Blastocyst stage transfer vs cleavage stage embryo transfer

ABSTRACT

OBJECTIVE: To evaluate the efficacy of blastocyst transfer in comparison with cleavage stage embryo in a similar cohort of women. **DESIGN:** Retrospective analysis. **SETTING:** University teaching hospital. **MATERIALS AND METHODS:** Women aged 35 or less undergoing *in vitro* fertilization/intracytoplasmic sperm injection between January 2005 and December 2006 were included in the study. When four or more grade 1 embryos were observed on day 3, extended culture till day 5 was undertaken. This policy was compared with a cohort of women who had at least three grade 1 embryos on day 3 and who had undergone a cleavage stage embryo transfer during the time period of January 2002–December 2004. Primary outcome evaluated was implantation rate and clinical pregnancy rate. **RESULTS:** Group 1 consisted of 50 women who underwent extended culture and blastocyst transfer. Group 2 comprised of 85 women who had cleavage transfer. The implantation rate for embryos transferred in group 1 was significantly higher than that for embryos transferred on day 3 (40.16% vs 11.43%). The clinical pregnancy rate was also significantly better with blastocyst transfer as compared with cleavage stage transfer (62% vs 29.76%). Significantly fewer embryos were required for transfer at the blastocyst stage compared with day 3 transfer (2.54 vs 3.45). **CONCLUSION:** In selected cases, blastocyst transfer with fewer embryos can be performed with high implantation and clinical pregnancy rates. This policy could lead to a reduction in the incidence of higher-order pregnancies.

KEY WORDS: *In vitro* fertilization/intracytoplasmic sperm injection, blastocyst stage embryo, cleavage stage embryo, pregnancy rates

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INTRODUCTION

Advances in the dynamics of embryo culture allow us to culture embryos to the blastocyst stage. Prolonging the duration of culture to day 5 allows chromosomally competent embryos to develop to the blastocyst stage and permits selection of embryos that have the potential for continued development under embryonic genomic control.[1] In addition, selection of day 5 embryos has the advantage of physiological synchronization with the uterine endometrium, thereby, perhaps, resulting in better pregnancy rates.[2]

The introduction of sequential culture media that takes into account the changing metabolic requirement of the embryo, as it develops from the zygote to the blastocyst stage, allows extended culture.[3,4]

Blastocyst transfer should enable transfer of fewer but higher-quality embryos resulting in increased implantation rates. This would maintain a high pregnancy rate while controlling the incidence of higher-order pregnancy.[5-7]

Although blastocyst transfer has been shown to be beneficial in good prognosis patients, similar benefits were not seen in an unselected group.[8] The aim of our study was to evaluate the efficacy of blastocyst transfer in comparison with day 3 embryo transfer.

MATERIALS AND METHODS

Women aged 35 years or less undergoing an *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycle between January 2002 and December 2006 were included in the study.

Cycle regulation was carried out in the cycle before the scheduled IVF with the oral contraceptive pill. Leuprolide acetate at a dose of 0.5 mg subcutaneously daily was used to achieve down regulation and continued till day of human chorionic gonadotrophin (hCG) administration.

Recombinant follicle stimulation hormone (FSHr) was started after pituitary down regulation was confirmed with dose being adjusted for age. (Women aged 30 or less received 150 international

units [IU] FSHr, between 30 and 35 received 225 IU and above 35 received 300 IU.) This dose schedule was modified according to parameters like body mass index, previous response and estimates of ovarian reserve. Follicular monitoring was initiated from the 6th day of stimulation. Women were scheduled for oocyte retrieval once at least three follicles reached a size of 18 mm or more. Transvaginal oocyte retrieval was planned 35 h after an injection of hCG 5000 IU was given. Oocyte retrieval was carried out under conscious sedation using intravenous pethidine, midazolam and fentanyl in titrated doses.

