A double embryo transfer on days 2 and 4 or 5 improves pregnancy outcome in patients with good embryos but repeated failures in IVF or ICSI

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Summary

The purpose of this paper was to evaluate the outcome of a double embryo transfer during the same cycle for patients who had had three or more implantation failures in IVF-ET or ICSI-ET programs after the transfer of good quality embryos in all attempts.

Forty-five women who had had previous unsuccessful attempts in IVF-ET or ICSI-ET programs after transfer of good quality embryos (Group A) were included in the study. Group A was divided into two subgroups, Group A1 consisted of 34 patients who underwent embryo transfer on day 2 and day 4 after pick-up and Group A2 consisted of ten patients who underwent embryo transfer on day 2 and day 5 after pick-up. Forty-two other women with a similar unsuccessful history in IVF-ET (Group B) were studied as controls. The patients in this group had a day 4 or 5 only transfer without having an additional day 2 transfer. The outcome of the procedure was compared in the two groups.

Double embryo transfer had beneficial effects on patients with good embryos but with previous failure attempts. These patients had a 38.2% clinical pregnancy rate and a 50% total pregnancy rate if the additional embryo transfer was done on day 4 and a 60% clinical and 60% total pregnancy rate if the additional embryo transfer was done on day 5.

Our data showed that excellent pregnancy rates can be obtained with a commercially available medium and double embryo transfers on days 2 and 4 or 5 after pick-up for patients with good quality embryos that have had previous failure attempts in an IVF-ET program. Due to the fact that endometrial maturation varies considerably in each patient, an adequate endometrial maturation and improved uterine receptivity seem to be the reason for improved pregnancy rates with double embryo transfers. It was also shown that morullae have high viability and high potential for implantation and pregnancy.

Key word: Double embryo transfer; IVF-ET; ICSI-ET; Pregnancy rate.

Introduction

Over the past years, a number of important advances have been introduced in assisted reproductive technologies, such as the use of gonadotropin releasing hormone (GnRH) agonists or antagonists or the use of recombinant gonadotropins for better manipulation of ovarian stimulation or the use of intracytoplasmic sperm injection (ICSI) to overcome fertilization problems. Still, the overall pregnancy rate has not increased [1]. An increased frequency of genetically abnormal embryos [2] or the improper synchronization of the transferred embryos with endometrial maturation [3-5] are two of the main considered problems that lead to a decreased implantation and pregnancy rate [1]. The incapability of culture media to sustain the proper development of human embryos in long-term cultures in vitro is an additional possible cause of a high rate of early embryo wastage [6, 7], a factor that can be more readily assessed compared to those previously mentioned.

Recently, blastocyst transfer has been used in selective groups of patients and research has focused on the development of more suitable culture media to promote in vitro human blastocyst development [6-8]. It is well known that culture conditions which do not support all stages of preimplantation development, irrespective of results of transfer trials with cleavage stage embryos, are intrinsically injurious to embryos, which are rescued only by early transfer to a more hospitable (in vivo) environment [9]. On the other hand, improved success rates following an 8-cell morulla or blastocyst stage transfer are conferred by the advantage of selecting developmentally competent embryos.

The routine day 2 transfer of three or four embryos has resulted in pregnancy rates of about 30-40%, which are comparable to those achieved by the transfer of blastocysts, in which case embryos will not be available for transfer in about 40% of the patients after five days of laboratory culture [10]. If morullae could be transferred with high success rates, the majority of these patients would have embryos transferred instead of canceling their cycle.

The purpose of this study was to evaluate the outcome of a double transfer during the same cycle for patients who had had three or more implantation failures in in vitro fertilization and embryo transfer (IVF-ET) or ICSI-

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ET programs after the transfer of good quality embryos in all attempts. For these patients, we performed a double embryo transfer, one on day 2 and one on day 4 or 5. The hypothesis tested was the failure of synchronization between the transferred embryos and the female reproductive tract in the previous attempts as the main cause of not achieving a pregnancy. Therefore, this study investigated whether the consequent extended culture conditions offer an advantage for the "implantation window", especially in patients with previous unsuccessful attempts and good quality embryos. In addition, the potential of morulla-stage embryos to be implanted with high success rates was investigated.

