Elective Single Embryo Transfers in Selective IVF-ET Cases

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Abstract: The culture of human embryos to the blastocyst stage in vitro has been an important advancement in ART, resulting in increased implantation rates and a reduction in the number of embryos transferred in IVF-ET cases. Higher-order multiple pregnancy rates have been significantly reduced by routinely transferring only two blastocysts; however, twinning rates still remain abnormally high.

The objective of this study was to compare the effects of transferring one versus two embryos on implantation, pregnancy and multiple pregnancy rates in IVF-ET cases which presented at least one high-grade expanded blastocyst (4AA) (Gardner and Schoolcraft, 1999) on Day 5.

The retrospective analysis presented here indicates that elective single embryo transfers coupled with blastocyst culture dramatically reduced the rate of multiple pregnancies and increased the percentage of embryos cryopreserved without significantly altering implantation, pregnancy or live birth rates in IVF-ET cases with at least one high-grade expanded blastocyst (4AA) on Day 5.

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Storm Stories – of the IVF Kind

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This hurricane season was unique in that four major storms hit the US and specifically Florida, in the space of 6 weeks. Orlando, which had not been hit by a hurricane in over 40 years, was hit by 3. This season gave several programs a chance to evaluate and re-evaluate emergency planning under real conditions. This article includes items suggested by Craig Sweet, MD, Ft. Myers, FL and Richard Cochran, PhD, Baton Rouge, LA.

Power

This is the key issue. All labs responding had emergency generators. This proved necessary as power outage was prolonged in many areas. Battery back-up would have been insufficient. All systems were reported to work as designed. One unexpected issue applied to gasoline powered generators. Most of these had tank capacities of less than 2 days. An unexpected issue was obtaining fuel once the tanks ran dry. This was affected by two components. First, massive evacuation of the population had resulted in a generalized shortage of fuel. Additionally, even after the storms had passed, most gas stations did not have power for their pumps. We all learned that first priority with an impending storm is to keep all vehicle

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Introduction

Blastocyst culture using sequential culture media has been a major advancement in human ART. Culturing human embryos to the blastocyst stage in vitro increases implantation rates, which in turn has led to a reduction in the number of embryos transferred (Gardner et al., 1998a; Gardner et al., 1998b; Behr et al., 1999; Marek et al., 1999; Wilson et al., 2002). Two blastocysts are now routinely transferred in most IVF-ET cases (Milki et al., 1999). Although this decrease in the number of embryos transferred has dramatically reduced the risk of higher-order multiple pregnancies, the incidence of twinning still remains extremely high (Marek et al., 1999; Wilson et al., 2002).

Twin pregnancies carry with them an increased risk of complications relative to singleton pregnancies. These include a significant reduction in gestation length and birth weights and a significant increase in spontaneous abortions, birth defects and perinatal mortality (Cunningham et al., 1993). In addition, there is an emotional and financial burden placed on patients with twin pregnancies. Most ART programs believe that a normal singleton pregnancy should be the goal of all IVF-ET cycles.

One approach to achieving this goal is through elective single embryo transfers, in which the patient is given a choice between transferring one or two embryos in IVF-ET cases which present ≥ 1 high-grade expanded blastocyst (4AA) on Day 5. This gives the patient the choice of a conservative approach (single embryo transfer)-- which reduces the risk of a multiple pregnancy but possibly also of any pregnancy at all -- or a more aggressive approach (double embryo transfer) which may decrease the risk of a failed IVF-ET cycle yet also may increase the probability of a multiple pregnancy.

Our ART laboratory has begun to offer elective single embryo transfers for good prognosis IVF-ET cases. The following study is a retrospective analysis of all IVF-ET cases that presented ≥ 1 high-grade expanded blastocyst (4AA) on Day 5 from August 1, 2003 to December 31, 2004. Patients who had IVF-ET cases that met these criteria were given the choice of a single embryo or double embryo transfer.

