

A comparative study between cleavage stage embryo transfer at day 3 and blastocyst stage transfer at day 5 in *in-vitro* fertilization/intra-cytoplasmic sperm injection on clinical pregnancy rates

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ABSTRACT

OBJECTIVE: To evaluate the efficacy of blastocyst transfer in comparison with cleavage stage transfer. **STUDY DESIGN:** A randomized, prospective study was conducted in Infertility clinic, Department of Obstetrics and Gynecology, Mahatma Gandhi Hospital, Jaipur on 300 patients aged 25-40 years undergoing *in-vitro* fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI) cycle from May 2010-April 2011. When three or more Grade-I embryos were observed on day 2 of culture, patients were divided randomly into two study groups, cleavage stage transfer and blastocyst transfer group having 150 patients each. Primary outcomes evaluated were, Clinical pregnancy rate and Implantation rate. The results were analyzed using proportions, standard deviation and Chi-square test. **RESULTS:** Both the groups were similar for age, indication and number of embryos transferred. Clinical pregnancies after blastocyst transfer were significantly higher 66 (44.0%) compared to cleavage stage embryo transfer 44 (29.33%) ($P < 0.01$). Implantation rate for blastocyst transfer group was also significantly higher ($P < 0.001$). **CONCLUSION:** Blastocyst transfer having higher implantation rate and clinical pregnancy rate lead to reduction in multiple pregnancies.

KEY WORDS: Blastocyst transfer, clinical pregnancy rates, embryo transfer, intra-cytoplasmic sperm injection, *in-vitro* fertilization

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 resulting in better implantation rates.^[2] The introduction of sequential 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 multiple pregnancy rates.^[5] Reasons for higher success rates with blastocysts are mainly related to embryo selection process. Embryos selected for transfer on day 5 are healthier and carry a lower risk of being aneuploidy, thereby increasing patients' chance of achieving an on-going pregnancy.^[6]

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

MATERIALS AND METHODS

Three hundred patients aged 25-40 years undergoing *in-vitro* fertilization (IVF)/

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intra-cytoplasmic sperm injection (ICSI) cycle between May 2010 and April 2011 were included in our study, meeting the inclusion criteria set namely, 2-20 years of infertility, having minimum five oocytes at oocyte pick up and endometrial thickness of 7 mm and more indicating good ovarian response, having normal uterine cavity and basal follicle stimulating hormone level (FSH < 10 mIU/ml). Complete patient work-up, baseline routine investigations, and hormonal analysis were done, and postmenstrual diagnostic hysteroscopy was done. Patient was put on long protocol, gonadotropin-releasing hormone agonists started on cycle day 21 daily doses given subcutaneously till cycle day 3. Hormonal evaluation—serum FSH, luteinizing hormone, estradiol (E2) and transvaginal sonography was done on day 3 to confirm down-regulation. Induction with recombinant FSH (rFSH) was started once pituitary down-regulation was confirmed. The dose schedule was modified according to parameters such as body mass index, previous response, and ovarian reserve estimates and was given for 5 days (days 3-7). Follicular monitoring was initiated on day 8 of the cycle, and further dose of rFSH was given according to follicle size and continued till day 11. Patients were scheduled for oocyte retrieval once at least 3 follicles reached 18 mm size and injection human chorionic gonadotropin (hCG) 10,000 IU was given. Transvaginal sonography guided oocyte retrieval was then planned 36 h after hCG, which was performed under short general anesthesia.

The retrieved oocytes were then incubated for 3-4 h in IVF-30 media and then depending on maturity of oocytes and previous IVF performance, IVF or ICSI was performed. Short incubation insemination for 2 h and group culture was followed for IVF.

Denudation of oocytes was carried out before ICSI was performed. Oocytes were incubated overnight in IVF-30 media in a carbon dioxide incubator and observed after 16-18 h postinsemination for fertilization. The fertilized oocytes were then transferred into a cleavage medium and incubated. Embryos were observed on day 2 and transfer was scheduled according to random allocation of patients into two groups based on availability of minimum three good quality embryos:

Group 1: Included patients undergoing embryo transfer on day 3, and Group 2 in which extended culture till day 5 was done in G2 plus media and blastocyst was transferred on day 5.