Material and Methods

Forty-five women that had had previous unsuccessful attempts in IVF-ET or ICSI-ET programs after transfer of good quality embryos (Group A) were included in the study. Group A was divided into two subgroups (A1 and A2). Group A1 consisted of 34 patients who underwent embryo transfer on day 2 and day 4 after pick-up and Group 2A consisted of ten patients who underwent embryo transfer on day 2 and day 5 after pick-up. One patient had no viable embryos on day 4 and she had only a day 2 transfer. The latter was excluded from the study. Forty-two other women with a similar unsuccessful history in IVF-ET (Group B) were also included in order to compare their results to those of the previous group. The patients in this group had a day 4 or 5 only transfer without having an additional day 2 transfer.

The extent of ovarian suppression was evaluated by an ultrasound scan and serum E_2 (< 40 pg/ml) before starting exogenous gonadotropin administration. On cycle day 21, a baseline ultrasound scan was performed, followed by pituitary down regulation with GnRH-α buserelin acetate (intranasal spray 100 μg x 5 daily). The stimulation protocol started with 100, 150 or 200 IU of recombinant FSH depending on the previous response of the patients to ovarian stimulation. The daily hormonal dose after the first four days of treatment was individualized according to the ovarian response (by E_2 level and ultrasound) and GnRH-α was continued until hCG (10,000 IU, IM) was administered. Oocyte retrieval and fertilization (Loutradis *et al.*, 1998a) or ICSI [12] were performed as previously described.

Embryos with fragmentations, impaired blastomeres or other obvious distortions were excluded. All the rest of the embryos that had more than one blastomere were considered for transfer. For each patient, a number of embryos were transferred on day 2 and the rest were cultured till day 4 or 5 in the same culture medium (modified Hams F10 without hypoxanthine) [13]. For the control group (Group B), all embryos were transferred on day 4 or 5 after culture in the same medium as above.

Differences in pregnancy rates and in the other clinical and biological parameters of the outcome of the patients in the groups studied were analyzed using the unpaired t-test.

Institutional review board approval was obtained.

Results

The mean age of the patients was 34.7 ± 5.0 for Group A1, 35.8 ± 4.6 for Group A2 and 36.2 ± 0.7 for Group B ($p > 0.5$, NS) (Table 1). They all had at least three previous failures in IVF-ET or ICSI-ET with good quality embryos. The hormonal profile, including day 3 FSH, day 3 LH, day 3 estradiol and prolactin was within normal range for all groups.

If we consider the total units of recFSH used as multiples of 100 IU ampoules, the total number of 100 IU ampoules used for ovarian stimulation was 19 ± 8 in Group A1, 22 ± 5 in Group 2 and 20 ± 4 in Group B ($p > 0.5$, NS). The duration of stimulation was 9.2 ± 0.2, 9.5 ± 1.2 and 10.0 ± 3 for the three groups, respectively ($p > 0.5$, NS). Serum estradiol on the day of hCG administration was 1,398 ± 762, 1,456 ± 567 and 1,422 ± 808 pg/ml for the three groups respectively ($p > 0.5$, NS) (Table 1).

