patients	Birth rate (%)	Percentage of all twins	Estimated twin Frequency ^a (%)
Age ≤32 years	+ First IVF cycle	25.0	30.2	29.9	15.8
Age ≤32 years	+ Two top quality embryos	37.7	33.3	29.4	10.5
Age ≤32 years	+ Not male indication	35.9	30.2	30.4	11.8
Age ≤32 years	+ First IVF cycle + two top quality embryos	18.6	32.2	31.0	17.5
Age ≤32 years	+ Two top quality embryos + 4/8 cleavage cells	20.5	39.0	33.6	14.3
Age ≤32 years	+ Two top quality embryos + not male indication	25.7	34.0	32.8	13.7
Age ≤32 years	+ Two top quality embryos + 4/8 cleavage cells + first IVF cycle	10.0	38.5	38.2	18.9
Age ≤32 years	+ First IVF cycle + two top quality embryos + not male indication	12.2	32.4	34.6	19.3
Age ≤38 years	+ First IVF cycle	44.0	28.7	26.5	11.6
Age ≤38 years	+ Two top quality embryos	69.0	31.4	26.1	3.1
Age ≤38 years	+ Not male indication	67.9	28.2	27.1	4.6
Age ≤38 years	+ First IVF cycle + two top quality embryos	32.9	31.7	27.2	13.8
Age ≤38 years	+ Two top quality embryos + 4/8 cleavage cells	38.1	36.3	29.4	9.1
Age ≤38 years	+ Two top quality embryos + not male indication	49.7	31.7	29.0	7.2
Age ≤38 years	+ Two top quality embryos + 4/8 cleavage cells + first IVF cycle	18.3	36.6	32.1	16.3
Age ≤38 years	+ First IVF cycle + two top quality embryos + not male indication	23.4	31.8	29.9	16.1
All ages	+ Two top quality embryos + 4/8 cleavage cells	41.5	34.5	28.9	8.9
All ages	+ At least three top quality embryos	21.2	35.6	28.9	16.3

^aEstimated total pregnancy rate in the whole population if single embryo transfer is performed in the particular group of patients. Expected monozygotic twins are not included.

single embryo transfer program. The age-related decline of fertility has mainly been attributed to oocyte factors. Fewer oocytes after controlled ovarian hyperstimulation with consequently fewer embryos in general leads to a poor quality of embryos transferred and a lower implantation rate. Fertilization and cleavage rates might be normal (14). Results from donor oocyte programs (16) have led to the opinion that endometrial factors play a minor role. In these programs the endometrium of the aging woman is stimulated by exogenous estrogens and might not be directly compared with FSH stimulation. A study with cryo-preserved embryos following controlled ovarian hyperstimulation, and embryo transfer in a subsequent estrogen-stimulated cycle would help to clarify the question.

Embryo quality might be even more important than age (17). There is no general consensus on how to define embryo quality. We consider fragmentation, shape and size of cells, symmetry, intercellular spatium and to some degree the nuclei for our embryo score. Cleavage rate stands as an indepen-

dent factor for embryo quality. Terriou *et al.* (17) suggested an embryo score based on four variables; cleavage as such as a prerequisite, division stage, irregular cells and less than 20% fragmentation. Van Royen *et al.* (18) included less than 20% fragmentation, absence of multinucleated blastomeres, and number of blastomeres in the embryo score, while De Sutter *et al.* (19) focused on blastomer symmetry, absence of multinucleation and less than 10% fragmentation. Embryo score has with no exception proved to be a valuable predictive tool for IVF outcome.

Some authors have shown that IVF rank four or more is correlated to a poor outcome (20). This was true also in this study, but the major decrease in birth rate appeared after the first IVF cycle while there was only a small decrease in twin ratio by IVF cycle rank. To improve the outcome and not the least, for psychological reasons it might be important for both the doctor and for the couple to perform two-embryo transfer after failures with single embryo transfer.

Tubal infertility has in many studies been associated with decreased birth and twin ratio. Templeton

et al. (20) reported an odds ratio of 0.8 for having twins in tubal fertility patients, and with his large study population this difference was significant. We had an odds ratio of 1.1 but it in comparison with a large proportion of male infertility, which showed an inverse relation for having twins. To our knowledge such an inverse relation has not been found previously. Male infertility was not associated with decreased birth rate and we find the reasons for the decreased twin rate obscure. Our findings are quite strong, but it cannot be excluded that the result is due to chance.

The results of this study demonstrate that factors that are associated with a high pregnancy rate are essentially the same as those associated with a high twin rate. The results confirm previous studies (21,22).

Hunault *et al.* (21) designed a twin pregnancy prediction model based on patients' age and embryo score. The trends in twin ratios were comparable to those in the present study. A theoretical pregnancy score was also estimated, in contrast to the present study. An estimation of pregnancy rates in single embryo transfer as compared to two-embryo transfer requires knowledge about implantation rates in the two situations. An assumption (21,22) that implantation rates are similar independent of number of embryos transferred is not true (23), but to determine the exact differences large randomized studies are required, which are not available at present.

