Interval double transfer improves treatment success in patients with repeated IVF/ET failures

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Abstract

Purpose To examine whether double transfer of embryos on day 2–3 and on day 5 after ovum pick-up (OPU) improves IVF/ET success rates in patients with multiple consecutive IVF failures

Methods Consecutive IVF failures patients (7.6 ± 0.5) undergoing IVF/ET cycles were treated with interval double transfer on day 2–3 and on day 5 after OPU. Matched patients, that had embryos transferred only once on day 2 or 3 served as controls.

Results Baseline and cycle characteristics were similar in the study group and controls. The total pregnancy rate was 44.6% in the study group and 24.2% in the controls ($p=0.001$), and the clinical pregnancy rates were 38.5% and 19.6%, respectively ($p=0.001$).

Conclusions Patients with multiple consecutive IVF/ET failures, treated with the interval double transfer approach had significantly improved cycle success rates compared with regular day 2 or 3 embryo transfer protocol.

Keywords Interval double-transfer · Repeated IVF/ET failures

Introduction

Both clinicians and patients are frustrated by multiple failures of in vitro fertilization (IVF) treatment cycles. Various treatment plans and a change of protocol have been suggested for “low responders” [1–6], but patients who fail treatment repeatedly despite good response and good quality embryos pose a special therapeutic challenge.

Published data on interval double transfer is limited. Earlier studies had demonstrated increased pregnancy rates following IVF/ET cycles with interval double transfer [7]. Loutradis et al. [8] demonstrated increased treatment success with an interval double transfer technique in women who had more than three failed treatment cycles with transfer of good embryos. The variability in the endometrial maturation process and increasing the receptivity “window” were cited by those authors as the main reasons for performing an interval double transfer cycle.

Recently Loutradis et al. [8] have reported an improvement of IVF/ET outcome in patient with repeated IVF/ET who underwent endometrial biopsy prior to the recent cycle. Their explanation for such improvement was that local injury to the endometrium inducing production of cytokines which enhance implantation.

Blastocyst transfer is an approach aiming to improve the IVF/ET in repeated IVF/ET failures. However, the results of this approach depend on the number of fertilized oocytes and the cleavage potential of the fertilized embryos. Recently Ledee-Bataille et al. [9] reported similar implantation rate of day 3 embryos versus day 5 embryos in prospective randomized study. Moreover, cancellation of

Capsule Patients with consecutive repeated IVF/ET failures, treated with the interval double transfer had significantly improved cycle success rates compared with regular day 3 embryo transfer.

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the entire treatment cycle can happen due to failure of the embryos to develop to blastocyst stage with unfavorable emotional and economic consequences. Thus, interval double transfer approach has the advantage of blastocyst transfer without exposing the all cycle to the risk of cancellation.

Increasing the likelihood of replacing embryos at the receptivity “window” of the endometrium by interval double transfer and possible local injury to the endometrium by double insertion of the catheter prompted us to use this method in special subgroup of patients. This subgroup of patients consisted women who had a minimum of three consecutive IVF/ET failures (average 7.6 ± 0.5), yet having good response to hormonal stimulation and at least five good embryos for transfer on day 2–3 after ovum pick-up.

Our goal was to further evaluate the efficacy of interval double transfer of a day 2–3 embryo followed by a 5 day blastocyst in this selected subgroup of patients.

Materials and methods

This is a retrospective report of IVF success rates in patients treated in our unit. Consecutive women who had a minimum of three previous IVF/ET failures (range 4–26, average 7.6 ± 0.5 , $n=65$) between April 2004 and May 2005 (Group 1) were included in our analysis. The controls were women after a minimum of three consecutive cycle failures (range 4–28, average 7.9 ± 0.1 , $n=66$) that were selected for analysis from our computerized database (Group 2) between March 2003 and January 2004. We only included patients who had undergone an adequate stimulation cycle with good response and with at least five good quality embryos for replacement in both groups. Women in the study group had also acted as their own controls during the preceding, unsuccessful treatment cycle. Patients treated for preimplantation genetic diagnosis were excluded from our analysis.

All participants were stimulated using a “short-flare up protocol”, the routine protocol in our unit and as follows: Down-regulation with subcutaneous triptorelin 100 $\mu\text{g/day}$ was carried out from cycle day 1. Ovarian stimulation with recombinant follicle-stimulating hormone (FSH) or purified urinary human menopausal gonadotrophin (hMG) started on cycle day 3. The dose of gonadotropins was individualized according to the patient’s age and previous stimulation history or response to stimulation. We have found similar success rates for the short-flare up protocol and the long protocol in our unit (Data to be published). This protocol is preferred in our unit for all patients due to easier monitoring, better patient compliance and reduced total dosage of gonadotrophins with similar results. Cycles were monitored by transvaginal ultrasonography and serum

estradiol and progesterone levels. Follicular maturation was completed by the administration of 10,000 IU hCG or 250 μg of recombinant hCG when at least two follicles reached a diameter of >17 mm. After embryo-transfer luteal support was carried out by using 600 mg/day of micronized progesterone.

