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## REDUCING THE RISK OF MULTIPLE BIRTHS BY TRANSFER OF TWO EMBRYOS AFTER IN VITRO FERTILIZATION

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### ABSTRACT

**Background** In vitro fertilization is associated with a high risk of multiple births, which is a direct consequence of the number of embryos transferred. However, other factors that contribute to the risk are not well defined.

**Methods** Using the data base established by the Human Fertilisation and Embryology Authority in the United Kingdom, we studied the factors associated with an increased risk of multiple births in 44,236 cycles in 25,240 women. The factors included the woman's age, the cause and duration of infertility, previous attempts at in vitro fertilization, previous live births, number of eggs fertilized, and number of embryos transferred.

**Results** Older age, tubal infertility, longer duration of infertility, and a higher number of previous attempts at in vitro fertilization were all associated with a significantly decreased chance of a birth and of multiple births. Previous live birth was associated with an increased chance of a birth but not of multiple births. The higher the number of eggs fertilized, the higher the likelihood of a live birth. When more than four eggs were fertilized, there was no increase in the birth rate for women receiving three transferred embryos as compared with those receiving two, but there was a considerable increase in the rate of multiple births when three were transferred (odds ratio, 1.6; 95 percent confidence interval, 1.5 to 1.8).

**Conclusions** Among women undergoing in vitro fertilization, the chances of a live birth are related to the number of eggs fertilized, presumably because of the greater selection of embryos for transfer. When more than four eggs are fertilized and available for transfer, the woman's chance of a birth is not diminished by transferring only two embryos. Transferring more embryos increases the risk of multiple births. (N Engl J Med 1998;339:573-7.)

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THE high rate of multiple births resulting from in vitro fertilization is a major health issue.<sup>1-3</sup> The medical, social, and financial consequences are considerable, chiefly because of the excessive morbidity among the survivors of high-order multiple births (triplets or more).<sup>4-8</sup> The problem exists because of the large number of embryos transferred in treatment cycles, frequently three or even more. Clinicians and couples are under considerable pressure to maximize pregnancy rates, but there is a view that multiple gestation is an unacceptable consequence of these pressures.<sup>5,9</sup> Some have suggested that no more than two embryos need be transferred,<sup>10,11</sup> arguing that doing so will not necessarily result in a reduction in pregnancy rates. Others have indicated that transferring only two embryos would be unduly restrictive and would in many instances reduce the chance of success.<sup>12-15</sup>

To address this issue, we examined the factors, including the number of embryos transferred, that predispose women undergoing in vitro fertilization to multiple gestation. This study extends a previous analysis of treatment outcomes,<sup>16</sup> and like the outcome study it was carried out with the data base established by the Human Fertilisation and Embryology Authority that has recorded all cycles of in vitro fertilization carried out in the United Kingdom since 1991.

### METHODS

#### Subjects

We previously studied the factors affecting the outcome of in vitro fertilization treatment, using data from 36,961 cycles in 26,389 women registered in the Human Fertilisation and Embryology Authority data base from 1991 to 1994. Using an extended

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data base of all 78,325 cycles (in 34,430 women) initiated between August 1991 and April 1995, we analyzed in the current study the factors affecting the likelihood of multiple births.

We excluded from this analysis 2478 cycles with donated eggs in 1689 women, 566 cycles with donated embryos in 398 women, 10,611 transfers of frozen embryos in 7113 women, 4608 unstimulated cycles in 3370 women, and 23,151 cycles in 15,051 women in which one embryo or no embryo was transferred. Because of overlap between the categories, the total numbers of excluded cycles and women were 34,089 and 9190, respectively. Thus, we analyzed 44,236 cycles in 25,240 women. Of these women, 63 percent had one treatment cycle, 20 percent had two, 8 percent had three, and 9 percent had more than three. Their mean ( $\pm$ SD) age was  $34 \pm 4$  years (range, 18 to 52), and the mean duration of infertility was  $6 \pm 4$  years (range, 0 to 20). The indications for in vitro fertilization were tubal disease (50 percent), unexplained infertility (35 percent), endometriosis (10 percent), and other conditions (12 percent); some women had more than one indication. The factors analyzed were the woman's age, the duration and cause of infertility, previous in vitro fertilization treatment, previous live births and whether these were the result of in vitro fertilization, number of eggs fertilized, and number of embryos transferred. The outcome measures were rates of births (live births and perinatal deaths) and multiple births per embryo transfer. Seventy-eight centers contributed both treatment and outcome data for the 44,236 cycles that formed the basis of this analysis. Permission to review the data was granted by the information committee of the Human Fertilization and Embryology Authority.

