CLINICAL ASSISTED REPRODUCTION

An IVF Fallacy: Multiple Pregnancy Risk Is Lower for Older Women

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Introduction: Multiple pregnancy is one of the most important and preventable complications of in vitro fertilization (IVF) and embryo transfer. The general clinical practice in many IVF clinics is to transfer four or five embryos to older women if available, since pregnancy rates are lower in women older than 35 years of age. However, it is not clear whether the risk for multiple pregnancy is also lower. **Objective:** Our objective was to investigate whether transferring a higher number of embryos actually improves pregnancy outcome in older women, without increasing the risk for multiple pregnancy and to investigate other factors that may affect the occurrence of multiple pregnancy.

Setting: The setting was university-based IVF program at The Toronto Hospital.

Design: The design was a retrospective case series.

Patients and Methods: The outcome of 1116 IVF cycles between January 1992 and December 1993 was investigated according to different age groups.

Main Outcome Measure: The main outcome measure was multiple pregnancies.

Results: Seventy multiple pregnancies resulted from a total of 242 pregnancies. Overall pregnancy and multiple pregnancy rates were inversely correlated with age. However, when the data were adjusted for the number of embryos transferred, this trend disappeared. The result of multiple regression analysis showed that the multiple pregnancy rate was higher without improving the pregnancy rate when the number of embryos transferred exceeded three, regardless

of the age of the patients, especially when more embryos were available than the number of transferred ones.

Conclusions: The number of embryos transferred should be limited to a maximum of three regardless of the age of patients, to reduce the high frequency of multiple gestations in an IVF program.

KEY WORDS: in vitro fertilization-embryo transfer; pregnancy; multiple pregnancy; age; endometrial thickness.

INTRODUCTION

The first in vitro fertilization and embryo transfers (IVF-ET) (1) were performed during natural cycles with only a single embryo being transferred. Later controlled ovarian hyperstimulation was used extensively, resulting in the availability of many embryos for transfer and higher pregnancy rates but many unwanted, multiple pregnancies, ranging from 11 to 44% (2,3) in various studies. High multiple pregnancy rates can be prevented in an IVF program by controlling the number of embryos transferred. However, in most IVF clinics, transferring up to three or four embryos to improve the pregnancy rate is common practice, and this may lead to high multiple pregnancy rates. The United States IVF-ET Register (4) for 1989 reported that 18% of women who had an embryo transfer became pregnant and 24% of those pregnancies were multiple. Apart from social concerns, multiple pregnancies are associated with more medical problems such as the increased risk of preterm labor, prematurity, perinatal mortality, anemia, congenital malformations, and preeclampsia (5).

It is known that pregnancy rates are low in older women and many authors (6,7) have reported that

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female age is inversely correlated with multiple pregnancy rates. It has, therefore, become the practice at many clinics to transfer more embryos in older women than in younger ones in order to improve the pregnancy rate. In this retrospective study, we questioned the rationale for this practice and examined other possible factors related to multiple pregnancies in each age group.

MATERIALS AND METHODS

We studied 973 of 1116 cycles in our IVF program between January 1992 and December 1993 after excluding patients whose cycles were canceled (6%), patients who had no oocytes retrieved (3%), and patients who had all their embryos cryopreserved (2%) to prevent severe ovarian hyperstimulation syndrome (OHSS). Patients with a low sperm count after sperm preparation were not excluded. A previous study by us (unpublished data) showed that the pregnancy rate in the group with male-factor infertility was similar to that for other diagnostic categories with normal sperm parameters when fertilization occurred and embryos were available for transfer. The remaining patients were divided into three groups: group 1, less than 32 years of age; group 2, between 32 and 35 years of age; and group 3, greater than 35 years of age. The primary indications for assisted reproduction were tubal factor in 51%, endometriosis in 12%, unexplained infertility in 11%, male factor in 9%, and polycystic ovary disease in 5% of the cycles. Of the 973 cycles, 12% had a mixed etiology. For the majority of women, a "flare-up" protocol was used, requiring daily subcutaneous injections of a gonadotropin releasing hormone agonist (GnRH-a; 1 mg leuprolide acetate; Lupron; Abbott Pharmaceutical Company Ltd., Point Claire, Quebec, Canada) and parenteral gonadotropins beginning on the eighth day following the last day of oral contraceptive pill ingestion. Patients younger than 35 years started with 2 ampoules of human menopausal gonadotropins (hMG; 75 IU/amp Pergonal; Serono, Mississauga, ON, Canada, or Humegon, Organon, The Netherlands) and patients older than 35 started with 3 ampoules. The standard protocol was modified in some cycles, when there was a history of poor response or a high risk for OHSS. Patients were followed by frequent transvaginal ultrasound examinations and estradiol measurements starting on the third day of gonadotropin administration until at least two follicles with a mean diameter of 18 mm and an estradiol level of approximately 1000 pmol per mature follicle were detected.

