Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers

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Objective: To evaluate the efficacy of blastocyst transfer among patients with at least three previous cleavage-stage embryo transfer failures and to compare pregnancy and implantation rates of blastocysts according to the day of embryo transfer (day 5 or day 6 after oocyte retrieval).

Design: Retrospective clinical study.

Setting: Private ART center.

Patient(s): One hundred forty-eight patients (with at least three failed cleavage-stage embryo transfers) undergoing blastocyst-stage embryo transfer.

Intervention(s): Embryos were grown for up to 6 days and only blastocyst-stage (cavitating) embryos were transferred on either day 5 or day 6 after oocyte retrieval.

Main Outcome Measure(s): Clinical pregnancy and implantation rates.

Result(s): Blastocysts transferred on day 5 implanted almost five times the rate of those transferred on day 6 (23% vs. 5%). Pregnancy rates were triple as high among the 73 day 5 patients compared to the 63 day 6 transfer patients (38% vs. 11%). The number of blastocysts formed and per embryo rates of blastocyst formation were both significantly higher for patients undergoing day 5 transfers: more blastocysts developed (3.0 vs. 2.1) and more were transferred (3.0 vs. 1.9). In addition, blastocyst formation rates were 46% and 33%, respectively, for both groups of patients.

Conclusion(s): Blastocyst transfer (preferably on day 5 after retrieval) appears to be a successful and improved alternative for patients with multiple failed IVF attempts. Moreover, with blastocyst transfer there should be a reduction in multiple pregnancy risk, because fewer embryos have to be transferred. (Fertil Steril 2005;83: 49–53. ©2005 by American Society for Reproductive Medicine.)

Key Words: Blastocyst transfer, day 5 blastocyst transfer, day 6 blastocyst transfer, IVF, implantation rate, pregnancy rate, multiple gestations, cleavage-stage embryo transfer failure

Implantation, although improved markedly since the birth of Louise Brown in 1978, represents one of the main challenges to IVF centers, especially among couples with previous failed attempts (1). Moreover, many studies have shown a decrease in implantation rates after repeated IVF unsuccessful cycles (2). One of the given reasons for this success limiting factor has been the practice of transferring embryos to the uterus on day 2 or day 3 of the cleavage stage (1). To overcome the low implantation rates among couples who have had multiple previous failed attempts two options have been proposed: to place more than two embryos at the cleavage stage, thus increasing the risk of high-order multiple pregnancies, or to transfer embryos in a stage other than cleavage stage with higher implantation chance.

Advances in embryo culture media, specifically the development of sequential media, to promote embryonic growth through genome activation, blastocoele development, and embryonic expansion, have allowed for selection of those embryos with the greatest implantation potential and have led to an increase in the practice of blastocyst stage embryo transfer after IVF during the past years (2–4). Higher pregnancy and implantation rates have been obtained with blastocyst transfer than with transfers of early cleavage-stage embryos (5, 6). Several groups of investigators (2, 3, 5, 6) have recommended embryo transfer on day 5 after retrieval as it has a higher rate of implantation and pregnancy associated with blastocysts. The more advanced developmental stage of blastocyst may be a possible reason for these higher rates (3, 7, 8).

On the other hand, it is well established that pregnancy rates are affected by the average embryo score and that the chance for a successful outcome diminishes with each successive attempt at IVF (2).

Multifetal pregnancy has been a great concern to patients, assisted reproductive technology practitioners, and society. The costs in terms of maternal morbidity, developmental problems in the offspring, and financial drain on the health care system have been well documented. Blastocyst transfer...
allows better selection of embryos and has been suggested as a method to reduce the incidence of high-order multiple pregnancies. In conclusion, high-order multiple pregnancies could be almost completely eliminated by transferring only two embryos in the blastocyst stage (9).

Transferring a reduced number of embryos minimizes multiple pregnancy rates. This should be a goal of assisted reproductive technology (ART) programs. To accomplish this objective, embryos with the greatest potential for implantation should be selected for embryo transfer (6).

