CLINICAL ASSISTED REPRODUCTION

The Transfer of Two Embryos Instead of Three to Reduce the Risk of Multiple Pregnancy: A Retrospective Analysis

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Purpose: Our purpose was to investigate whether a reduction in the number of embryos transferred from three to two would help reduce the incidence of multiple pregnancies and yet leave the pregnancy rate unaffected.

Methods: Women were treated in a routine clinical in vitro fertilization program and the results analyzed retrospectively.

Results: There was no reduction in the pregnancy rate when two embryos were transferred compared with three. Indeed, there was actually an increase in pregnancy rate after the transfer of two embryos in those cases with one or more embryos remaining after the transfer.

Conclusions: The transfer of only two embryos compared to three in women younger than 40 years of age does not compromise the chance of pregnancy. Triplets were not seen in the limited series of patients when only two embryos were transferred, but the incidence of twins remained the same. Further consideration should be given to strategies that enable the transfer of single embryos without compromising the pregnancy rate.

KEY WORDS: embryo; multiple; number; pregnancy; transfer.

INTRODUCTION

There has been considerable debate about the optimal number of embryos to transfer within an in vitro fertilization (IVF) program, to maximize the chance of

pregnancy but to minimize the risk of multiple pregnancy and unfavorable obstetric outcome (1,2). The need to regulate the number transferred has even been debated (3,4), with some countries favoring an upper limit of three embryos for transfer and the transfer of four embryos in exceptional circumstances (5). In essence, reports have indicated that the transfer of only two embryos can yield pregnancy rates similar to those with three embryos (6,7), although there appear to be certain caveats. These would include the importance of having at least one of the two transferred embryos being of good quality (8) and a reduced implantation rate per embryo in older patients, which means a flexible approach should probably be considered in women older than 40 years (9,10).

The present study has retrospectively analyzed the results of the unit from January 1, 1993, to June 30, 1997, to determine whether the transfer of two embryos compared with three is associated with (a) a similar pregnancy rate per embryo transfer and (b) a reduction in the proportion of pregnancies that are of multiple gestation.

MATERIALS AND METHODS

Cycle Selection

Consecutive IVF treatment cycles from January 1, 1993, to June 30, 1997, were included and categorized according to the woman's age at embryo transfer. Cycles involving micromanipulation and donated gametes or embryos were excluded. The decision to transfer two or three embryos was made according to the prevailing policy of the unit.

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IVF Protocol

Women received treatment with Lucrin (leuporelin acetate; Abbott Australasia, Kurnell, Australia), 0.75 mg daily, or Synarel (nafarelin acetate; Searle, Division of Monsanto Australia, Sydney, Australia), 800 µg daily, in either a flare or a pituitary desensitization regimen (11). Ovarian stimulation was achieved by the administration of human menopausal gonadotropin [Humegon, Organon (Australia) Pty. Ltd., Sydney, Pergonal, Serono Australia Pty. Ltd., Frenchs Forest, NSW, Australia) or purified follicle-stimulating hormone (FSH) (Metrodin or Metrodin HP; Serono Australia Pty. Ltd.) until the leading follicle reached a diameter in excess of 17 mm. Ovulation was then initiated by the administration of 10,000 IU of human chorionic gonadotropin (Pregnyl; Organon), and oocytes were collected 34–36 hr later under ultrasound guidance. Follicles were flushed with HEPES-buffered Earle's balanced salt solution (EBSS) if the oocytes were not forthcoming. Embryos were transferred 2 days after oocyte collection using a K-Soft-4000-ATS transfer set (Cook IVF, Brisbane, Australia), and the luteal phase was supported by the administration of 600 mg progesterone vaginally from the day of embryo transfer until the 14th day after oocyte collection.