Relevant data on the demographics and treatment history as well as the indications for IVF treatment were recorded. Current cycle data, such as the number of oocytes collected and the number of embryos replaced during both day 2–3 and 5 and their quality were recorded, as was the performance of IVF or ICSI. Embryo replacement took place on day 2–3 after ovum pick-up (48- to 72-h embryos) in all the participating subjects.

Only patients who had good response to stimulation protocol and had at least five good quality embryos being available for the interval double transfer were included in the analysis. Women in the control group had to fulfill the same criterion as an indication of a successful stimulation protocol and had at least five good quality embryos available for transfer on day 2–3. Good quality embryos were defined as four cells on day 2 or 8 or more cells on day 3, grade 1–2 defined as embryos with 0–20% of fragmentation. Between March 2003 and January 2004, our routine protocol for embryo transfer consisted of transfer of embryos only on day 2 or 3. All patients treated after March 2004 and fulfilling the inclusion criteria were treated with the interval double transfer protocol.

Positive pregnancy was defined as a β -hCG level >10 mIU/mL 12 days following embryo-transfer, and clinical pregnancy was identified by the appearance of a gestational sac approximately 4 weeks after embryo replacement. Data are reported using the mean $\pm$ standard deviation.

Statistics

The statistical analysis was carried out using the SPSS software package (SPSS Inc., Chicago, IL). Differences between parameters in the different patient groups were evaluated using the Fisher exact test and the *t* test where appropriate. Differences between proportions were evaluated using the chi-square test. In order to increase the statistical power of the study a large control group was chosen without excluding patients from our computerized database who fulfilled the inclusion criteria.

Results

A total of 65 women were treated by the double transfer technique (Group 1) and 66 patients with matching characteristics were selected from our computerized data-

Table 1 Demographic and previous treatment data of the study and control groups

	Age, y (mean±SD)	Previous cycles, <i>n</i> (mean±SD)	Indication for IVF			
			Male factor (%)	Tubal factor (%)	Unexplained infertility (%)	Others (%)
Study group (<i>n</i> =65)	34.3±0.7	7.6±0.5	24 (37)	18 (28)	17 (26)	6 (9)
Controls (<i>n</i> =66)	34.7±0.1	7.9±0.1	25 (37)	19(28)	20 (30)	2 (3)
<i>P</i> value	NS	NS	NS	NS	NS	NS

base to serve as controls. Data on their demographics and previous number of cycles as well as the indication for IVF treatment are given in Table 1. These data confirm that there was no significant age difference nor was the number of unsuccessful previous cycles or indications for IVF significantly different between the two groups. The women in both groups had experienced failed IVF treatment cycles repeatedly, with an average of >7 previous IVF/ET cycles.

There was no difference in the total number of embryos that were replaced being 3.7 ± 0.02 in the study group and 3.6 ± 0.02 in the control group ($p=0.16$) (Table 2). Moreover there was no difference in the average number of embryos currently replaced in the study group (3.7 ± 0.02) compared to the average number of replaced embryos (3.5 ± 0.07) in their preceding cycle ($p>0.1$). The average number of embryos replaced in the study group on day 2–3 was 2.6 ± 0.7 , and 1.2 ± 0.5 on day 5.

Pregnancy as defined above (β -hCG level >10 mIU/ml) was documented in 44.6% of the study group women and in 24.2% of the controls ($p=0.001$). Clinical pregnancy was documented in 38.5% and 19.6% in the study and control groups, respectively ($p=0.001$). Live birth rate was 29.2% and 15.1% in the study and control groups, respectively ($p=0.001$).

The rates of multiple gestations were not different significantly being 6.0% in the control group (4.5.0% twins, 1.5% triplets,) and 7.6% in the study group (6.1% twins, 1.5% triplets) ($p>0.05$).

Discussion

Clinicians in IVF units often encounter challenging cases of repeated IVF/ET failures. The enigma of why failure had occurred is sometimes exacerbated by the facts that the stimulation protocols are adequate, a good number and quality of oocytes and embryos are available for transfer and the endometrium is judged to be ready for implantation. Over the years, many strategies have been devised for “low responder” cycles. A change in the dosage or the addition of relatively new drugs have been suggested for such cases [1–3], and changes in laboratory techniques have been called for as well [5, 6]. Endometrial failure and implantation failure are usually blamed in cases where a successful stimulation and embryo-transfer cycle is followed by failure to achieve pregnancy. These two possibilities have been the subjects of a great deal of research [9, 10]. Inagaki et al. [11] have suggested that a delicate balance of cytokines and growth factors might bring about improved endometrial receptivity.

