Impact of day 3 or day 5 embryo transfer on pregnancy rates and multiple gestations

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Objective: To test the hypothesis that day 5 ET (D5ET) is superior to day 3 ET (D3ET) in pregnancy outcome and that it also reduces multiple gestations.

Design: Retrospective cohort study.

Setting: Assisted reproductive technologies program at Wilford Hall Medical Center.

Patient(s): Patients electing for either D3ET or D5ET.

Intervention(s): Participants meeting inclusion criteria for D5ET elected either D3ET or D5ET.

Main Outcome Measure(s): Cycles were compared by day of transfer and further stratified by patient age (<35 years and 35–40 years). The number of oocytes retrieved, embryos on day 3, embryos transferred, pregnancy rate, implantation rate, and twin and high order multiples (≥triplets) rates were compared.

Result(s): Of the 274 patients who met our inclusion criteria, 153 underwent a D3ET and 121 underwent a D5ET. The D5ET group had a significantly lower mean age and number of embryos transferred and a higher implantation rate (56% vs. 42%) than the D3ET group. Patients who were 35–40 years old had a significantly higher live-birth rate (68% vs. 40%). Although not statistically significant, the D5ET groups had higher clinical pregnancy (73% vs. 65%) and twin pregnancy (33% vs. 25%) rates.

Conclusion(s): Blastocyst transfer resulted in fewer embryos transferred, with a trend toward improved clinical pregnancy and higher twin pregnancy rates. Live-birth rates were improved in patients 35–40 years of age. Younger patients opting for D5ET should do so with a commitment toward single ET. (Fertil Steril 2009;91:1717–20. © 2009 by American Society for Reproductive Medicine.)

Key Words: Embryo transfer, blastocyst transfer, multiple gestation

The goal of IVF and intracytoplasmic sperm injection (ICSI) is to attain the highest pregnancy rate possible while simultaneously limiting the number of high order multiple (HOM) gestations (defined as three or more implanted embryos). Embryos are typically transferred to the uterus in either the day 3 zygote or day 5 blastocyst stages when certain criteria are met (1). In theory, allowing day 3 embryos to mature for two additional days allows for self-selection of the “best-quality” embryos, resulting in fewer embryos transferred, higher implantation rates, and decreased HOM rates (2, 3). Existing studies have shown varied results (4). While most have shown similar implantation and pregnancy rates, the impact on (HOM) gestations remains unclear (5–9). Ultimately, the answers to these clinically relevant issues will help providers accurately counsel couples as to the optimal day of transfer.

To investigate this question, we performed a retrospective cohort study to determine whether ET on day 3 versus day 5 shows a significant difference in implantation, number of clinical pregnancies, and number of multiple gestations among women undergoing IVF or ICSI at Wilford Hall Medical Center, San Antonio, Texas. We hypothesized that blastocyst transfer is superior to a day 3 ET (D3ET) in pregnancy outcome and that it also reduces multiple gestations.

MATERIALS AND METHODS

This study was approved as an exempt retrospective cohort study by the Institutional Review Board at Wilford Hall Medical Center. The charts of 1015 cycles in women ages 21–41 years of age who underwent IVF at Wilford Hall Medical Center between July 1998 and May 2006 were reviewed.
All patients underwent a standard IVF protocol for controlled ovarian stimulation with daily injections of gonadotropins. This regimen was continued until at least two follicles 16 mm in mean diameter and two follicles >12 mm in mean diameter were seen on serial transvaginal ultrasound examinations and the E2 level was >500 pg/mL. Ovulation was triggered with hCG, followed by transvaginal ultrasound-guided oocyte retrieval 35.5–36.5 hours after hCG administration. P (administered IM before clinical pregnancy and then per vagina) for luteal phase support was initiated the day after oocyte retrieval. All cycles were supplemented with corticosteroids and antibiotics in all patients (methylprednisolone 16 mg/day and tetracycline 250 mg every 6 hours beginning the day of oocyte retrieval and continuing until the day after ET). Gametes were prepped in P-1 medium (Irvine Scientific, Santa Ana, CA) plus 0.3% human serum albumin. After confirming fertilization the following day, embryos were cultured in P-1 plus 10% synthetic serum substitute (SSS) until day 3. Embryos not selected for transfer on day 3 were placed in Global medium (IVFonline, Guelph, ON, Canada) plus 10% SSS for extended culture.

Data collection was limited to patients meeting our minimum criteria for consideration of blastocyst transfer (day 5 ET [D5ET]). Our predetermined inclusion criteria for a D5ET consideration incorporated six or more total embryos with at least three in the 7- or 8-cell stage displaying <10% fragmentation. These “good-prognosis” patients were counseled on day 3 as to the potential risks and benefits of both a D3ET and a D5ET. These patients selected to either proceed with D3ET or delay until day 5 based on physician counseling and patient preference. The IVF cycles were then compared by D3ET versus D5ET and stratified into two groups based on patient age: <35 years and 35–41 years. Each of the four age groups were evaluated for number of oocytes retrieved, embryos produced, embryos on day 3, embryos transferred, implantation rate, pregnancy rate (positive hCG), number of clinical pregnancies (gestational sac seen on transvaginal ultrasound), live-birth rate, and multifetal gestations.

Data analysis was performed using the Student’s t-test, χ²-test, or Fisher’s exact tests. Relative risks (RRs) with 95% confidence intervals (CIs) were determined. P < .05 was considered statistically significant. Outcome rates were determined per cycle.