The purpose of this study was to compare the implantation and pregnancy rates between embryos that require 5 days to develop to the expanded blastocyst stage and embryos that require 6 days in patients with multiple IVF previous failures with transfers on day 2.

On the basis of preliminary results, we offered routinely fresh blastocyst transfer to the segment of our patient population with at least three failed IVF attempts.

**MATERIALS AND METHODS**

**Patient Selection**

Patients undergoing IVF at a private ART center between January 2000 and December 2002, all of them with three or more previous unsuccessful IVF cycles of cleavage-stage embryo transfers, were included in the study. There was no pregnancy in the previous cycles. Transfer of fresh, non-donor IVF was considered.

Institutional Review Board approval was not required because of the retrospective nature of the study.

Ovarian stimulation protocol included the use of a GnRH agonist (leuprolide acetate [LA], Procrin, Abbot Laboratoríos S.A., Madrid, Spain) administered in either the long (down-regulation) or short (flare) protocols and the subsequent addition of FSH (Gonal F. Serono Europe Ltd. London, UK). Exogenous FSH was administered daily until the lead follicles averaged 18–20 mm in diameter, measured by serial ultrasound monitoring. Human chorionic gonadotropin (Profasi HP, Lab Serono S.A. Madrid, Spain) (10,000 IU) was administered 36 hours before transvaginal oocyte retrieval. After retrieval, oocytes were fertilized by either conventional insemination or intracytoplasmatic sperm injection (ICSI).

**Embryo Culture and Transfer**

Fertilization was performed 3–6 hours after retrieval in IVF medium (Vitrolife AB, Gotheburg, Sweden) with either ICSI or conventional insemination as appropriate for the presence or absence of male factor infertility. At 24 hours after retrieval, normal fertilization was confirmed by the presence of two pronuclei.

Embryos were initially cultured in IVF medium (Vitrolife AB), being transferred from this medium to G1.2 and G2.2 media (Vitrolife AB) on days 1 and 3, respectively. Embryos were transferred on either day 5 or 6, depending on the degree of expansion of the blastocyst.

Only cavitating blastocyst-stage embryos were transferred to patients. Embryo transfers were performed when at least one blastocyst had expanded sufficiently that a distinct inner cell mass could be identified within a well-developed blastocoel filling the embryo. As a result, embryos were transferred on day 5 or 6 after the oocyte retrieval according to their rate of development.

The luteal phase was supported by vaginal progesterone (P) at a dose of 600 mg daily, 1 day after oocyte retrieval. A serum pregnancy test was performed 17 days after oocyte retrieval.

The implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. A viable pregnancy was defined as a pregnancy in which there was fetal cardiac activity detected by ultrasound at 6 weeks.

**Statistical Analysis**

Patient age, the number of oocytes retrieved, the percentage of oocytes fertilized, the number of developing embryos to the blastocyst stage, and the number of embryos transferred were compared between patients receiving transfers on day 5 and day 6. Blastocyst formation rates per fertilized oocytes, the percentage of transferred blastocysts that were expanded as the time of transfer, and the per patient implantation rates were also compared.

Data were analyzed using the \( \chi^2 \) and Student’s \( t \) tests where appropriate. A probability of <.05 and <.01 was considered statistically significant and highly significant, respectively.

**RESULTS**

One hundred forty-eight patients with at least three day 2 embryo transfer failures were involved in the study. The average patient age was 34.05 years (range, 25–43 years). The duration of infertility ranged from 24–120 months (average, 54.48 months). More than 1 out of 10 patients had had 5 or more previous failed attempts (Table 1).

Although the chance of blastocyst implantation seems to be higher than that of cleavage-stage embryos, one to four embryos were transferred per patient in our series depending on the number and quality of the blastocysts available. As stated before, at the period of the study the Spanish law still allowed the transfer of four embryos. Moreover, the known bad prognosis of couples with many previous transfer failures caused our team to perform such transfers.

The results are summarized in Table 2. A total of 73 patients were treated with day 5 blastocyst transfer and 63 were treated with day 6 transfer. Previous failed attempts
made no difference in pregnancy rates. In 12 patients (8.11%) transfer was cancelled due to failure of embryos to form any blastocyst. No difference was found between day 5 and day 6 transfer patients concerning either etiology of infertility or frequency of severe male factor infertility.