The timing of maximal endometrial receptivity after ovum pick-up has not been studied in depth. This “window of opportunity” may span just a few hours or days after pick-up. Faced with many patients after multiple treatment cycles, diagnosed as “implantation failure”, we hypothesized that there will be a better chance of aiming at this time frame and “window of opportunity” by implementing the interval double transfer technique. Our current results

Table 2 Treatment and outcome data of the study and control groups

	Study group <i>n</i> =65 (mean±SD)	Controls <i>n</i> =66 (mean±SD)	<i>P</i>
Number of oocyte retrieved	14.2±4.9	15.1±4.3	NS
Percent of good quality embryos on day 2 or 3 ^a	87.1	85.9	NS
Embryos transferred (<i>n</i>)	3.7±0.02	3.6±0.02	NS
β -hCG positive rate (%)	29 (44.6%)	16 (24.2%)	0.001
Clinical pregnancy (%)	25 (38.5%)	13(19.6%)	0.001
Rate of multiple pregnancy (%)	7.6	6.0	NS

NS Not statistically significant

^a Good quality embryos were defined as four cells on day 2 or 8 or more cells on day 3, grade 1–2

clearly show that this approach improves success in IVF/ET treatment cycles. Both groups of patients have comparable ages and similar characteristics of the stimulation cycle. Moreover, there was no significant difference in the number of embryos replaced nor was there a difference in the numbers of previous cycle failures.

The findings of a pregnancy rate of 44.6% and a clinical pregnancy rate of 38.5% in our study group are significant for various reasons. Treatment cycle rates among patients who have had three or more consecutive failures have been much lower according to reports in the literature [12, 13]. An increase in the number of cycle failures has been demonstrated to further increase failure rates [12]. The pregnancy rate in our unit in the seventh treatment cycle is 19.6%, declining to 15.1% at the eighth cycle. As such, the results of the current study group (i.e., women with an average of 7.6 previous failures) of a 44.6% pregnancy rate seem very promising.

Our findings are in agreement to those of Loutradis et al. [8] who report a 50% pregnancy rate and a 38% clinical pregnancy rate, but higher than the pregnancy rate (25%) reported by Abramovici et al. [7]. Loutardis et al. found that if the second transfer was performed on day 5 as opposed to day 4, there was additional success in outcome (i.e., a 60% pregnancy rate). The success rates of our study group are comparable to those achieved in what is considered to be the 'optimal patient' [14, 15], and considerably remote from the rates of success previously reported for patients with failed multiple cycles (i.e. 10–20%) [12]. Machtinger et al. reported similar results for sequential transfer cycles in a similar group of patients. In their study, pregnancy rates of 30.3% and 17.1% were reported for double transfer vs. day 3 transfer cycles [16].

Additionally, we used our study group as their own controls during the cycle prior to the study cycle. The increase in IVF success after the interval double transfer technique cannot be attributed to an increase in the number of embryos replaced. Our data show that women in the study group had the same total number of embryos replaced in their previous cycle.

We hypothesize that the reinserting of the catheter may affect the endometrial cavity by inducing factors, which may enhance implantation. Indeed recently, Barash et al. [17] have reported that the application of pipelle may enhance implantation rate in patients with repeated IVF/ET failures. In the above cited study however, endometrial samples were taken on days 8, 12, 21 and 26 of the cycle preceding IVF–ET cycle whereas in our work catheter placement was carried out during ET. Similar mechanism might explain the enhanced implantation rate.

Furthermore, one may raise the question the blastocysts per se are responsible for the significant improvement in implantation rate. This issue can not be ruled out or proved by our current study. Current data on higher implantation

rate of blastocysts is controversial. Recently Bungum et al. [18], reported similar implantation rate of day 3 embryos versus day 5 embryos in prospective randomized study. However, others have found a significantly higher implantation rate for embryos transferred at the blastocyst stage than that for embryos transferred on day 3 [19, 20]. Cancellation of the entire treatment cycle can happen due to failure of the embryos to develop to blastocyst stage with unfavorable emotional and economic consequences. Thus, interval double transfer approach has the advantage of blastocyst transfer without exposing the all cycle to the risk of cancellation.

However the fact is that the double transfer approach enhances implantation is not doubtful according to our findings. The accurate mechanism for these findings needs to be further investigated. It is important to note that this technique is suitable for patients having an adequate amount of good quality embryos to be replaced on both days of transfer and thus not suitable for poor responders.

Conclusion

In conclusion, our findings show that patients after multiple cycle failures treated with the interval double transfer technique had significantly improved cycle success rates compared with the regular day 2 or 3 embryo replacement protocol. This protocol can be implemented without the need to increase the number of embryos to be replaced and without the risk of high order pregnancies in this special group of patients.

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