### RESULTS

Two hundred seventy-four patients met our inclusion criteria, with 153 receiving D3ET and 121 receiving D5ET. These two transfer groups were further stratified by age. There were no patients older than 40 who met the inclusion criteria. For the D3ET group, 82 were <35 years old and 71 were 35–40 years old. For the D5ET group, 70 were <35 years old and 51 were 35–40 years old. The average age of the D3ET group as a whole was greater than that of the D5ET group (33.3 ± 2.9 vs. 31.8 ± 3.9 years; P = .002), but the two groups were similarly matched when stratified by age: D3ET (<35) = 30.2 ± 2.7 versus D5ET (<35) = 30.0 ± 3.1 years and D3ET (35–40) = 37.0 ± 1.6 versus D5ET (35–40) = 36.4 ± 1.4 years.

The outcome categories reaching statistical significance are listed in Table 1, with the D5ET group showing a higher number of oocytes retrieved and total number of embryos cultured and with the same group subsequently having fewer embryos transferred with a significantly higher implantation rate. The same (statistically significant) results were seen in both of the age categories. For the remainder of the outcome categories, we observed similar rates of positive pregnancy tests and number of clinical pregnancies.

Patients in the 35- to 40-year-old group had a significantly higher live-birth rate (67.6% vs. 40.0%; RR = 1.69; 95% CI, [1.17–2.45]), whereas no difference was seen in patients <35 years old (67.8% vs. 67.5%; RR = 1.01; 95% CI, [0.82–1.24]). Although the numbers did not reach statistical significance, there was a higher rate of twin pregnancies in the D5ET group in both age categories. There were a total of seven triplets in the D3ET group (4.7%) and three triplets in the D5ET group (2.5%; Table 2).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Retrieval and transfer data.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>No. of patients</td>
<td>153</td>
</tr>
<tr>
<td>Age</td>
<td>33.3 ± 2.9</td>
</tr>
<tr>
<td>Mean no. of oocytes retrieved</td>
<td>18.5</td>
</tr>
<tr>
<td>Mean no. of embryos transferred</td>
<td>13.7</td>
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<tr>
<td>Mean no. of embryos transferred</td>
<td>2.52</td>
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</tbody>
</table>

a P < .0001.  
b P < .0007.  
c P < .0006.  

Beesley, Impact of day 3 or day 5 ET. Fertil Steril 2009.
higher rates of twin gestations in both of the D5ET age
groups. The trends that were observed suggest that statisti-
cal significance may have been reached with a larger study
population. A potential weakness in this study is the inher-
tent bias of the retrospective design, specifically with regard
to patient counseling. Although our criteria for determining
candidates eligible for D5ET were predetermined, it is pos-
sible that individual physician counseling was influenced
by factors that were not measured. These biases may
have influenced the patients’ ultimate selection of day of
transfer. This can only truly be overcome with a prospective
randomized trial.

Our results indicate that patients 35–40 years of age opting
for D5ET should do so with a commitment toward single ET.
Overall, blastocyst transfer resulted in fewer embryos trans-
ferred, higher implantation rates, and improved clinical preg-
nancy rates. The trade-off was a higher twin pregnancy rate.
Single ET in this group may therefore result in similar overall
clinical pregnancy rates without this higher multiple gesta-
tion rate (12). Patients <35 years old opting for D5ET
showed no overall benefit compared with D5ET and even
had a higher multiple gestation rate. This was likely due to
the fact that the only patients electing single ET were those
with only one embryo to transfer. D5ET should not be per-
formed unless a single ET is the ultimate goal. Only a con-
certed effort toward single ET will accurately assess clinical
efficacy.

REFERENCES
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Ginsburg ES. The number of 8-cell embryos is a key determinant for
selecting day 3 or day 5 transfer. Fertil Steril 2000;73:558–64.
2. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Helsa J.
A prospective randomized trial of blastocyst culture and transfer in

TABLE 2

Post-transfer outcomes.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>&lt;35 Years old</th>
<th>35–41 Years old</th>
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<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 5</td>
<td>Day 3</td>
</tr>
<tr>
<td>Clinical pregnancy rate, %</td>
<td>76.0 (0.90–1.19)</td>
<td>76.0 (0.90–1.19)</td>
<td>80.7 (0.79–1.09)</td>
</tr>
<tr>
<td>Implantation rate, %</td>
<td>42.1 (1.13–1.57)</td>
<td>56.4 (1.13–1.57)</td>
<td>53.1 (0.92–1.32)</td>
</tr>
<tr>
<td>% Twin (2 FHB)</td>
<td>24.8 (0.92–1.96)</td>
<td>33.1 (0.92–1.96)</td>
<td>28.9 (0.80–1.91)</td>
</tr>
<tr>
<td>% HOM (3+ FHB)</td>
<td>4.6 (0.14–2.05)</td>
<td>2.5 (0.14–2.05)</td>
<td>7.2 (0.12–1.85)</td>
</tr>
<tr>
<td>% Live born</td>
<td>58.8 (0.96–1.38)</td>
<td>67.5 (0.96–1.38)</td>
<td>67.5 (0.82–1.24)</td>
</tr>
</tbody>
</table>

Note: RRs with 95% CIs for blastocyst transfer are shown.

Beesley. Impact of day 3 or day 5 ET. Fertil Steril 2009.