Table 1. — *Age and hormonal profile of the study patients.*

	Group A1 (n = 34)	Group A2 (n = 10)	Group B (n = 42)
Age (yrs.)	34.7 ± 5.0*	35.8 ± 4.6	36.2 ± 0.7
Estradiol (pg/ml)	36.5 ± 10.5	52.6 ± 9.7	45.7 ± 9.9
PRL (ng/ml)	12.1 ± 4.3	13.6 ± 3.9	15.1 ± 5.5
FSH (mIU/ml)	5.6 ± 2.6	6.9 ± 1.8	6.6 ± 0.3
LH (mIU/ml)	6.7 ± 5.8	7.9 ± 1.2	8.7 ± 3.8
Serum estradiol on the day of hCG (pg/ml)	1,398 ± 762	1,456 ± 567	1,422 ± 808

Mean ± standard deviation for all values.
$p > 0.5$ (NS) for all values in the table.

The number of eggs collected in Groups A1 and A2 was 524 and the number of those that fertilized was 406 (77.4%). From these, 147 embryos were transferred in 44 patients on day 2, 182 were additionally transferred in 34 patients (Group A1) on day 4 and 65 were additionally transferred in ten patients (Group A2) on day 5. Twelve embryos did not divide any further and were not transferred. Thus, the mean number of embryos transferred per patient was 3.3 for day 2 transfers, 2.6 for day 4 and 2.5 for day 5 ($p > 0.5$, NS). The cell stage at the time of transfer is shown in Table 3. The vast majority of embryos transferred on days 4 and 5 were in the morulla stage (Table 2).

Table 2. — *Biological and clinical outcomes in the three groups.*

	Group A1	Group A2	Group B
Eggs collected	402	122	482
Fertilization rate (%)	78.5	76.5	75.0
No. embryos transferred on day 4/5	2.6 ± 0.6	2.5 ± 0.7	2.7 ± 0.5
Pregnancy rate (%)*	59	60	24
Clinical PR (%)*	38	60	19
Miscarriage rate (%)	24	0	13

*$p > 0.5$ between groups A1 and B and A2 and B.
$p > 0.5$ for all other values; unpaired t-test and z-test for proportions.
For miscarriage rate the numbers were too small for statistics.

The number of eggs collected in group B was 482 with a 75% fertilization rate (362 embryos). The mean number of embryos transferred on days 4 or 5 combined was 2.7 per patient (Table 2). The cell stage is shown in Table 3. Again, the vast majority of transferred embryos were in the morulla stage. These values are comparable to those observed for groups A1 and A2 after transfer on days 4 or 5.

Pregnancy rate (PR) was 59% with a 38% clinical PR for Group A1, 60% with the same clinical PR (no miscarriages) for Group A2 and 24% with 19% clinical PR for Group B ($p < 0.05$ for groups A1 and B as well as for groups A2 and B). The miscarriage rate was 24% for Group A1, 0% for Group A2 and 13% for Group B (samples too small for statistical analysis) (Table 3).

Table 3. — *Cell-stage of the embryos transferred in the three groups.*

	Group A day 2 ET (n = 147)	Group A1 day 4 ET (n = 182)	Group A2 day 5 ET (n = 65)	Group B day 4/5 ET (n = 350)
No. of cell embryos	5			
No. of 3-cell embryos	22			
No. of 4-cell embryos	76			
No. of 6-cell embryos	44			
No. of 8-cell embryos	18			
No. of morullae		152	45	252
No. of blastocysts		12	20	98

Discussion

The present study shows that double embryo transfer has beneficial effects on patients with good embryos but with previous failure attempts. Our data show that these patients had a 38.2% clinical pregnancy rate and a 50.0% total pregnancy rate if the additional embryo transfer was done on day 4 and a 60.0% clinical and 60.0% total pregnancy rate if the additional embryo transfer was done on day 5. Day 5 had a 0% miscarriage rate which is notable, although the sample was small. It seems, therefore, that by using this commercially prepared culture medium (Ham's F10 without hypoxanthine), the cleavage rate on days 4 and 5 is adequate to provide an appropriate number of good quality embryos and, by transferring the embryos to the patients at two different time points after pick-up, we increase her chances to receive them on the optimum day of embryo transfer.