At this stage, human chorionic gonadotropin (hCG; 5000 IU; Profasi; Serono, Mississauga, ON, Canada) was given. Oocyte retrieval was scheduled 36 hr following hCG administration, using an ultrasound-guided transvaginal approach with local anesthesia infiltration in the lateral fornices of the vagina. Aspirated oocytes were examined under a stereomicroscope, graded according to the appearance of a cumulus-corona complex (8) and inseminated with approximately 50,000 sperm per oocyte. Oocytes were examined for the presence of two pronuclei at 18 hr after insemination and embryo quality and morphology were assessed by scoring them from 1 to 4 (8) using an inverted microscope a few hours before transfer. Up to three embryos (rarely four or five embryos) were transferred 48 hr after oocyte retrieval. All embryos in 19 (2%) patients with a high risk (9) for severe ovarian hyperstimulation were cryopreserved to avoid conception. Luteal phases were supported with progesterone vaginal suppositories, 50 mg twice daily, and hCG, 1500 IU every third day for 2 weeks, except for the patients with a high risk of OHSS, who were given only twice the amount of progesterone. The serum β -subunit of hCG was determined 1 week after the last hCG injection. If it was positive, the patient was scheduled 2 weeks later for ultrasound determination of the presence of a gestational sac. We defined the embryonic implantation rate as the number of gestational sacs in the first ultrasound examination divided by the number of embryos transferred.

Statistical Analysis

SPSS PC statistical software (SPSS Inc., MI) was used for the analysis. Chi-square, multiple regression analysis, ANOVA, one-way ANOVA, Duncan multiple-comparison test, Student's *t* test, variance analysis, and Fisher's exact chi-square test were used whenever appropriate. Values are presented as mean $\pm$ standard deviation except where indicated.

When a power analysis is performed, one can see that the trend of multiple pregnancy rates in the three age groups would have been the same even if the number of cycles in the study group had been 46 times higher (5750 cycles). By assuming that there had been 5750 cycles in the study and the same trend of occurring multiple gestations shown in Tables I and II had continued, a statistically significant ($P = 0.05$) difference between age groups would have been achieved. It would have taken 7 years to collect these data at our IVF clinic.

Table I. IVF Data on Patients Who Had Three Embryos Transferred

	Age group (years)			P
	<32	32-35	>35	
Number of cycles	147	217	149	
Pregnancies	55 (37.4%)	79 (36.4%)	48 (32.2%)	0.60 ^a
Chemical	6 (10.9%)	11 (13.9%)	6 (12.5%)	
Single	33 (60.0%)	41 (51.9%)	24 (50.0%)	
Twins	7 (12.7%)	19 (24.1%)	12 (25.0%)	
Triplets	8 (14.5%)	5 (6.3%)	4 (8.3%)	
Ectopic	0 (0.0%)	2 (2.5%)	2 (4.2%)	
Unknown	1 (1.8%)	1 (1.3%)	0 (0.0%)	0.57 ^a
Implantation rate	16.3 ± 27.9	14.9 ± 25.1	13.7 ± 25.4	0.69
Average embryo grade	3.0 ± 0.6	3.1 ± 0.7	2.9 ± 0.6	0.19

^a Chi-square.

RESULTS

The overall pregnancy and multiple pregnancy rates were examined to show how these rates changed with the increasing number of embryos transferred. Of the 973 cycles with at least one oocyte retrieved, 248 cycles occurred in patients younger than 32 years. There were 410 cycles in the women between 32 and 35 years and 315 cycles in women older than 35

years. The distribution of the cycles with regard to stimulation protocol and the reasons for IVF among the age groups were not different. The majority of patients were stimulated with a GnRH-a "flare up" protocol with hMG and hCG (91, 94, and 92%, respectively; $P > 0.05$). The reason for IVF in the majority of cycles was tubal obstruction (52, 56, and 55%, respectively; $P > 0.05$). Older patients, as expected, used more ampoules of hMG than groups 2 and 1

Table II. IVF Data on Patients Who Had Three Embryos Transferred and More than Three Embryos Available