Materials / Methods

Oocytes were co-incubated with 200,000 motile sperm/mL in 5-well culture dishes with 500μ of G1.3 media (Vitrolife) + 5% HSA (Vitrolife) overlaid with NidOil (Nidacon) or OVOIL (Vitrolife) at a density of 1-5 oocytes/well. Denuding and fertilization checks were performed at 18 hrs post-insemination. All 2PN embryos were then washed and cultured in 20μ drops of G1.3 media + 5% HSA overlaid with NidOil or OVOIL for 48 hrs. On Day 3, all viable embryos were washed and transferred to 20 mL drops of G2.3 media (Vitrolife) + 5% HSA overlaid with NidOil or OVOIL and cultured to Day 5 or Day 6. All such embryos were cultured at a density of 3-5 embryos/drop in a 37°c incubator with 6% CO2 and 94% air.

Using 18 cm Wallace embryo transfer catheters (Irvine) containing ≤ 15μ of Embryo Glue (Vitrolife), we performed embryo transfers on Day 5 if ≥ 1 expanded blastocyst with a visible ICM (≥ 4BB) was present. (Only those with at least one ≥ 4AA blastocyst are included in this retrospective analysis, however.) All IVF-ET cases which did not present ≥ 1 expanded blastocyst with a visible ICM (≥ 4BB) were transferred on Day 6. Cryopreservation was performed on all expanded/hatching/hatched blastocysts with a visible ICM (≥ 4BB) on Day 5 or Day 6.

Serum bhCG tests were performed at 10 days post-transfer. A positive bhCG was defined as ≥ 5.0 mIU/mL. Fetal cardiac activity was determined by transvaginal ultrasound at 5 weeks post-transfer. The implantation rate was defined as the number of fetal sacs with cardiac activity/ number of embryos transferred. Multiple pregnancies were defined as > 1 fetal heartbeat on ultrasound. Statistical analysis was performed using either Chi square or Student’s t-test.
Results
The present study retrospectively examined 86 Day 5 blastocyst transfers. All of the IVF-ET cases included in the study presented at least one high-grade expanded blastocyst with a visible ICM on Day 5. The best blastocyst in each case was graded 4AA or higher using the grading scale of Gardner (Gardner and Schoolcraft, 1999). During the study period, 56.2% (86/153) of our embryo transfers met these criteria. [At the other extreme, 6.1% (10/163) of the Day 5 cases yielded no viable embryos for transfer on Day 5 or 6.]

In the 86 cases with > 1 fully expanded / partial or fully hatched AA blastocysts examined here, the patients were given the choice of transferring either one or two blastocysts. This decision was made in consultation with the physician and embryologist. ICSI or assisted hatching was not performed on any of the IVF-ET cases included in this study. Donor oocytes were utilized in 10.7% (3/28) of the single embryo transfer group and in 10.3% (6/58) of the double embryo transfer group.

There was no significant difference in the patients' ages, number of prior IVF cycles, number of oocytes retrieved, fertilization, cleavage or blastulation rates between the single embryo and double embryo transfer groups (Table 1).

The transfer of one versus two embryos also did not significantly alter the implantation, pregnancy or live birth rates (Table 2, Graph 1). A reduction in the number of embryos transferred from two to one did dramatically reduce the multiple pregnancy rate from 60.9% (28/46) to 9.5% (2/21) (Table 2, Graph 1). The single embryo transfer group also had a significantly higher percentage of supernumerary blastocysts to cryopreserve (Table 3).

Discussion
The present study demonstrates that elective single embryo transfers can dramatically reduce the multiple pregnancy rate while not significantly altering pregnancy and implantation rates in IVF-ET cases with at least one AA fully expanded / partially or fully

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<thead>
<tr>
<th>TABLE 1: ANALYSIS OF THE IVF-ET CASES</th>
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<tbody>
<tr>
<td><strong>No. Transferred on Day 5</strong></td>
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<tr>
<td>Transfers</td>
</tr>
<tr>
<td>Patients' Age</td>
</tr>
<tr>
<td>Prior IVF Cycles</td>
</tr>
<tr>
<td>Oocytes Retrieved</td>
</tr>
<tr>
<td>% Fertilization (2PN)</td>
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<tr>
<td>% Cleavage</td>
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<tr>
<td>% Blastulation</td>
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Results are expressed as the mean ± one standard deviation or as a percent of the total oocytes or 2PN embryos. IVF-ET cases utilizing donor oocytes were excluded from the patients' age category. Results were compared using either Chi square or Student's t-test.

