**Original article**

**Embryo Transfer – Cleavage Stage Vs Blastocyst Stage – A Comparative Study**

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**ARTICLE INFO**

**ABSTRACT**

Background: In vitro fertilisation (IVF) is a complicated procedure, whose success depends on several factors. Besides the status of the embryo and endometrium, embryo transfer (ET) plays a key role. However, there are few prospective comparative studies in India, comparing day 3 versus day 5 embryo transfer. Aims: To compare implantation and pregnancy potential of day 3 versus day 5 embryo transfer in women undergoing an in-vitro fertilization or Intracytoplasmic sperm injection (ICSI). Settings and Design: Prospective comparative study. Material and methods: Study included 270 women from January 2014 to December 2016. After retrieval of oocytes and their fertilization, patients were randomly assigned to undergo embryo transfer either on day 3 (Group A) or day 5 (Group B). Demographic data, clinical profile, implantation and pregnancy rates were compared between the two groups. Statistical Analysis: Comparisons between groups were made by using Fisher’s exact test or chi-square analysis. P ≤ 0.05 was considered as significant. Results: The distribution of patients were similar between the two groups (n=135 and 135 respectively) and there was no statistically significant difference in terms of duration of infertility (p=0.208), number of oocytes retrieved (p=0.136), number of oocytes fertilized (p=0.619), and number of embryos transferred (p=1). Both the groups had statistically similar implantation rates, clinical pregnancy rates, live birth and perinatal death rates (p=0.33, p=0.272, p=0.532, and p=0.701, respectively). Conclusion: Present study demonstrates that blastocyst stage transfers have no advantages over cleavage stage transfers. Both had similar implantation rates, pregnancy rates, live births and perinatal death rates.

**Introduction**

Inspite of several advances in in-vitro fertilisation (IVF), implantation rates (IR) remain low and only a small percentage of patients achieve pregnancy. There are various components that effect the results. The first pregnancy confirmed at the time of assisted conception involved the transfer of a blastocyst. However, due to problems faced in culturing human embryos for a period of 5 days, it became a common practice to transfer embryos at cleavage stages, i.e. day 2 or 3, despite relatively low implantation rates of 10–20%[1]. In contrast, few studies have reported unusually high implantation rates after blastocyst culture and embryo transfer [2]. Prolonged culture of human embryos has been advocated to extend the efficiency of IVF treatments by improving the choice of embryos with the utmost implantation potential [2]. Due to current progress in the choice of culture media used for IVF/intracytoplasmic sperm injection (ICSI) many clinics have been encouraged to postpone embryo transfer to day 5 [3,4]. It has been suggested that transferring embryos at the blastocyst stage rather than selection at an earlier stage without knowing their developmental capacity, might enhance the implantation rate by a better embryo selection, thereby reducing the necessity to transfer more embryos [5,6,7] . The most competent embryos reaching the blastocyst stage are selected for transfer and therefore the embryos that have arrested their development are identified and aren’t transferred. It is thought that the expression of genes of human embryos is switched on around the 8-cell stage immediately before compaction [8]. Therefore, nutrient requirements of embryos are more complex media after they reach the 8-cell stage. The present study was done to determine whether transferring blastocyst-stage embryos would result in higher implantation and pregnancy rates than transferring cleavage-stage embryos.

**Aims and Objectives**

To compare implantation and pregnancy rates of cleavage stage vs blastocyst stage embryo transfer in women undergoing an in-vitro fertilization or ICSI.
Material and Methods

This was a prospective study done from 1st January 2014 to 31st December 2016 at Krishna Medical Centre, Lucknow. Ethical approval was taken before initiation of this study. All procedures carried out in this study were in agreement with the ethical standards of the Institutional and National Research Committee. All patients undergoing oocyte retrieval were informed about the study and were enrolled into the study after they signed an informed consent if they wished to participate in the study.

Inclusion criteria

The subject inclusion criteria were as follows:

- Patient's age < 40 years
- Patients from whom at least four embryos were formed on day 1 of fertilization.
- Patients who gave written informed consent to participate in this study and were ready for follow-up.