	Age group (years)			P
	<32	32-35	>35	
Number of cycles	123	179	106	
Age	29.4 ± 1.5	33.5 ± 1.2	37.3 ± 1.2	<0.001
Range	(23-31)	(32-35)	(36-40)	
Total hMG ampoule	19.2 ± 9.1	19.8 ± 7.7	23.9 ± 7.0	<0.001
Range	(6-62)	(7-68)	(12-48)	
E ₂ on hCG day	12,583 ± 7,395	10,710 ± 5,830	9,100 ± 6,053	<0.003
Range	(1,045-46,304)	(2,104-35,557)	(2,075-41,976)	
Cycle day of hCG	13.3 ± 1.7	13.1 ± 1.8	13.3 ± 1.5	0.74
Range	(11-19)	(9-28)	(19-18)	
Endometrial thickness on hCG day	9.3 ± 2.3	9.4 ± 2.3	9.2 ± 2.5	0.68
Range	(5-19)	(5-18)	(4-17)	
Total number of eggs retrieved	12.8 ± 6.5	11.2 ± 5.2	9.9 ± 5.2	<0.006
Range	(4-34)	(3-29)	(3-35)	
Fertilization rate (%)	61.6 ± 34.0	57.3 ± 35.6	57.8 ± 43.0	0.59
Range	(0-100)	(0-100)	(0-100)	
Total No. of embryos	6.6 ± 2.8	6.7 ± 2.9	6.0 ± 2.4	0.10
Pregnancies	49 (35.0%)	68 (38.0%)	34 (32.1%)	0.45 ^a
Chemical	2 (4.7%)	8 (11.8%)	5 (14.7%)	
Single	26 (60.5%)	36 (52.9%)	17 (50.0%)	
Twins	7 (16.3%)	17 (25.0%)	7 (25.6%)	
Triplets	7 (16.3%)	4 (5.9%)	4 (11.8%)	
Ectopic	0 (0.0%)	2 (2.9%)	1 (2.9%)	
Unknown	1 (2.3%)	1 (1.5%)	0 (0.0%)	0.60 ^a
Multiple pregnancy rate	11.4 ± 31.9	11.7 ± 32.3	10.4 ± 30.6	0.94
Implantation rate	17.5 ± 28.6	15.6 ± 25.4	13.7 ± 26.2	0.57
Average embryo grade	3.1 ± 0.6	3.1 ± 0.7	3.0 ± 0.6	0.23

^a Chi-square.

(25.2 ± 9.6 , 21.2 ± 9.7 , and 20.6 ± 10.7 , respectively; $P = 0.012$) but had lower estradiol levels than those in groups 2 and 1 (7692 ± 5681 , 9081 ± 5848 , and 10570 ± 7355 , pmol/L, respectively; $P < 0.001$). There was no difference between age groups with respect to endometrial thickness on the day of hCG or in fertilization rates. However, patients in group 3 had fewer oocytes retrieved compared to groups 2 and 1 (7.1 ± 4.8 , 9.0 ± 5.9 , and 10.1 ± 6.7 , respectively; $P < 0.001$). The age of patients was inversely and very weakly correlated with the total number of oocytes retrieved but not with the total number of embryos available ($r = -0.19$, $P = 0.001$, and $r = -0.07$, $p > 0.05$, respectively). The older patients (group 3) had more cycles with four or more embryos transferred than the younger patients. In 30 cycles (12%) of the first, 70 (17%) of the second, and 50 (16%) of the third age group, patients failed to fertilize and no embryos were transferred ($\chi^2 = 2.0$, $P = 0.37$). Pregnancy rates in each age group were 26, 29, and 19%, respectively ($P = 0.009$). However, multiple pregnancy rates per cycle (respectively, 7.3, 8.3, and 5.7%; $P = 0.41$) and multiple pregnancy rates per pregnancy were similar in each group (respectively, 27.4, 29.3, and 30.5%; $P = 0.97$). Figure 1A shows the fertilization, implantation, total pregnancy, and multiple pregnancy rates in each patient age group.

Different variables in patients who had at least one embryo transferred among the three age groups were compared. There were no differences with respect to stimulation protocol, indication for IVF, day of hCG, or endometrial thickness on the day of hCG administra-

tion (data not shown). On the other hand, the patients older than 35 years had fewer oocytes retrieved, lower estradiol levels on the day of hCG, lower overall implantation rates, and lower rates of pregnancy and used more ampoules of hMG for their ovulation induction than did the younger patients (data not shown). While 31.8% of the patients with an endometrium on ultrasound of 8 mm or thicker became pregnant, only 21.3% of the patients with a thinner endometrium became pregnant ($P = 0.02$). The pregnant group used less hMG for stimulation than the nonpregnant group and had higher estradiol levels and a significantly greater number of eggs retrieved. Endometrial thickness on the day of hCG administration was also higher in patients who conceived than in those who did not (10.5 ± 2.3 and 9.8 ± 2.7 mm, respectively; $P < 0.009$), confirming our previously published results (10). The average embryo quality per transferred embryo in pregnant patients was slightly higher than in nonpregnant ones (respectively, 3.1 ± 0.7 and 2.9 ± 0.7 ; $P = 0.001$).