For patients in the day 5 embryo transfer group, a mean ± SD of 14.3 ± 5.4 mature oocytes was obtained and 8.71 ± 4.62 2PN embryos were formed, whereas the day 6 embryo transfer group did not show any statistically significant difference when compared with the former group (14.0 ± 7.0 oocytes retrieved and 7.89 ± 4.38 embryos formed, respectively).

The blastocyst formation rate was 46.3% for the day 5 embryo transfer group and 32.9% for the day 6 embryo transfer group (P = .002), with 91% of patients having at least one blastocyst to transfer and 73% having at least two blastocysts for transfer. In our study, 8.11% of patients (12 of 148) failed to develop a blastocyst.

Significantly fewer blastocysts were transferred among day 6 embryo transfer patients than among the day 5 embryo transfer group (1.9 vs. 3.0; P = .002).

Statistical analysis of data revealed that implantation rates differ significantly. It was higher for the day 5 embryo transfer compared with the day 6 embryo transfer group (23.33% vs. 4.86%; P = .001) (Fig. 1).

The clinical pregnancy rates were significantly higher in the day 5 embryo transfer group compared to day 6 transfer set of patients (38.36% vs. 11.11%; P = .001) (Fig. 2).

Five of 28 pregnancies in the day 5 transfer group were multiple (twins), whereas no twinning was observed among the day 6 transfer group (Table 1). All multiple pregnancies were nonidentical.

DISCUSSION

Patients with multiple failed IVF attempts represent a challenge to IVF teams. Different studies have shown a decrease

### TABLE 1

<table>
<thead>
<tr>
<th>Patients characteristics and previous ART profiles.</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Mean age (years) (min-max)</td>
<td>34.05 (25–43)</td>
<td></td>
</tr>
<tr>
<td>Infertility duration (months) (min-max)</td>
<td>54.48 (24–120)</td>
<td></td>
</tr>
<tr>
<td>Infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>131</td>
<td>88.59</td>
</tr>
<tr>
<td>Secondary</td>
<td>17</td>
<td>11.49</td>
</tr>
<tr>
<td>Number of previous failed transfers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>107</td>
<td>72.30</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>15.54</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>10.14</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>2.03</td>
</tr>
</tbody>
</table>


### TABLE 2

<table>
<thead>
<tr>
<th>Summary statistics for cycles of IVF with blastocyst transfer on either day 5 or day 6 postoocyte retrieval.</th>
<th>Day 5 transfer group</th>
<th>Day 6 transfer group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>73</td>
<td>63</td>
<td>NS</td>
</tr>
<tr>
<td>Age (y)a</td>
<td>34.1 ± 3.4</td>
<td>33.5 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocytesa</td>
<td>14.3 ± 5.4</td>
<td>14.0 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>No of cleavage embryosa</td>
<td>8.71 ± 4.62</td>
<td>7.89 ± 4.38</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization (%)a</td>
<td>61.9 ± 18.2</td>
<td>56.3 ± 15.5</td>
<td>NS</td>
</tr>
<tr>
<td>Blastocyst numbera</td>
<td>3.0 ± 1.8</td>
<td>2.1 ± 1.1</td>
<td>.003</td>
</tr>
<tr>
<td>Blastocyst formation (%)a</td>
<td>46.3 ± 19.1</td>
<td>32.9 ± 20.7</td>
<td>.002</td>
</tr>
<tr>
<td>Number transferreda</td>
<td>3.0 ± 1.1</td>
<td>1.9 ± 1.0</td>
<td>.002</td>
</tr>
<tr>
<td>Implantation rate (%)b</td>
<td>23.33 (22.1)</td>
<td>4.86 (3.6)</td>
<td>.001</td>
</tr>
<tr>
<td>Pregnancy rate (%)c</td>
<td>38.36 (28/73)</td>
<td>11.11 (7/63)</td>
<td>.001</td>
</tr>
<tr>
<td>Twinning rate (%)</td>
<td>17.86 (5/28)</td>
<td>0 (0/7)</td>
<td></td>
</tr>
</tbody>
</table>

aMeans ± one standard deviation.  
bMeans (medians in parentheses).  
cProportions (percentages in parentheses).