Gamete and Embryo Culture

Oocytes were identified using a dissecting microscope fitted with a warming stage, washed in HEPES-buffered EBSS containing 10% maternal serum and then placed into 12 × 17-mm polystyrene tubes (Falcon No. 2003; Becton Dickinson, USA) containing EBSS supplemented with 10% maternal serum and maintained in a desiccator at 37°C perfused continually with premixed gas composed of 90% N₂:5% O₂:5% CO₂ (Air Liquide WA Pty. Ltd., Coogee, Western Australia). Semen was produced by masturbation and processed within 1 hr of production. Depending on the sperm concentration and motility, sperm were isolated either by a swim-up method from semen into medium or by centrifugation through a two-layer 90%:45% Percoll gradient (12) and then washed twice in the IVF medium by centrifugation. Oocytes were inseminated with 100,000 sperm per ml 4–6 hr after collection and pronuclei observed 16–18 hr later. Fertilized oocytes were cultured for a further 24 hr in EBSS supplemented with 10% maternal serum prior to transfer in the same medium.

Diagnosis of Pregnancy

Pregnancy was defined as the presence of a fetal heart seen using ultrasound at 8 weeks of gestation. This definition was chosen to avoid the inadvertent exclusion of multiple pregnancies that might have miscarried at a later stage.

Data Analysis

Statistical analysis was by χ^2 , with *P* less than 0.05 being considered statistically significant.

RESULTS

The patients were divided into two main categories, namely, those with no embryos remaining after transfer and therefore having the number of embryos transferred dictated by circumstance and those with one or more embryos remaining and thus making a choice on the number of embryos transferred.

There was no significant difference in the proportion of women becoming pregnant when either two or three embryos were transferred and no embryos were remaining (Table I). Similarly, there was no difference in the proportion of embryos implanting (Table II). The low number of pregnancies does not permit conclusions to be drawn about the incidence of multiple pregnancies (Table III).

Regarding those women with at least one embryo remaining after transfer, there was a small but statistically significant increase in the overall pregnancy rate for women with two embryos transferred compared to those with three. However, the differences were not significant within the various age groups. There was a significant increase in the implantation rate for women aged 25–39 years having two embryos replaced (Table II), but no decrease in the multiple pregnancy rate (Table III). Interestingly, there were only two sets of triplets and both of these followed the transfer of three embryos.

DISCUSSION

Assisted reproduction is a major contributor to the occurrence of multiple pregnancies in both the United Kingdom (13) and the United States (14), resulting in a considerable increase in demands on neonatal intensive care facilities and hospital costs. While ovulation induction appears responsible for just as many

Table I. Pregnancy Rates per Embryo Transfer when Either Two or Three Embryos Are Transferred

Age (years)	No embryos remaining		≥1 embryo remaining	
	2 embryos	3 embryos	2 embryos	3 embryos
25–29	2/11 (18%)	1/4 (25%)	33/95 (35%)	8/32 (22%)
30–34	6/41 (15%)	3/19 (16%)	71/239 (30%)	21/100 (21%)
35–39	8/36 (22%)	5/17 (29%)	46/195 (24%)	21/107 (20%)
≥40	0/18 (—)	0/11 (—)	3/36 (8%)	1/25 (4%)
Total	16/106 (15%)	9/51 (18%)	153/565 (27%)*	51/264 (19%)*

* $P < 0.02$.

multiple pregnancies as IVF and gamete intrafallopian transfer (13, 14) and therefore deserves an equal amount of effort in monitoring the response to ovarian stimulation drugs, IVF has been the focus of most of the attention to minimize the risk of multiple pregnancy. Selective reduction has been proposed as a way of helping the pregnancy continue, and that seems to be well received by the small proportion of patients who do take that option (15, 16). However, this approach is rejected by the majority of patients, and so prevention of multiple pregnancies seems to be the best long-term solution. The transfer of only two embryos has been proposed as a way of eliminating triplets that have an increased perinatal mortality than their twin counterparts (16), and this has been echoed in the present study. Nevertheless, care must still be taken when applying this as a general principle. There are groups of patients (such as those older than 40 years of age) who may do better with a higher number of oocytes or embryos transferred (9, 17) because of the increased number of embryos that have chromosomal anomalies (18). The use of age as an arbiter, though, is not without limitation, as there are subgroups of these older women who may have an adequate gonadal reserve and have a similar chance of pregnancy as the younger women (19).