The differences between groups with respect to implantation, total pregnancy, and multiple pregnancy rates became smaller when the data were adjusted by considering only the patients with three embryos transferred. The values are shown in Table I. There were no differences between groups with respect to any of those variables. Figure 1B shows the multiple pregnancy rates per pregnancy versus the number of embryos transferred. There was no difference among age groups 1 to 3 with respect to average embryo quality (respectively, 3.0 ± 0.6 , 3.1 ± 0.7 , and $2.9 \pm$

Fig. 1. (A) The mean fertilization, implantation, pregnancy, and multiple pregnancy rates in 973 patients (vertical bars represent standard errors of means). (B) The mean multiple pregnancy rates in 242 pregnancies according to the number of embryos transferred.

0.6; $P = 0.19$). We did not observe any difference between the patients with multiple pregnancies or single pregnancies with respect to the average quality of embryos transferred (respectively, 3.2 ± 0.7 and 3.0 ± 0.7 ; $P = 0.13$).

Table II shows the data on patients who had three embryos transferred and more than three embryos available to transfer. Patients over 35 years old used more hMG ampoules, had higher estradiol values on hCG day, and had fewer oocytes retrieved than patients in the other two groups. The implantation rate and multiple pregnancy rate were similar in all groups. As shown in Table II, the triplet rates in the three groups were 16.3, 5.9, and 11.8% respectively. By keeping the same trend and assuming that there had been three times more cycles in the study, this difference would have been statistically significant. However, the difference would have been made by group 2, which was not the oldest group. By combining groups 1 and 2 and making two study groups (younger than 35 and older than 35), the triplet pregnancy ratio would have been 11.3% in the group younger than 35 and 14.3% in the group older than 35 ($P = 0.67$). By using the same trend to reach a statistically significant value ($P = 0.048$), the total number of cycles in the study would have been 22 times more (2750 cycles).

Multiple regression analysis revealed that the most important parameter affecting pregnancy rate was the number of embryos transferred, followed by, consecutively, the thickness of the endometrium on the day of hCG administration, the age of the female partner (inversely related), the total number of embryos available, and the total number of oocytes retrieved. Surprisingly, when the patients who had more embryos available than the number of embryos transferred were examined separately, or when the patients with three or more embryos transferred were included in the statistical analysis, the only important parameter affecting pregnancy was found to be endometrial thickness (data not shown). Age did not enter the equation and was not related to the risk of multiple pregnancy. When we repeated multiple regression analysis for the patients who conceived with respect to the occurrence of multiple pregnancy, the only parameter of significance in the regression equation was the number of embryos transferred.

DISCUSSION

The introduction of IVF and other assisted reproductive techniques has led to an enormous increase in the

number of multiple pregnancies compared with the normal fertile population (11,12). Because multiple pregnancies are associated with obstetrical problems, many clinics practice different prevention or reduction techniques for multiple pregnancies while trying not to compromise the success rate of IVF. Multifetal pregnancy reduction carries its own risks and is associated with a high rate of miscarriage (13,14). Therefore other solutions to prevent the high incidence of multiple pregnancy and its complications are being sought. Rutherford *et al.* (15) reported that patients were at high risk for multiple pregnancy when over five embryos were available for transfer, when tubal disease was the main cause of infertility, and when fertilization rates were over 60%. All of these factors may contribute to the decision to transfer more embryos. The number of embryos transferred seems to be the most important factor in the occurrence of multiple pregnancy (16,17), although other factors, such as the age of the female partner, the quality of embryos, the IVF indication, the number of oocytes retrieved, the cleavage rate, replacement techniques, the uterine receptivity, and the quality of the luteal phase, have also been suggested (6,16,17). Statistical analysis of the data of IVF patients has the potential for many errors, since most of the parameters are not independent. For example, the age of patients and the total number of retrieved oocytes, the number of embryos available, and the number of embryos transferred are all dependent on each other. In many studies comparing the pregnancy rates when one or two embryos were transferred to those when three or more embryos were transferred, data were not adjusted according to the number of embryos available or any other dependent variables which might affect the implantation rate, such as endometrial thickness and pattern. When a patient is given four embryos instead of two or three, this is usually not random. There are likely several factors, including a larger number of embryos available for transfer, affecting the decision-making process of both the medical staff and the infertile couple. Multiple regression analysis is also difficult to interpret when most of the parameters are dependent. It is known that the multiple pregnancy rate is lower in patients over 35 (7). This observation has led some clinicians to transfer more embryos in older women. Although not statistically significant, our data also showed lower total and multiple pregnancy rates in patients over 35 years old. However, when the data were adjusted to account for the number of embryos transferred and the endometrial thickness, this trend completely disappeared. In our clinic, transferring three embryos is the routine proce-