**Statistical Analysis**

The relation between age and birth rate was modeled by the method of fractional polynomials,<sup>17</sup> on the basis of a logistic-regression analysis that found that a linear relation with age was the most parsimonious. All other factors influencing the probability of a birth or of multiple births were evaluated by logistic-regression analysis with adjustment for age.

Age and duration of infertility were analyzed as continuous variables. Twelve years was taken as the maximal duration; the few women who had been infertile for more than 12 years were assigned a value of 12. The number of eggs fertilized (two only, three or four, or more than four) and previous unsuccessful attempts at in vitro fertilization (no previous attempt, one to three previous attempts, or four or more previous attempts) were analyzed as categorical variables. Both forward and backward selection methods were used to obtain the smallest number of explanatory variables that provided a well-fitting model; the difference in deviance was used to assess whether any additional term was necessary in the model. The confidence intervals for the odds of a birth and of multiple births according to the number of eggs fertilized and the number of embryos transferred were derived from the logistic-regression analysis with use of the method of calculating the floating absolute risk.<sup>18</sup>

**RESULTS**

Older age, the presence of tubal infertility, four or more previous attempts at in vitro fertilization, and longer duration of infertility all significantly reduced both the odds of a birth and the odds of multiple births (Table 1). Having had a previous live birth (whether the result of in vitro fertilization or not) increased the odds of a birth but not of multiple births.

The effect of the number of embryos transferred was analyzed in relation to the number of eggs fertilized (and thus the number of embryos available for transfer) (Table 1). When only two eggs were fertilized and available for transfer, the odds of a birth, whether single or multiple, were reduced. When more than two eggs were fertilized, the transfer of three

**TABLE 1. FACTORS AFFECTING THE RESULTS OF IN VITRO FERTILIZATION.\***

VARIABLE	ODDS OF A BIRTH (95% CI)	P VALUE	ODDS OF MULTIPLE BIRTHS (95% CI)	P VALUE
Maternal factors†				
Age (per additional year)	0.9 (0.9–1.0)	<0.001	0.97 (0.95–0.99)	0.013
Tubal infertility (vs. no tubal infertility)	0.7 (0.7–0.8)	<0.001	0.8 (0.7–0.9)	<0.001
No. of previous attempts at IVF (vs. none)				
1–3	0.8 (0.8–0.9)	<0.001	1.0 (0.9–1.1)	0.85
4 or more	0.6 (0.5–0.7)	<0.001	0.6 (0.4–0.8)	<0.001
Duration of infertility (per additional year)	0.98 (0.98–0.99)	<0.001	0.98 (0.97–0.99)	0.02
Previous live birth (vs. none)				
Not IVF	1.1 (1.0–1.2)	<0.001	Not included in model	
IVF	1.6 (1.4–1.8)	<0.001	Not included in model	
No. of eggs fertilized and embryos transferred‡				
2 Eggs, 2 embryos	0.5 (0.4–0.5)	<0.001	0.5 (0.4–0.7)	<0.001
3 or 4 Eggs, 2 embryos	0.6 (0.5–0.7)	<0.001	0.7 (0.6–0.9)	0.008
3 or 4 Eggs, 3 embryos	0.7 (0.7–0.8)	<0.001	1.3 (1.1–1.4)	0.008
>4 Eggs, 2 embryos	1.01 (0.9–1.1)	—	1.0 (0.9–1.1)	—
>4 Eggs, 3 embryos	1.0 (0.9–1.1)	0.78	1.6 (1.5–1.8)	<0.001

\*CI denotes confidence interval, and IVF in vitro fertilization.

†The odds for each factor were adjusted for all other factors in the table.

‡The odds for each factor were adjusted for the above factors that significantly affected the outcome of IVF treatment.