A further benefit of transferring only two embryos is to make more embryos available for cryopreservation,

which helps maximize the chance of pregnancy from the original oocyte collection (20). This is illustrated by the data from Concept Fertility Centre during the study period where a total of 992 transfers of frozen/thawed embryos was done, resulting in 149 (15%) pregnancies. The only way to arrive at a clear answer of whether the transfer of two embryos is useful is to undertake a prospective, randomized trial. Unfortunately, such studies take a long time to initiate and complete and so it remains important to analyze comprehensively and critically the information we do have. It is, after all, our only current source of guidance in deciding what is and what is not useful. Nevertheless, we must be aware of the limitations of retrospective analysis in terms of unintentional bias. For instance, it is hard to see how pregnancy rates in those patients with one or more embryos remaining should be higher when two embryos are transferred compared with three (Table I), although the prudent approach would be to use these data to indicate that two embryo transfers are no worse.

The transfer of only two embryos does appear to reduce the incidence of triplets but not necessarily that of twins as shown in this study and elsewhere (14). The logical progression would be then to transfer only one embryo but sadly the pregnancy rates appear markedly reduced. This applies to both patients in whom there was only one embryo for transfer (21), which

Table II. The Implantation Rate of Embryos Transferred

Age (years)	No embryos remaining		≥1 embryo remaining	
	2 embryos	3 embryos	2 embryos	3 embryos ^a
25–29	2/22 (9%)	1/12 (8%)	44/190 (23%)	11/96 (12%)*
30–34	6/82 (7%)	4/57 (7%)	91/478 (19%)	31/300 (10%)*
35–39	8/72 (11%)	7/51 (14%)	54/390 (14%)	26/321 (8%)*
≥40	0/36 (—)	0/33 (—)	0/33 (—)	1/75 (1%)
Total	16/212 (8%)	12/153 (8%)	192/1130 (17%)	69/792 (9%)**

^a Versus corresponding two embryos: * $P < 0.02$; ** $P < 0.0001$.

Table III. The Proportion of Pregnancies Achieved Which Were of Multiple Gestation

Age (years)	No embryos remaining		≥1 embryo remaining	
	2 embryos	3 embryos	2 embryos	3 embryos
25–29	0/2 (—)	0/1 (—)	11/33 (33%)	2/8 (14%) ^a
30–34	0/6 (—)	1/3 (33%)	20/71 (28%)	9/21 (40%) ^a
35–39	0/8 (—)	2/5 (40%)	8/46 (17%)	5/21 (24%)
≥40	—	—	0/3 (—)	0/1 (—)
Total	0/16 (—)	3/9 (33%)	39/153 (24%)	16/51 (31%)

^a All pregnancies were twin gestations except for one set of triplets in each of the marked groups.

might be compounded by a reduced ovarian response in the original cycle, and those women receiving donated embryos (22). During the period of the present study, 6 (9.6%) of 62 cycles having one embryo transferred into a woman younger than 40 years of age resulted in pregnancy, which is significantly reduced. Furthermore, 0 of 19 women 40 years of age or older conceived after the transfer of only one embryo. An interesting way of achieving single embryo transfers that have a good chance of implantation is the transfer of human blastocysts (23), with the supernumerary blastocysts appearing to do well after cryopreservation (24). However, work is still required to determine the optimal method for the extended culture and transfer of blastocysts, as the use of certain protocols has been shown to markedly affect the proportion of embryos reaching the blastocyst stage in culture and also implanting (25).

In summary, the transfer of only two embryos does not affect the pregnancy rate and limits the occurrence of grand multiple pregnancies. However, the prevalence of twin gestations is not affected greatly and so efforts are now needed to move to successful single embryo transfers.

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