The percentages of developed embryos in our study were compared to the results of embryos developed in other special culture systems. Although these investigators showed that 45% of blastocysts come from G1 or other medium, in our culture system the proportion of embryo development was 52% morullae and 1% blastocytes on day 4 and 31% morullae and 8% blastocytes on day 5. However, in this study we show that by transferring morulla-stage embryos on day 4 and day 5, the implantation rates were not significantly different from other studies in which they transferred blastocyst stage embryos [5].

The positive impact of transferring fully expanded blastocysts on the outcome of IVF-ET has been previously observed [14]. However, it remains to be seen whether fully expanded blastocysts grown in complex media such as Ham's F-10 without hypoxanthine are indeed developmentally superior to slower-developing blastocysts. Nevertheless, from this study it is clear that such developmentally advanced embryos are not required for establishing viable pregnancies, since, in our study, the vast majority of embryos transferred on day 4 (91%) and on day 5 (80%) were morullae. These data, confirmed by earlier observations [15, 16] show that morullae have a high viability and may lead to high implantation and pregnancy rates. Double transfer on day 2 and on days 4 or 5 provides an adequate number of embryos for transfer: 2.5 morulla-stage embryos per transfer. Furthermore, pregnancy rates were similar to those of previous reports with day 5 transfers [6].

When compared to the patients in our study that have had previous failure attempts and had only day 5 transfer, the patients with double ET had significantly higher pregnancy rates (43.2% clinical and 52.3% total for double ET compared to 19.0% clinical and 23.8% total for ET on day 5 only; $p < 0.05$). The findings of this study show that the patients that had only day 5 transfer could have increased pregnancy rates if they have had an additional embryo transfer on day 2. It seems, therefore, that the optimum time for best synchronization of embryo stage and endometrial maturity varies from one patient to the other and double transfers can achieve this implantation window for a large number of patients.

Because of the previous attempts, the quality of produced embryos was excellent in all cases. Most likely, the previous failures were due to the quality of the endometrium, which plays an important role in the outcome of the procedure. The fluctuating estradiol concentrations may have had adversely affected the endometrium when we transferred the embryos on day 2. This speculation agrees with fluctuating or not physiological concentrations of estradiol which have been shown to contribute to failure of implantation [17] and other physiological events of proliferating endometrium [18, 19]. "Non physiological" concentrations may vary from patient to patient. On the other hand, for two decades, routinely transferred day 2 embryos offered a pregnancy rate of approximately 30-40%. In our study, from the 44 embryo transfers, two patients produced embryos which did not develop past the 8-cell stage. The two patients had only day 2 transfer and one became pregnant. In our previous study, we found important variations in the timing of the implantation window among patients receiving an identical hormonal treatment. The presence of pinopodes as "markers" for the "nidation window" on the luminal epithelial surface of the human uterus did not exceed 48 hours. Fully developed pinopodes existed for one day only which may correspond to the short period of optimal endometrial receptivity. Furthermore, the timing of the presence of fully developed pinopodes varied from patient to patient [4]. However, it is obvious that the synchronization of uterine receptivity with the day of embryo transfer may be the "key" to success for patients with good quality embryos and previous failures on IVF-ET.

Concluding, our data showed that excellent pregnancy rates can be obtained with a commercially available medium and double embryo transfers on days 2 and 4 or 5 after pick-up for patients with good quality embryos that have had previous failure attempts in an IVF-ET

program. Due to the fact that endometrial maturation varies considerably in each patient, an adequate endometrial maturation and improved uterine receptivity seems to be the reason for improved pregnancy rates with double embryo transfers. We thus assume that differences in the implantation window from patient to patient may explain the increased pregnancy rates achieved. This way, the chance for transferring the embryos on the proper day is increased. This study, therefore, shows that the maturity of the endometrium is at least as important as the quality of the embryos for achieving a pregnancy in an IVF-ET program. Finally, it was also shown that morullae have a high viability and a high potential for implantation and pregnancy.

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