Exclusion criteria:

- Patient's age > 40 years.
- Patients from whom less than four embryos were formed on day 1 of fertilization.
- Patient who developed Ovarian Hyperstimulation Syndrome (OHSS).
- Patients who declined to play a part in this study.
- Patients who refused for follow up.

Patient selection and randomization

During the period from January 2014 to December 2016, a total of 300 patients undergoing standard IVF or ICSI were analysed for the study. Eligible women were told about the details of the study. After the exclusion of 18 patients who refused to participate in the study, the remaining 282 patients gave written informed consent and were then equally divided using a random list generated from computer, into either the Cleavage stage (day 3 embryo) which was assigned as Group A or Blastocyst stage (day 5 embryo) which was assigned as Group B (n= 141, n=141, respectively). Once a patient was randomized, she remained within the same group throughout the study. 6 patients were discontinued from the study due to loss to follow up, 4 patients were discontinued due to developmental arrest at blastocyst stage, and 2 patients who developed OHSS were also excluded. Therefore, 135 patients in Group A and 135 patients in Group B were included in the final analysis. Collected data were assessed using the SPSS version 21.0. Comparisons between groups were made by using Fisher’s exact test or chi-square analysis. P ≤ 0.05 was considered as significant.

Controlled ovarian hyperstimulation and oocyte retrieval

For IVF cycle stimulation, the gonadotropin at a daily dose of 375 IU was started after keeping track of patient’s follicles sizes on cycle-day 2/3. The dose of gonadotropin differed according to the follicular response. Gonadotropins were started as usual and the antagonist was started when the follicle reached a size of 14 mm or from the 6th day of stimulation onwards at a dose of 0.25 mg/day till the day HCG injection had to be given. There was routine monitoring via trans-vaginal sonography (TVS) and hormonal profiling of FSH, LH, oestrogen and progesterone levels of patients. When at least four follicles had reached a diameter of 18mm – 20 mm or more, 5000/10,000 IU of human chorionic gonadotrophin (HCG) was administered as a single dose to induce final follicular maturation.

Oocyte retrieval was performed 35-36 hrs later by vaginal ultrasound-guided follicle aspiration. The follicular aspirate was emptied into 60 mm Falcon dishes and cumulus-oocyte complexes were transferred into another dish with standard IVF medium. After the assessment of every cumulus-oocyte complex for cumulus-corona cell morphology, the complexes were incubated in standard incubators.

Sperm preparation

According to the World Health Organization (WHO) guidelines (WHO, 2010), the semen analysis was done in a tube, 1.5 mL of 90 % SpermGrad density solution was added first and then slowly 1.5 mL of 45 % SpermGrad solution was added on top of it. Finally, 1.0 mL of these men is slowly layered on top. The tubes were then centrifuged for 10–20 minutes at 300–600g. The two top layers were removed and care was taken so that there were no residues on the tube wall. The sperm pellets were transferred with as little possible of the 90 % solution to a sterile conical tube with 5 mL of equilibrated spermwash medium. Centrifugation was done for 10 minutes at 300–600g. After aspirating and discarding the supernatants, the wash was repeated. After the second wash, pellets were combined and re-suspended in 1 mL of equilibrated spermwash medium. The washed sample was then analyzed for motility and concentration.

Insemination procedure

In cases of traditional IVF, spermatozoa at a concentration of 150,000 × 106/ml were added to the oocytes.

Intracytoplasmic sperm injection

In cases of microinjection (ICSI), denudation of cumulus cells was achieved by exposure of the oocytes to HYASE (Vitrolife) for a period less than 30 seconds. Denudation of cumulus cells was carried out by the use of glass denuding pipettes immediately before the injection. The oocytes were washed four times after denudation. ICSI was carried out by commercially available ICSI pipettes.

Assessment of fertilization

Fertilization was checked 18–20 h after the insemination procedure. The oocytes were considered fertilized when two definite pronuclei were detectable. Cleavage and classification of morphology was first analyzed after 24 hours.