Attempts to predict the likelihood of pregnancy for couples undergoing IVF have demonstrated that the probability of success in IVF decreases with each unsuccessful attempt (10).

The initial reason for transferring day 2 or 3 cleaving embryos to the uterus was clearly related to culture media, which presumably would not support long-term growth. The percentage of blastocysts developing in standard media had generally been low. Therefore, if in vitro conditions were optimal, uterine transfer of blastocysts would be routine (3, 4, 11).

The first successful attempt to produce higher quality embryos in greater numbers was through co-culture (5). Later on, sequential culture media have been designed specifically for the first 2 days after fertilization (early cleavage) and the third and fourth day of embryo development (morula and blastocyst). The results have been as good as with co-culture (4, 12). A better in-depth understanding of the dynamic physiology of the early human preimplantation embryo has led to the development of new culture systems (13, 14).

A review of the recent literature indicates an emerging consensus on the potential advantages of blastocyst production and transfer (1, 3, 6, 7, 12, 14, 15).

The success of embryo transfer on day 5 or 6 has been shown to be associated with the degree of blastocyst development at the time of transfer (3, 10). Previous reports (3, 6, 14, 16) have shown higher implantation and pregnancy rates when transferring embryos on day 5 compared with day 6, suggesting that the viability may be higher for faster developing embryos. Blastocyst transfer permits the selection of presumably higher quality embryos after embryonic genomic activation has occurred. Extending the duration of culture may provide a mechanism for a better selection of the healthiest embryos to be transferred. Nevertheless, if limited cleavage-stage embryos are available, extended culture will not improve embryo quality and risk exists of either no transfer or a poor-quality embryo transfer. Concerning endometrial receptivity, no differences were found in ultrasound characteristics of the endometrium between day 5 and day 6 embryo transferred patients.

Fewer blastocysts are needed to be transferred to achieve pregnancy, therefore reducing the chance of multiple pregnancies. Furthermore, the number of supernumerary embryos for freezing is reduced, allowing an increase of storage capacity at the laboratory and reducing expense for the patient (2, 16).

Compared with early cleavage-stage embryo transfer, blastocyst transfer facilitates decision making for clinicians. The higher implantation rates seen with blastocyst transfer makes the choice to transfer only two embryos easy (4, 12).

In the present study, more than two embryos were transferred due to the prior poor prognosis of patients. To reduce or even eliminate the chance of multiple pregnancies after blastocyst transfer, limiting the number of embryos to be transferred is counseled.

Due to the larger diameter of blastocysts the rate of ectopic pregnancy might be decreased after blastocyst transfer. Moreover, uterine receptivity may be enhanced for day 5. Day 5 is the day on which embryos normally reside in the uterine cavity, and uterine contractions decrease with progression into the luteal phase (12).

In animal models, embryo expulsion rates decrease as the interval between ovulation and transfer increases and fewer uterine contractions are associated with improved pregnancy rates (9, 11, 14).

One of the drawbacks of the blastocyst transfer is the possible failure to achieve embryo transfer. Some patients, therefore,
might have no embryos for transfer. In our study, 8.11% of patients failed to develop a blastocyst. This figure is in agreement with the findings of other studies that used sequential culture media in patients for whom a number of oocytes had been retrieved (1, 7). Improvement of culture media might help prevent this outcome (2). Possibility of identical twinning after blastocyst transfer has also been mentioned as a drawback to this procedure (7). All the multiple pregnancies observed in this study were nonidentical.

In conclusion, blastocyst transfer appears to be a successful and improved alternative for patients with multiple failed IVF attempts. Moreover, with blastocyst transfer there will be a reduction in multiple pregnancy risk, because fewer embryos are needed to be transferred. Blastocysts transfer could also aid in evaluating embryo quality.

REFERENCES