The retrieved oocytes were incubated for 3–4 h in a fertilization medium and then depending on the situation (indication, number of oocytes, previous IVF performance) a decision for IVF or ICSI was made. Short incubation insemination (2 h) and group culture was followed for IVF.

Denudation of the oocytes was carried out (both mechanical and enzymatic) before ICSI was performed. The oocytes were incubated overnight in a miniincubator with triple gas mixture and observed after 16–18 h pos insemination/ injection for fertilization. The fertilized oocytes were transferred into a cleavage medium (SAGE cleavage medium), incubated and observed for cleavage on day 3.

When four or more grade 1 embryos were observed on day 3, extended culture (SAGE blastocyst medium) till day 5 was undertaken. This policy was carried out between the years of January 2005 and December 2006 (study period of 2 years). This cohort was included in group 1, with a sample size of $n = 50$.

The number of blastocysts transferred was determined by availability of embryos, the patient age and the patient's previous clinical history. Not more than three blastocysts were transferred on any occasion. All embryo transfers were performed using the Sydney IVF catheter (k-jets-7019-SIVF; Cook IVF, Eight Miles Plains, Queensland, Australia) or Edward-Wallace catheter (Smiths Medical, Hythe, Kent, U.K).

Luteal support was given in the form of micronised vaginal progesterone pessaries in a dose of 400 mg twice daily for 18 days postoocyte retrieval. In addition, 100 mg IM progesterone was administered twice weekly.

Serum beta-hCG was performed on the 18th day following oocyte retrieval and, if positive, a transvaginal ultrasound was performed 10 days later to detect and confirm intrauterine pregnancy.

To evaluate its efficacy, this policy was compared with a cohort of women (group 2, $n = 85$) who had at least three grade 1 embryos on day 3 and who had undergone a cleavage stage transfer during the time period of January 2002–December 2004 (study period of 3 years).

Outcome measures

Primary outcome was implantation rate and clinical pregnancy rate per oocyte retrieval.

The secondary outcomes included:

1. Fertilization rate
2. Cleavage rate
3. Multiple pregnancy rates
4. Mean number of embryo transferred

Implantation rate was defined as number of gestational sacs determined by ultrasound by number of embryos transferred.

Clinical pregnancy rate was defined as presence of a gestational sac with a fetal pole with cardiac activity on transvaginal ultrasound at 6 weeks.

Fertilization rate was defined as total number of fertilized oocytes by total number of mature oocytes retrieved.

Cleavage rate was defined as total number of day-3 embryos by total number of fertilized oocytes.

Statistical analysis

Data were analyzed with SPSS software (SPSS Inc., Chicago, IL, USA) subjected to analysis of variable (ANOVA), χ^2 and paired sample *t*-test with significance ($P < 0.5$, < 0.01 and < 0.001).

RESULTS

Women in whom blastocyst transfer was carried out were included in group 1 while group 2 consisted of women who underwent day 3 cleavage stage transfer.

There was no significant difference in mean age between the two groups [Table 1].

Women in group 1 had a significantly higher oocyte yield compared with women in group 2. The fertilization rates and cleavage rates were similar in both the groups. The mean number of embryos transferred was significantly lower in group 1.

The clinical pregnancy rate in group 1 was significantly higher than group 2 ($n = 31/50$ [62%] vs $n = 25/85$ [29.76%]).

The implantation rate in group 1 was also significantly higher than group 2 ($n = 49/122$ [40.16%] vs $n = 35/306$ [11.43%]).

