A Prospective Randomized Multicenter Study to Evaluate the Best Day for Embryo Transfer: Does the Outcome Justify Prolonged Embryo Culture?

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Purpose: This study aimed to evaluate the best day for embryo transfer in a prospective unrestricted randomized multicenter trial.

Methods: Data were collected on a preformed Excel-sheet which contained random numbers from 1 to 5 for each subsequent patient as a preprogrammed day for embryo transfer. Information was requested on patient's age, indication for sterility treatment, stimulation protocol used, numbers of oocytes retrieved, fertilized oocytes, cryopreserved embryos, and cell stage of embryos transferred.

Results: A total of 329 embryo transfers were performed, resulting in 106 clinical pregnancies (32.2%). Pregnancy rates achieved were 20.0% on day 1, 30.4% on days 2 and 3, and 50.0% on days 4 and 5 ($p = 0.03$).

Conclusions: Within the scope of the present randomized multicenter trial, embryo transfers performed on days 4 and 5 enhanced the pregnancy rate significantly, compared to those of days 1, 2, and 3.

KEY WORDS: Blastocyst; culture; day; embryo transfer; pregnancy rate.

INTRODUCTION

Advantages of prolonged embryo culture include synchronization between embryos and endometrium, better selection of viable embryos, improved implantation rates, reduction of the number of embryos transferred, decreasing multiple pregnancy rate (PR), and availability of preimplantation genetic diagnosis (1). Despite various studies reporting an improvement of pregnancy rates delaying the embryo transfer (ET) to the blastocyst stage, preference for ET is still

given to day 2 or 3. Some in vitro fertilization (IVF) centers reason the maintenance of this procedure by the fear that some embryos might not develop continuously, but arrest at an early cleavage stage (2).

Regarding recent data (3,4), implantation rates for embryos transferred at the blastocyst stage were twice as high as observed for embryos transferred on day 3. Hence, prolonged culture until day 5 could lead to fewer embryos suitable for transfer, but transferring embryos at the blastocyst stage might enhance the implantation rate by better embryo selection, thereby reducing the need to transfer more embryos (5). Furthermore, it provides greater opportunities for the selection of more viable and genetically normal embryos.

Within the last years, knowledge for the embryo's metabolic requirements and developmental needs at "difficult" cell stages has increased and several new culture systems and media have been specially

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designed for the development of human blastocysts in vitro (6–8). These latest findings enhance the probability of gaining high-quality blastocysts.

However, data presented so far either compare 2 days of ET or describe blastocyst transfers, mainly in retrospective analysis. The purpose of our study was to evaluate the best day for embryo transfer in a prospective randomized multicenter setting. Therefore we compared the pregnancy rates resulting from ET on days 1–5.

PATIENTS AND METHODS

Study Design

In December 2000 this study was initiated as a prospective unrestricted randomized trial. Fifty-two clinics, all being members of the International Association of Private Assisted Reproductive Technology Clinics and Laboratories (A PART), were informed and invited to participate. Ten clinics agreed to take part, sending their data until March 2001.

Data were collected on a preformed Excel sheet with a random number from 1 to 5 for each subsequent patient. Each number represented a preprogrammed day for embryo transfer, irrespective of other criteria and the center's normal policies, but the clinics had the option of excluding a day in advance. Information was requested on the center's I.D., patient's initials and age, indication for sterility treatment, i.e., tubal or male, stimulation protocol used, numbers of oocytes retrieved, fertilized oocytes, frozen embryos, and cell stage of embryos transferred.

Patients

Until March 2001 we obtained data from 5 centers, but only the A PART clinics in Austria, Israel, and Egypt sent sheets suitable for statistical evaluation. After the exclusion of seven patients due to fertilization failure, the information received allowed analysis on 329 cycles (Table I). There were minor differences between the centers in terms of age of the patients, stimulation protocols used, and numbers of embryos transferred.