Embryo grading

Embryos for day 3 and blastocysts for day 5 transfer were moved into standard culture media. The number of blastomeres, the degree of cytoplasmic fragmentation, and uniformity of blastomere size for every embryo was recorded on day 2 and day 3 for day 3 transfers and further monitoring was done on day 4 and day 5 for day 5 transfers. The following classification [9] was used for embryo assessment on the day 3 of culture. The embryos were classified as:

- Grade 1: embryos with equal sized blastomeres and no cytoplasmic fragments;
- Grade 2: embryos with equal sized blastomeres and minor cytoplasmic fragments or blebs;
- Grade 3: embryos with unequal sized blastomeres and no or few cytoplasmic fragments.

According to the size of blastocele cavity, blastocysts were graded as early full or expanded.
Embryo transfer and Luteal phase support

Once the randomization was done, the two best quality embryos were transferred into the uterus on day 3 or day 5. Luteal phase support was given by daily vaginal administration of 90 mg progesterone gel starting on the day after the oocyte retrieval and continuing until the day of the pregnancy test (i.e. day 14 after embryo transfer). A positive pregnancy test was defined by a plasma β-HCG value greater than 10 IU/L. A clinical pregnancy was described as an intrauterine gestational sac with a heartbeat, 3 weeks after a positive HCG test.

Results

A total of 270 patients who fulfilled the inclusion criteria were included in this study. Embryo transfer was performed in 135 patients in day 3 group (Group A) and in 135 patients in day 5 group (Group B). Demographic details are given in Table 1. No statistically significant differences were seen between the two groups. The distribution of patients was similar between the two groups (n = 135 and 135 respectively) and there was no statistically significant difference in terms of age and BMI.

Table 1. Demographic details

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (Day 3)</th>
<th>Group B (Day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>31.3 (22.0-39.0)</td>
<td>31.2 (22.5-39.3)</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>23.6 (17.9-29.5)</td>
<td>22.9 (16.9-30.0)</td>
</tr>
</tbody>
</table>

Table 2 shows the distribution of patients with respect to duration and causes of infertility. Group A and Group B were statistically similar with respect to the duration of infertility (p=0.208) and different causes of infertility, which included tubal factor (p=0.601), polycystic ovarian disease (p=0.429), male factor (p=0.409), endometriosis (p=0.672) and others/combined (p=0.736).

Table 2. Distribution of patients with respect to duration and causes of infertility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Overall (n=270)</th>
<th>Group A (Day 3) (n=135)</th>
<th>Group B (Day 5) (n=135)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility duration (years)</td>
<td>5.5±3.9</td>
<td>5.8±3.6</td>
<td>5.2±4.2</td>
<td>0.208</td>
</tr>
<tr>
<td>Infertility cause</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal factor</td>
<td>86 (31.9%)</td>
<td>45 (33.3%)</td>
<td>41 (30.3%)</td>
<td>0.601</td>
</tr>
<tr>
<td>Polycystic ovarian disease</td>
<td>49 (18.3%)</td>
<td>27 (20%)</td>
<td>22 (16.3%)</td>
<td>0.429</td>
</tr>
<tr>
<td>Male factor</td>
<td>26 (19.3%)</td>
<td>31 (23.0%)</td>
<td>35 (26.1%)</td>
<td>0.469</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>67 (49.6%)</td>
<td>32 (23.7%)</td>
<td>35 (25.9%)</td>
<td>0.672</td>
</tr>
<tr>
<td>Others/combined</td>
<td>42 (31.1%)</td>
<td>20 (14.8%)</td>
<td>22 (16.3%)</td>
<td>0.736</td>
</tr>
</tbody>
</table>

Table 3 shows the clinical characteristics of embryo transfer. When we compared the two groups in terms of the number of collected oocytes per cycle, number of fertilized oocytes, and number of transferred embryos per cycle, there was no statistically significant difference between the two groups (p=0.136, p=0.619 and p = 1 respectively). However, the number of grade 1 embryos were significantly greater in Group B (p=0.001). Grade 3 embryos were significantly more in Group A (p=0.001). There was no statistically significant difference between two groups in Grade 2 embryos. In Group B, majority of blastocyst were of early grade (48.9%).