dures when three or more embryos are available. We transfer more embryos when patients are older or have had a poor outcome in previous IVF cycles or fewer embryos in cases when we have only one or two good-quality embryos. Therefore we chose three as the optimal number of embryos transferred to adjust our data to do the statistical analysis. When we transferred only two embryos, the multiple pregnancy rates were 7.1% in group 1 (under 32 years of age), 6.3% in group 2 (32 to 35 years of age), and 0 in group 3 (over 35 years of age; $P = 0.19$). Pregnancy rates were 21.4, 34.4, and 8.2%, respectively ($P = 0.004$). The lower overall and multiple pregnancy rates in the older age group can be explained because most of these patients did not have more than two embryos, while the younger patients with several embryos elected to have only two embryos transferred to avoid multiple pregnancy. The quality of embryos has been found to affect the implantation rate, resulting in higher total and multiple pregnancy rates (11). We did not observe any difference in the average quality of embryos transferred in multiple pregnancies versus single pregnancies. Therefore, embryo quality did not seem to be very important to the occurrence of multiple pregnancy. Although there is no prospective study comparing IVF outcome with two or three embryos transferred, clinics which switched to a two embryo transfer protocol appear to have good results (18,19). Nijs *et al.* (18) reported a 36% total pregnancy rate and 26% multiple pregnancy rate in 1990 when they routinely transferred up to two embryos. These results were not different from their data in 1989 when three embryos were transferred, and they eliminated triplets and quadruplets. Besides these reports, the 20.8% pregnancy rate in an IVF program during spontaneous cycles with only one embryo transferred is also very encouraging (20). Our pregnancy rate when three embryos were transferred was higher (although not significantly) than that when four embryos were transferred (35 and 23%, respectively). This observation reflects the patient selection criteria for replacing more embryos. In the group with four embryos replaced, there were more patients with previous repeated unsuccessful cycles and fewer patients with only tubal problems than in the group with three embryos transferred. Multiple regression analysis showed that the only important parameter affecting the occurrence of pregnancy was the endometrial thickness, when only the patients who had more embryos available than were transferred were included in the equation. This finding also suggests that the lower pregnancy rate when we transferred two

embryos (22%; $P = 0.04$) compared to three embryos may be a result of only two embryos being available.

The age of the female partner is reported to be inversely correlated with fertility in both natural (21) and stimulated IVF cycles (22). The higher rate of chromosomal abnormalities (23–25) in older patients may explain the lower pregnancy rates. However, the older patients who became pregnant after IVF in our study appeared to behave like their young counterparts. Therefore, we may speculate that the majority of oocytes of any given patient may be either normal or abnormal. Therefore, if a patient conceives, she may be at risk for multiple pregnancy, increasing with the number of embryos transferred, regardless of her age.

Unfortunately, we do not know the exact live birth rates. However, all pregnancies in the study were clinical pregnancies. This means that fetal heartbeats were detected by ultrasound. After detecting a fetal heartbeat, the abortion rate is quite low, approximately 5%. Therefore, the pregnancies resulting in abortion would not change the conclusion of this study. Although the chance of vanishing excess embryos after a fetal heartbeat is detected is not as great as that in the earlier stages of pregnancy, when only the gestational sac is shown, it might still occur in the earlier stages of pregnancies. It would have been interesting to know the live birth rates in our study. This means that there is still a question awaiting an answer.

CONCLUSIONS

According to the results of multiple regression analysis, patients who have the greatest chance to become pregnant through IVF treatment are patients younger than 35 who have an endometrium of at least 8-mm thickness and two or three embryos replaced, with more embryos available than the number transferred. Our data support the concept that the number of embryos transferred in an IVF program should be limited to three. The age of the female and multiple pregnancy risk, therefore, do not seem to be associated. The false impression of low multiple pregnancies in older patients comes from the low pregnancy rate in general in this age group. Therefore, we conclude that women over the age of 35 should not be treated differently than their younger counterparts with regard to the number of embryos transferred, unless there is an individual indication to transfer more.

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