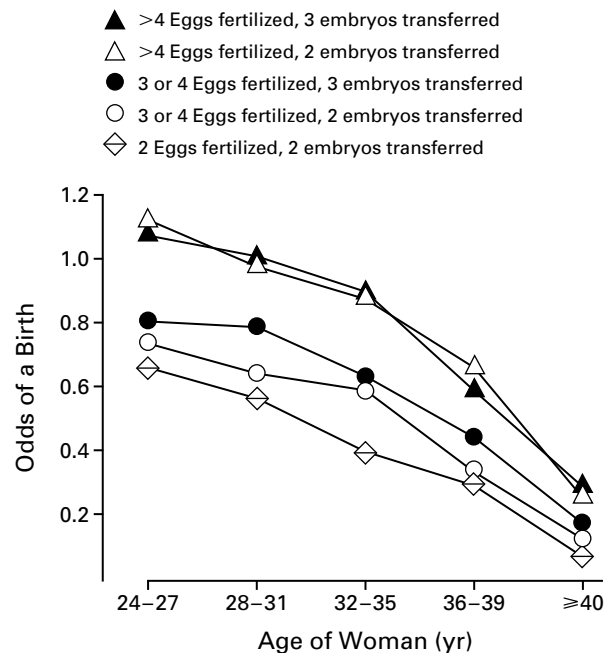
**TABLE 2.** NUMBER OF SINGLE, TWIN, AND TRIPLET BIRTHS ACCORDING TO THE NUMBER OF EGGS FERTILIZED AND OF EMBRYOS TRANSFERRED, UNADJUSTED FOR AGE OR OTHER FACTORS.

VARIABLE	No. OF LIVE BIRTHS				TOTAL No. OF TRANSFERS
	0	1	2	3 OR MORE	
2 Eggs, 2 embryos					
No.	3,918	434	82	2	4,436
Percent of transfers	88	10	2	0	
Percent of births	—	84	16	0.4	
>2 Eggs, 2 embryos					
No.	8,297	1647	586	8	10,538
Percent of transfers	79	16	6	0	
Percent of births	—	73	26	0.4	
>2 Eggs, 3 embryos					
No.	23,171	3980	1755	356	29,262
Percent of transfers	79	14	6	1	
Percent of births	—	65	29	6	

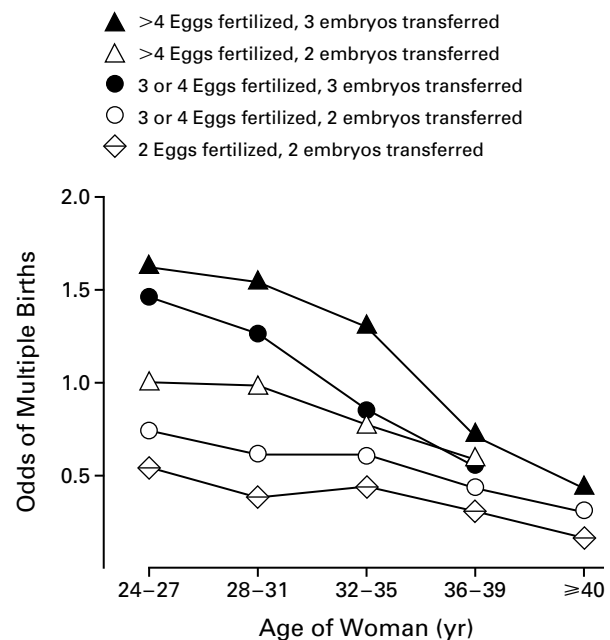
embryos did not significantly increase the chance of a birth (over the transfer of two embryos), but it did increase the chances of both twin and triplet births (Table 2).

The odds of a birth were then calculated in relation to the age of the woman, the number of eggs fertilized, and whether two or three embryos were transferred. In all age groups, if more than four eggs were fertilized, the odds of a birth were no greater when three embryos were transferred than when two embryos were transferred (Fig. 1). However, the possibility of multiple births did increase (Fig. 2). Overall, when age was taken into account, the odds of a birth increased significantly with the number of eggs fertilized, whether two or three embryos were transferred (Fig. 3).