Table 1: Comparison of blastocyst group and cleavage group (patient profile, laboratory parameters and clinical outcome)

Parameters	Group 1 (n = 50), mean (SD)	Group 2 (n = 85), mean (SD)	*P*-value
Age (years)	31.08 (4.46)	31.24 (3.53)	NS
M-II oocyte (metaphase II)	14.96 (6.38)	12.63 (4.39)	0.03 S
Fertilization rate	491/748 (65.64%)	713/1059 (67.32%)	0.103 NS
Cleavage rate	469/491 (95.51%)	689/713 (96.63%)	0.583 NS
No. of embryo transferred	2.54 (0.54)	3.45 (1.09)	0.000 S
Clinical pregnancy rate*	62% (31/50)	29.76% (25/85)	0.000 S
Implantation rate*	40.16% (49/122)	11.43% (35/306)	0.000 S

*Value in percentages, S = Significant, NS = Non significant

Table 2: Comparison of multiple pregnancy rates between group 1 and group 2

Parameters	Group 1 (n = 50)	Group 2 (n = 85)	*P*-value
Singleton	18	19	NS
Twins	11	5	NS
Triplets	2	1	NS

There was no significant difference between the two groups in relation to singletons, twins or triplets [Table 2].

DISCUSSION

Human blastocysts developed *in vitro* have been reported to achieve high implantation rates prompting us to evaluate the efficacy of blastocyst transfer.[9] We followed a strategy proposed by Racowsky *et al.* allowing extended culture only when four or more grade 1 embryos had developed on day 3, thereby reducing the risk of not having embryos available for transfer on day 5.[6]

The results of this study conducted over a 2-year period were compared with the results of the preceding 2 years. Because extended culture was practiced only if an adequate number of good quality day 3 embryos were available, a similar cohort was selected from the preceding 2 years, namely women with at least three grade 1 embryos on day 3. This ensured proper matching and similarity in comparison.

Although the number of oocytes retrieved was significantly more in the blastocyst transfer group, there were no differences in the age, fertilization rates or cleavage rates between the two groups. There was a significantly higher implantation and clinical pregnancy rate in the blastocyst transfer group [40.16% vs 11.43%, $P = 0.00$; 62% vs 29.8%, $P = 0.00$).

In view of an anticipated improvement of implantation rate in the blastocyst transfer group, we limited the embryo transfer numbers to three or less. This is reflected in the significant difference between the two groups in terms of number of embryos transferred. (group 1 = 2.45 vs group 2 = 3.45, $P = 0.00$.) Nonetheless, there was no significant difference between the two groups in terms of higher order pregnancies, which is a reflection of the higher implantation rate in the blastocyst group. The results of this study will give us the confidence to limit transfer to two or lesser blastocysts.

Reasons for higher success rates with blastocyst are mainly related to an embryo selection process. Embryos selected for transfer on day 5 are healthier and carry a lower risk of being aneuploid, thereby increasing a patient's chances of achieving an ongoing pregnancy.[10]

Studies support the idea that blastocyst transfer with its higher implantation and pregnancy rates permits a single/double embryo transfer policy, thereby resulting in a reduction of multiple pregnancies.[11,12]

Because the study groups were over different periods of time, the influence of changing practices cannot be eliminated. We were unable to incorporate the results of frozen embryo transfers in the two groups as the processes of freezing were dissimilar (slow freeze for the day 3 transfers vs vitrification for the blastocysts). Hence, cumulative pregnancy rates could not be evaluated. In spite of its pitfalls, this study demonstrates the benefits of blastocyst transfer. A randomized controlled trial will provide robust data and eliminate bias and data from this trial will help in calculation of sample size.

Our study indicates that in younger patients (less than 35 years), a threshold of four good quality embryos on day 3 is an indication for extended culture to day 5.

When assisted reproductive technology (ART) treatment is self-financed, couples are often unable to afford more than a single cycle of treatment. This prompts the care giver to consider transferring more than two to three embryos. To avoid neonatal morbidity and mortality associated with higher order pregnancies, fetal reduction is then resorted to.

In younger women with a good response and with four or more grade 1 embryos on day 5, extended culture can be offered. The good clinical pregnancy and implantation rates

observed will confidently allow transfer of not more than two good quality blastocysts and allow women to enjoy the benefits of limiting numbers for transfer.

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