Blastocyst Culture

Embryos were cultured in G1.2TM-medium (Vitrolife Fertility Systems, Gothenburg, Sweden) to be transferred into CCMTM-medium (Vitrolife Fertility Systems, Gothenburg, Sweden) at the eight cell

Table I. Characteristics of Individuals Studied

No. of cycles	329
Mean ($\pm$ SD) age of patients	32.1 $\pm$ 5.2
Mean ($\pm$ SD) no. of oocytes retrieved	10.1 $\pm$ 6.6
Mean ($\pm$ SD) no. of oocytes fertilized	7.0 $\pm$ 5.3
Mean ($\pm$ SD) no. of embryos transferred on day 1	1.7 $\pm$ 0.8
Mean ($\pm$ SD) no. of embryos transferred on days 2 and 3	2.7 $\pm$ 0.9
Mean ($\pm$ SD) no. of embryos transferred on days 4 and 5	2.4 $\pm$ 0.6
No. of pregnancies	106 (32.2%)

stage. Standardized culture conditions were strictly controlled.

Statistics

Statistical analysis was performed with the Stat View SE program (Abacus Concepts, Berkeley, CA) using the contingency table test to compare the days of embryo transfer in terms of pregnancy rates. *p* values <0.05 were considered to be statistically significant.

RESULTS

A total of 329 embryo transfers were performed, resulting in 106 clinical pregnancies, i.e., an overall pregnancy rate of 32.2%. Comparing the PRs—separated per day—days 2 and 3 and days 4 and 5, respectively, were grouped together to obtain groups large enough to calculate statistical probability. Pregnancy rates achieved were 20.0% on day 1, 30.4% on days 2 and 3, and 50.0% on days 4 and 5 (*p* = 0.03) (Fig. 1). Prolonged embryo culture until days 4 and 5 resulted in a blastocyst development of 59.3%.

Regarding the treatment procedures in detail, there were minor differences between the centers, which

Fig. 1. Comparison of pregnancy rates resulting in embryo transfers performed on day 1 versus days 2 and 3 versus days 4 and 5.

however did not influence the overall results. Further information is given in Table I.

DISCUSSION

Within the scope of this study, embryo transfers performed on days 4 and 5 enhanced the pregnancy rates significantly, compared to those of days 2 and 3. This increased overall success seen with blastocysts might be attributable to the synchronous transfer of embryos at a stage when they are naturally designed to reach the uterus. In addition, better embryo selection, improved embryo–uterine synchrony, and decreased cervical mucus may have accounted for the better outcome (9). Moreover, delaying embryo transfer may be useful for the preimplantation diagnosis of genetic diseases (10).

However, as culture conditions are still “imperfect”, the longer culture lasts, the fewer embryos suitable for transfer remain (11). This consideration often results in embryo transfer on day 2 or 3. Certainly the fate of an embryo that has not been transferred until day 3 and degenerated or arrested development during prolonged culture cannot be known. Nevertheless, it is hard to imagine that such an embryo would have developed normally when other embryos from the same cohort under the same culture conditions and during the same time had formed morphologically good blastocysts (12).

Provided a patient had good blastocysts to transfer, she had the same chance of becoming pregnant, no matter how many embryos reached the blastocyst stage (13,14). As a result, there appears to be a very close relation between the success of blastocyst transfer and blastocyst quality (15). A precaution for enhanced pregnancy rates seems to be careful selection of embryos with a very high potential for implantation. As early cleavage divisions do not accurately predict the potential for further development, culturing for a prolonged period is a way of tackling this problem (11,16). Nevertheless, this procedure includes the risk of early cleavage arrest.

In comparison it is widely accepted that the number of embryos transferred is closely related to success rate (11) and as the “take-home-baby rate” per treatment cycle in IVF still does not approach natural fecundity, many centers try to improve their rates by transferring more embryos (12). Still, increasing the number of transferred embryos to more than two results in a higher rate of multiple birth, without necessarily improving the overall success rate (17,18). As a

method to reduce this incidence of higher-order multiple gestation blastocyst transfer has been suggested (19).

It also remains questionable whether delayed embryo transfer at day 3 or 6 is a suitable alternative for patients undergoing ovarian stimulation regimes who produce few oocytes or for patients who only produce one or two oocytes in a natural cycle (6,20).

Focussing on the patient’s interests, the decision on the day of transfer should still be made individually. Therefore, the number of oocytes retrieved and the embryos morphology have to be taken into account, as well as early cleavage, a strong biological indicator of embryo potential (3,6,21–24).

CONCLUSIONS

The ultimate aim of assisted reproduction can be seen in imitating or even exceeding natural fecundity with a maximal rate of singleton pregnancies. Blastocyst transfer should be considered as a step towards this goal.

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