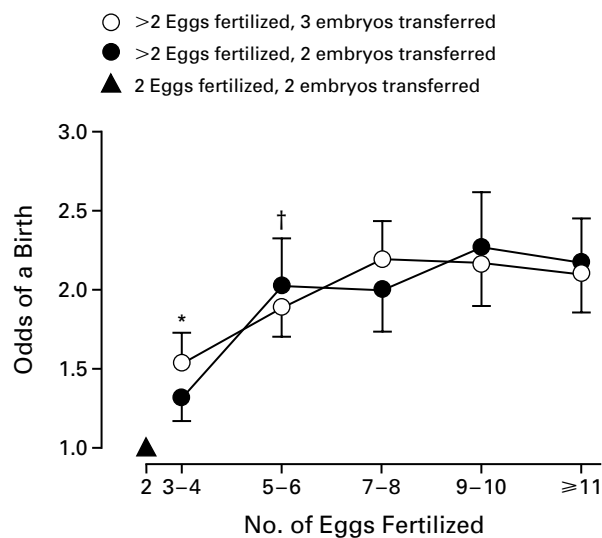
We used these data to construct a table to aid in clinical decision making (Table 3). For this purpose we assumed that each woman had tubal infertility, one to three previous attempts at in vitro fertilization and embryo transfer, and no previous pregnancies. For ages 30, 35, and 40 years, the rates of births (per embryo transfer) and of multiple births (as a percentage of all births) were related to the number of eggs fertilized (and hence available for transfer) and the number of embryos transferred. When three or four eggs were fertilized, the transfer of three embryos, as opposed to two, resulted in a small increase in the birth rate, but at the expense of an increase in the rate of multiple births. However, when more than four eggs were fertilized, the transfer of three embryos did not improve the overall birth rate, but it did result in an increase in the rate of multiple births by 4 to 11 percentage points, depending on age.



**Figure 1.** Odds of a Birth in Relation to the Woman's Age, Number of Eggs Fertilized, and Number of Embryos Transferred. The odds of a birth were calculated as compared with those of a 30-year-old woman with more than four eggs fertilized and two embryos transferred (odds for this woman, 1.0).



**Figure 2.** Odds of Multiple Births in Relation to the Woman's Age, Number of Eggs Fertilized, and Number of Embryos Transferred. The odds of multiple births were calculated as compared with those of a 30-year-old woman with more than four eggs fertilized and two embryos transferred (odds for this woman, 1.0).



**Figure 3.** Odds of a Birth in Relation to the Number of Eggs Fertilized and Number of Embryos Transferred, as Adjusted for Age. The odds of a birth were calculated as compared with those of a woman with two eggs fertilized. The asterisk denotes  $P < 0.001$  for the comparison between two eggs and three or four eggs fertilized, regardless of whether two or three embryos were transferred. The dagger denotes  $P < 0.001$  for the comparison between three or four eggs and five or six eggs fertilized, regardless of whether two or three embryos were transferred. The I bars indicate 95 percent confidence intervals.

### DISCUSSION

In this large study, we found that an increase in the number of embryos transferred invariably resulted in higher rates of multiple births, without necessarily improving the overall success rates of in vitro fertilization. Some studies have suggested that the pregnancy rate after the transfer of two embryos dif-

fers little from that when more embryos are transferred.<sup>9-11</sup> Other studies have suggested that the number of embryos transferred, particularly in older women, can be increased with little risk of multiple births. This assertion has been questioned,<sup>19,20</sup> and it is certainly true that in the United Kingdom women over 40 years of age who are undergoing in vitro fertilization and embryo transfer still regularly give birth to twins and triplets.

As previously described in a detailed analysis using the same data base,<sup>16</sup> a number of patient characteristics, chiefly the woman's age, diminish the likelihood that a pregnancy will occur after in vitro fertilization. It is now apparent that many of these same characteristics also diminish the chances of multiple gestation. These include treatment for tubal infertility, many previous attempts at in vitro fertilization and embryo transfer, and long duration of infertility. However, in this study, we found that in addition to these patient characteristics, the number of eggs fertilized, and hence the number of embryos available for transfer, was an important factor in determining outcome.