Strandell *et al.* (22) designed a model to calculate a fertility index. Age, number of previous IVF cycles, tubal infertility and embryo score were included in the model and were introduced into a formula and twin ratio could be estimated if two-embryo transfer was replaced by single embryo transfer. There was, however, no estimation on the percentage of patients that would be selected for single embryo transfer to achieve specified twin ratios.

We made no comparison with three-embryo transfer in this study. It has never been our policy to routinely transfer three embryos, and they only account for slightly more than 10% of embryo transfers in this population. A large part of these transfers are situations when no high quality embryos were at hand. Studies have however shown that there are few advantages by routinely transferring more than two embryos, but with disastrous consequences in multiple pregnancy rates (4).

Many IVF clinicians agree that single embryo transfer will be more used in the future (1,2,4,5). But there is also a negative attitude and fear that a general introduction of single embryo transfer will have

negative consequences both for the couple and the doctor and fear that it will deteriorate outcome of the IVF treatment (24). This study did not include any prediction or actual results of single embryo transfer in different patient groups. Previous studies on single embryo transfer must, however, be reviewed in this context.

There are several studies that report results of single embryo transfer (19,23,25–28). Retrospective, non-randomized studies (19,25,26,28) can however not evaluate differences between single embryo transfer and two embryo transfer, even in selected groups of patients. Too many factors are involved in the decision to transfer one or two embryos, such as IVF cycle rank, previous successful or failed IVF treatments, reproductive history, duration of infertility, age, etiology of infertility, the couples' desire, the ET doctor, embryo characteristics etc. This will make all comparisons in nonrandomized studies impossible. It is only possible to evaluate if IVF outcome is acceptable in those patients who underwent single embryo transfer. A prerequisite is that single embryo transfer because of insufficient number of embryos, perhaps of poor quality, must be excluded.

De Sutter *et al.* (19) recommended elective single embryo transfer in patients less than 37 years of age with at least two good quality embryos and who had their first or second IVF treatment. There was an ongoing pregnancy rate of 28% and twinning rate of 0.7% with single embryo transfer in 579 transfers, but two-embryo transfers constituted 2319 transfers in the group eligible for single embryo transfer. Vilska *et al.* (25) analyzed 74 cases of single embryo transfer, performed because of medical reasons or the couples' wish, and had 29.7% pregnancy rate with no twins. Dhont (26) studied patients below 37 years of age with at least two high quality embryos and conducting their first or second IVF treatment. One third of the subjects agreed to single embryo transfer and pregnancy rate was 37.9%. In a similar study by De Neubourg *et al.* (28), a 40% pregnancy rate with single embryo transfer was reported.

Both the prospective, randomized studies that are available up to date used carefully selected patients and are relatively small. Ongoing pregnancy rates were higher with two-embryo transfer in both studies. Gerris *et al.* (23) had an ongoing pregnancy rate of 38.5% in single embryo transfer, but also a very high ongoing pregnancy rate (74.1%) with two-embryo transfers. Women below 34 years of age with their first treatment cycle were asked ($n = 194$) and 110 women agreed to participate. Out of

these 53 fulfilled the inclusion, i.e. had two top quality embryos, and were randomized. Thus, only 27.3% of eligible women actually participated in the study. Martikainen *et al.* (27) used the presence of at least four good quality embryos as inclusion criteria. There was no age limit or less than 36 years, and IVF cycle rank differed, depending of the IVF clinic in this multicenter study, where 11.1% of eligible women agreed to participate. Clinical pregnancy rate in the single embryo transfer group was 32.4 and 47.1% with two-embryo transfer, but the difference was not significant. One clear conclusion can be drawn from these single embryo transfer studies, i.e. in a carefully selected group of patients an "acceptable" pregnancy rate, 30% or more, can be achieved.

Patients must be convinced to accept a single embryo transfer program and this will be a challenge for the clinician. Besides convincing clinicians and patients that the risk with multiple pregnancies is unacceptable, improvements in implantation rates and a good cryo-preservation program are needed (29, 30). Some authors claim that blastocyst transfer improves IVF outcome (2). If this can be confirmed by several groups in large comparative studies it might convince the couple to accept single blastocyst transfer, as the birth rate will be acceptable. Others try to improve the selection of embryos or use other options. At present it is evident that cryo-preservation will give a high cumulative pregnancy rate (30). Future studies must determine if a cumulative pregnancy rate with repeated cycles with frozen embryos will lead to pregnancy rates similar to those in two-embryo transfer.

CONCLUSION

This study aimed at making a selection possible by defining groups of patients that are both at high risk for a twin pregnancy and having high ongoing pregnancy rates. A number of groups might according to the results of this study be chosen for single embryo transfer.

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