Random allocation of patients was done equally so that study population in both groups was comparable. The number of blastocysts/embryos transferred was determined by the availability of embryos, patients' age and previous

clinical history. Not more than three embryos/blastocysts were transferred on any occasion. All transfers were performed using Edward–Wallace catheter. Luteal support was given in the form of micronized vaginal progesterone in dose of 200 mg thrice a day for 18 days postretrieval. In addition, injection hCG 2000 IU was given intramuscular on days 5th, 8th, and 11th after retrieval. Serum beta-hCG was performed on day 15 following embryo transfer, and if positive then transvaginal sonography was performed 15 days later to detect and confirm intra-uterine pregnancy. Positive cases were followed till 6 weeks to check for fetal cardiac activity.

Outcome measures

Primary outcome

- Implantation rate
- Clinical pregnancy rate.

Secondary outcome

- Fertilization rate
- Cleavage rate
- Multiple pregnancy rates
- Mean number of embryos/blastocysts transferred.

Implantation rate

The implantation rate is usually defined as the percentage of embryos transferred that implants and develops to the stage of ultrasound documented fetal heartbeat.

Clinical pregnancy

defined as the presence of the gestational sac with a fetal pole with cardiac activity on transvaginal ultrasound at 6 weeks.

Fertilization rate

It is the percentage of eggs fertilized out of eggs inseminated or injected.

Cleavage rate

total number of day 3 embryos by total number of fertilized oocytes.

Study analysis

The results were analyzed using proportions, standard deviation, and Chi-square test.

RESULTS

A total of 300 patients was randomized for the study and allocated into two groups each of 150 patients. As shown in Table 1, no significant difference was found for age, duration of infertility, type of infertility, ratio of ICSI: IVF cases, and mean E2 level at hCG injection. Table 2 shows that no significant difference between both the study groups in terms of indication for IVF.

As shown in Table 3, both the groups had a comparable mean number of oocytes at the retrieval, same proportion of mature oocytes and fertilized oocytes, and a comparable mean number of embryos per transfer.

Table 4 shows higher clinical pregnancies per oocyte retrieval was observed in blastocyst transfer group than in day 3 embryo transfer group (44% and 29.33%) ($P < 0.01$),

Table 1: Demographic profile

Variable	Day 3 transfer (n=150)	Day 5 transfer (n=150)
Age (years, mean±SD)	32.46±4.3	32.04±4.4
Range	25-40	25-40
Duration of infertility (years)	8.9±5.2	7.7±4.7
Type of infertility		
Primary	101	100
Secondary	49	50
Number of cases with ICSI	46	55
Number of cases with IVF	104	95
Estradiol at hCG injection (pg/ml, mean±SD)	1158.3±890.2	1327.52±917.62

No statistically significant difference was demonstrated between the two groups. SD=Standard deviation, ICSI=Intra-cytoplasmic sperm injection, IVF=In-vitro fertilization, hCG=Human chorionic gonadotropin

Table 2: Clinical profile of patients

Infertility indication (n)	Day 3 transfer (n=150)	Day 5 transfer (n=150)
Tubal	53	42
Endometriosis	10	9
Anovulation	15	21
Male	42	44
Mixed	15	19
Unexplained	15	15

No statistically significant difference was demonstrated between the two groups

Table 3: Results after oocyte aspiration with respect to the number of oocytes and embryos

Variable	Day 3 transfer (n=150)	Day 5 transfer (n=150)
Oocytes at OR (n)	1094	1141
Mature oocytes (n)	922	1007
Fertilized oocytes (n)	599	658
Total day 3 embryos (n)	447	549
Embryos transferred (n)	309	290
Number of oocytes at OR (mean±SD)	7.3±2.1	7.6±2.3
Number of mature oocytes (mean±SD)	6.1±1.6	6.6±2.1
Number of two-pronucleate embryos (mean±SD)	3.9±1.7	4.3±1.5
Number of embryos per transfer (mean±SD)	2.04±0.74	1.93±0.48
Fertilization rate (%)	64.96	65.34 ($P > 0.05$) NS
Cleavage rate (%)*	74.62	83.43 ($P < 0.001$) HS

No statistically significant difference was demonstrated between the two groups. *Cleavage rate was significantly higher in blastocyst transfer. NS=Not significant, HS=Highly significant, SD=Standard deviation, OR=Odds ratio

and higher implantation rate per embryo transfer in blastocyst transfer group (35.17%) than in day 3 embryo transfer group (21.35%) ($P < 0.01$).