In spite of the wide variation in practices with respect to in vitro fertilization, it appears that in almost all circumstances, and certainly when more than four eggs have been fertilized, the number of embryos available is more important in determining outcome than the number of embryos actually transferred into the uterus. When more than four eggs had been fertilized, the birth rate was similar whether three or two embryos were transferred. Transfer of more embryos, however, increased the risk of multiple births. A 30-year-old woman choosing to have two embryos transferred rather than three reduced her risk of multiple birth from 39 percent to 29 percent and nearly avoided altogether the risk of triplets (Table 3). Sim-

**TABLE 3.** RATES OF BIRTHS PER EMBRYO TRANSFER AND OF MULTIPLE BIRTHS AS A PERCENTAGE OF ALL BIRTHS, ACCORDING TO MATERNAL AGE, NUMBER OF EGGS FERTILIZED, AND NUMBER OF EMBRYOS TRANSFERRED.

MATERNAL AGE AND No. OF EMBRYOS TRANSFERRED	2 EGGS FERTILIZED		3 OR 4 EGGS FERTILIZED		>4 EGGS FERTILIZED	
	BIRTH RATE	MULTIPLE-BIRTH RATE	BIRTH RATE	MULTIPLE-BIRTH RATE	BIRTH RATE	MULTIPLE-BIRTH RATE
percent (95 percent confidence interval)						
30 yr						
2	11.4 (10.4-12.5)	17.3 (13.8-21.4)	14.1 (12.8-15.6)	22.3 (18.3-26.9)	21.3 (20.0-22.5)	28.6 (25.6-31.7)
3	—	—	16.2 (15.1-17.3)*	33.9 (30.1-37.9)†	21.1 (20.1-22.1)	39.4 (36.6-42.3)‡
35 yr						
2	8.9 (8.1-9.8)	15.2 (12.1-18.9)	11.1 (10.0-12.3)	19.8 (16.1-24.1)	17.0 (15.9-18.1)	25.6 (22.5-28.9)
3	—	—	12.8 (11.9-13.7)*	25.5 (22.4-28.8)‡	16.9 (16.0-17.7)	32.6 (30.0-35.4)‡
40 yr						
2	6.9 (6.2-7.7)	13.3 (10.2-17.3)	8.7 (7.8-9.7)	17.5 (13.6-22.4)	13.5 (12.4-14.5)	22.8 (18.7-27.5)
3	—	—	10.0 (9.3-10.8)*	18.5 (14.9-22.9)	13.3 (12.5-14.2)	26.5 (23.4-29.9)

\* $P < 0.02$  for the comparison with two embryos transferred.

† $P < 0.001$  for the comparison with two embryos transferred

‡ $P < 0.03$  for the comparison with two embryos transferred.

ilarly, for a 40-year-old woman there was no advantage in transferring three rather than two embryos. All that was achieved was an increase in the rate of multiple births from 23 percent to 27 percent.

These results come from centers with wide variations in clinical practice. The low success rate when only two eggs were fertilized reflects the lack of choice among embryos for transfer, whereas the increased choice when more than four eggs were fertilized is reflected in higher pregnancy rates. Thus, the ability to select embryos remains an important determinant of the outcome of in vitro fertilization, despite the limitations of grading the quality of embryos according to morphologic criteria, as is done in current clinical practice. When three or even more embryos selected from a larger number are transferred, there will inevitably be a higher rate of multiple births.

The data presented here are also consistent with the suggestion that uterine receptivity is likely to be an all-or-nothing effect.<sup>21</sup> When the uterus is receptive and the embryos have good implantation potential, as seems more likely when they are chosen from a large number, transfer of three will result in a higher chance of twins or triplets than transfer of two. On the other hand, if the uterus is not receptive, a pregnancy will not occur, no matter how many embryos are transferred. Centers in which the embryos have a higher potential for implantation may be more likely to reduce the number of embryos transferred in order to minimize the rate of multiple births, whereas centers with lower pregnancy rates may continue to transfer three embryos in order to increase their pregnancy rates. However, this pattern does not explain the significantly higher rates of multiple births when three embryos were transferred, nor was there any evidence that this effect was a contributing factor when the transfer policies of the individual centers were reviewed.

The implications of these results for clinical practice are clear. When more than four embryos are fertilized and available, transfer of only two embryos will not diminish the woman's chance of becoming pregnant, but it will reduce her chance of multiple births. This is true for women of all ages. When only three or four embryos are fertilized and the woman is about 40 years old, there may be a slight increase in the pregnancy rate when three embryos are transferred, but the risk of multiple births will also increase. When only two embryos are available for transfer, these issues do not arise, but the couple should be prepared to accept a much lower pregnancy rate.

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