<table>
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<tr>
<th>TABLE 2: PREGNANCY AND IMPLANTATION RATES FOLLOWING DOUBLE EMBRYO VERSUS ELECTIVE SINGLE</th>
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<tr>
<td><strong>No. Transferred on Day 5</strong></td>
</tr>
<tr>
<td>% Positive hCG</td>
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<tr>
<td>% Fetal Cardiac Activity</td>
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<tr>
<td>% Live Births / Ongoing Pregnancies</td>
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<tr>
<td>% Implantation</td>
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<tr>
<td>% Multiple Pregnancies</td>
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All pregnancy rates are expressed per transfer. Implantation rates are expressed as the number of fetal sacs with cardiac activity/number of embryos transferred. *Results differ from two blastocysts at p < 0.001.
hatched blastocyst on Day 5.

Elective single embryo transfers dramatically reduced the multiple pregnancy rate from 60.9% for double embryo transfers to 9.5%. The two multiple pregnancies in the single embryo transfer group were due to monozygotic twinning. Several investigators, including our laboratory, have demonstrated an increase in the rate of monozygotic twinning in Day 5 and/or Day 6 blastocysts transfers (Sheiner et al., 2001; Tarlatzis et al., 2002; Milki et al., 2003; Roots et al., 2003). Our current rate of monozygotic twinning in all Day 5 blastocyst transfers over the past 6 years is 2.7%. Whether the 9.5% monozygotic twinning rate seen in the single embryo transfer group represents a significant increase or is simply a statistical anomaly due to the relative low number of transfers (28) requires further investigation.

Elective single embryo transfers did not significantly reduce the implantation, pregnancy or live birth rates in the present study. A trend did exist toward the spontaneous abortion rate being higher in the single embryo transfer group (20.8%) versus the double embryo transfer group (14.0%) (Table 2). This trend might be due to the possibility that a second “helper” embryo was not present to assist the first with any of the steps involved in implantation, or that a “backup” embryo was not present if the first conceptus was spontaneously aborted during the pregnancy. Again, a larger group of elective single embryo transfers needs to be examined to determine if this trend becomes statistically significant.

Elective single embryo transfers also significantly increased the percent of embryos available for cryopreservation (33.5%) compared to double embryo transfers (26.5%). Of course, these results were expected, since in elective single embryo transfers fewer blastocysts are transferred, so more blastocysts remain for cryopreservation, relative to double embryo transfers.

One prospective, randomized clinical trial comparing elective single blastocyst transfers to double blastocyst transfers has appeared in the recent literature (Gardner et al., 2004). The study found no signifi-
cant difference in ongoing pregnancy rates for elective single blastocyst transfers compared to double blastocyst transfers, while demonstrating that elective single blastocyst transfers reduced the multiple pregnancy rate from 47.4% (two blastocysts) to 0.0% (one blastocyst).

Conclusions
We have demonstrated in this study that elective single embryo transfers can dramatically reduce the rate of multiple pregnancies without reducing the implantation, pregnancy and live birth rates in IVF-ET cases that present at least one high-grade expanded blastocyst (4AA) on Day 5. This should make elective single embryo transfers utilizing blastocyst culture an important approach to reduce the multiple pregnancy rate in many IVF-ET programs. More experimentation will be necessary to determine if elective single embryo transfers can be successfully utilized in all IVF-ET cases in an attempt to virtually eliminate the risk of multiple pregnancies.

An added benefit to elective single embryo transfers may be to increase the number of high-grade blastocysts available for cryopreservation. This should increase the implantation, pregnancy and live birth rates for frozen embryo transfers following elective single embryo transfers.

One disadvantage of the selective single embryo transfer regime utilized in this study is that it is dependent upon the extended culture of embryos to the blastocyst stage on Day 5, which carries with it the inherent risk of there being no viable embryos to transfer by Day 5 or 6. Here, in this study we found this risk to be minor, with a cancellation rate after oocyte retrieval of only 6.1%.

Acknowledgments
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References
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