Table 5 shows that although multiple pregnancy rate in blastocyst transfer group were higher than day 3 embryo transfer group, association was not significant (48.48% and 31.81%) ($P > 0.05$). Pregnancy rate in blastocyst transfer group was significantly higher than day 3 embryo transfer group (44% and 29.33%). Day 3 embryo transfer group reported 2% of pregnancies as missed abortions and 1% as ectopic while day 5 blastocyst transfer group had no ectopic pregnancy and 1.33% pregnancies ended up as missed abortions.

Hence, women in blastocyst transfer group had significantly higher clinical pregnancy rates, implantation rate, and cleavage rates.

DISCUSSION

Blastocyst transfer is gaining popularity, now-a-days due to its higher clinical pregnancy rates and implantation rates. In this study, no significant difference was found between

Table 4: Results after oocyte aspiration with respect to outcome

Variable	Day 3 transfer (n=150)	Day 5 transfer (n=150)	χ^2	df	P
Positive beta-hCG	48	68			
Percentage of per OR	32	45.33	5.6	1	<0.01 (S)
Clinical pregnancies (n)	44	66			
Clinical pregnancy rate (%)	29.33	44	6.3	1	<0.01 (S)
Embryos transferred (n)	309	290			
Total gestational sacs on USG (n)	66	102			
Implantation rate	21.35	35.17	14.12	1	<0.001 (HS)
Total multiple pregnancies	14	32			
Multiple pregnancy rates	31.81	48.48	2.36	1	>0.05 (NS)

S=Significant, HS=Highly significant, NS=Not significant, hCG=Human chorionic gonadotropin, OR=Odds ratio, USG=Ultrasound

Table 5: Pregnancy outcome

Variable	Day 3 transfer (n=150) (%)	Day 5 transfer (n=150) (%)
Positive beta-hCG	48	68
Clinical pregnancies	44 (29.33)	66 (44)
Missed	3 (2)	2 (1.33)
Ectopic	1 (0.66)	0
Single	30	34
Twin	10	30
Triplets	4	2

$\chi^2=5.20$, df=1, $P < 0.01$ (significant). hCG=Human chorionic gonadotropin

both the study groups in terms of age, duration of infertility, indication of infertility, and type of infertility. This was in agreement with the study conducted by Van der Auwera *et al.*^[7] The mean number of embryos transferred in both groups showed no significant difference (2.04 and 1.93, $P > 0.05$).

In our study, the proportion of clinical pregnancies occurring as a result of blastocyst transfer was higher 66 (44%) as compared to cleavage stage embryo transfer which was 45 (30%), the association was statistically significant and similar results were seen in the study by Van der Auwera *et al.*^[7] and Mangalraj *et al.*^[8] Although the fertilization rate was higher with blastocyst transfer (65.34%) compared to day 3 embryo transfer (64.96%) in our study, but association was not significant ($P < 0.05$) and same association was observed in the study of Mangalraj *et al.*^[8]

Among both the study groups, cleavage rate (74.62% and 83.43%) and implantation rate (21.35% and 35.17%) were significantly higher in blastocyst transfer/group 2 over day 3 embryo transfer/group 1. The results of our study were similar to the study by Van der Auwera *et al.*^[7] and Mangalraj *et al.*^[8] Although the proportion of multiple pregnancies in our study was higher with blastocyst transfer (48.48%) than with embryo transfer (31.81%), but the association was not significant ($P > 0.05$).

Thus, in younger women with good ovarian response and three or more grade-I embryos on day 3, 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 benefits of limiting numbers for transfer. Hence, by transferring fewer embryos at blastocyst stage in selected group of good prognostic patients, physicians are practicing preventive medicine as there are fewer multiple gestations and ultimately greater pregnancy success is achieved.

CONCLUSION

This study has shown that in younger patients with good ovarian response extended culture to day 5 can be offered as blastocyst transfer is found to have good clinical pregnancy rates. 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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