Discussion

The results of the present study are in overall agreement with the majority of previous published studies (Rienzi et al[10], Levron et al[11], Utsunomiya et al[12]). A prospective study done by Coskun et al[13] revealed that day 3 and day 5 transfers gave statistically similar overall implantation rates (21% vs. 23%), pregnancy rates (39% vs. 39%) and twinning rates (11.9% vs. 15%) in a group of 201 infertile women. The rate of twinning was statistically similar for the day 3 and day 5 transfers (36.8% vs. 30.4%) in the study done by Papnikolau et al [14] which is similar to the findings of the present study. Hatrnaz et al [15] had reported comparable overall results of pregnancy rates and implantation rates between day 3 and day 5 transfers in their study. To summarize, this study did not show statistically significant differences in implantation rates, clinical pregnancy rates, and live birth and perinatal death rates between the two groups. This inability can be attributed to the relatively small sample size of our study population.

Table 4. Pregnancy outcomes of the embryo transfer

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (Day 3) (n=135)</th>
<th>Group B (Day 5) (n=135)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collected oocytes per cycle</td>
<td>9.4±3.3 (3-14)</td>
<td>9.1±3.4</td>
<td>0.136</td>
</tr>
<tr>
<td>Fertilized oocytes per cycle</td>
<td>6.6±3.3 (2-8)</td>
<td>6.7±3.2</td>
<td>0.619</td>
</tr>
<tr>
<td>Transferred embryos per cycle</td>
<td>1.4±0.5 (1-2)</td>
<td>1.4±0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Embryo grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36 (13.3%)</td>
<td>7 (5.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>160 (54.8%)</td>
<td>74 (63.7%)</td>
<td>0.137</td>
</tr>
<tr>
<td>3</td>
<td>74 (27.4%)</td>
<td>54 (40.0%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Transferred embryos

<table>
<thead>
<tr>
<th>Type</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-cell</td>
<td>14 (5.2%)</td>
<td>14 (10.3%)</td>
<td>-</td>
</tr>
<tr>
<td>3-cell embryo</td>
<td>10 (6.7%)</td>
<td>8 (6.2%)</td>
<td>-</td>
</tr>
<tr>
<td>4-cell embryo</td>
<td>87 (32.2%)</td>
<td>87 (64.4%)</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;4-cell embryos</td>
<td>151 (56%)</td>
<td>106 (11.8%)</td>
<td>135 (100.0%)</td>
</tr>
</tbody>
</table>

Blastocyst grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>45 (34.3%)</td>
<td>20 (29.3%)</td>
<td>0.701</td>
</tr>
</tbody>
</table>

Table 4 displays the pregnancy outcomes of embryo transfer in Group A vs Group B. The implantation rate and clinical pregnancy rates were 48.1% and 43.7% in Group A and 54% and 50.4% in Group B respectively (p=0.33 and p=0.272). The results were not statistically significant. Miscarriage rate was 4.4% and 3.7% in Group A and Group B which was not statistically significant. Live birth rates (p=0.532) and perinatal death rates (p=0.701) were statistically similar in both the groups. The rate of twinning was similar in Group A and Group B which was 4.4% and 6.6%.
Conclusion

There is no difference in either implantation, pregnancy or delivery rates between cleavage stage and blastocyst stage embryo transfers when patients are appropriately randomized. In conclusion, the efficacy of blastocyst stage ET is similar to cleavage stage ET. Although due to small sample size, the power of the present study is limited, increasing the in-vitro period to 5 days does not seem to provide more information in selecting embryos with the highest chance of implantation. Hence, the results of this study do not support the use of blastocyst transfer in order to ameliorate implantation and pregnancy rates for good prognosis patients. Also, the results obtained do not justify a general conclusion that embryos from all patient categories show a comparable implantation potential on day 3 and day 5. Despite dissimilarity in design, selection of patients and culture conditions, the results of this study are in overall agreement with the majority of previous published studies.

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Conflict of interest: The authors declare that they have no conflict of interest.

Research involved human participants. Written informed consent was taken prior to start of the study. The patients consented to participate in this study and for publishing their data.

Ethical approval: The study was approved by the Institutional Ethics Committee. All procedures performed in this study were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. The idea of the paper was of Dr. Malvika Misra. Material preparation, procedures in the study and data collection were performed by Dr. Malvika Misra and Dr. Chandravati. The data analysis and manuscript preparation was done by Dr. Malvika Misra and Dr. Shubhi Srivastava. All authors have read and approved the final manuscript.

References


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