Comparison of Pregnancy, Implantation, and Multiple Gestation Rates for Day 3 Versus Day 5 Embryo Transfers

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Purpose: To compare pregnancy, implantation, and multiple gestation rates resulting from day 3 and day 5 embryo transfers after in vitro fertilization emphasizing a subset of patients who met criteria for day 5 transfer but elected to undergo day 3 transfer.

Method: A retrospective analysis of day 3 and day 5 embryo transfers from January 2001 to June 2002 were evaluated in a community teaching hospital setting. A total of 331 patients ≤ 40 years old were included. Using Student's *t* test, χ^2 test, and Fisher's exact test, we compared the pregnancy, implantation, and multiple gestation rates.

Results: Pregnancy, implantation, and multiple gestation rates were not significantly different between the subgroup who met criteria for day 5 embryo transfer but elected day 3 transfer. There was no significant difference between similar parameters in the overall comparison of day 3 versus day 5 embryo transfers.

Conclusions: Blastocyst transfers have similar multiple gestation rates, pregnancy rates, and implantation rates when compared to day 3 embryo transfers.

KEY WORDS: Blastocyst; day 3 embryos; embryo transfer; in vitro fertilization; multiple gestation.

INTRODUCTION

In vitro fertilization (IVF) programs attempt to achieve the highest pregnancy rate with least risk of multiple gestations. Multiple gestations are critical to prevent because of their increased risk of both perinatal and maternal morbidity and mortality (1). Despite recommendations from the American Society of Reproductive Medicine to limit the number of embryos transferred, 37% of all deliveries from assisted reproductive technologies are multiple gestations (2). In an effort to select potentially better quality embryos with the highest pregnancy rates and reduced risks of multiple gestations, the movement towards prolonged

culture and transfer on day 5 has been recommended by some authors (3–6). Unfortunately, controversy still exists as to the benefit of blastocyst transfers as other researchers have found no difference in outcome between day 3 versus day 5 transfers (7,8).

The purpose of our study is to determine whether pregnancy, implantation, and multiple gestation rates differ in day 3 versus day 5 embryo transfers at our institution with specific focus on a comparison of day 3 embryo transfer candidates who met criteria for a day 5 embryo transfer but elected to undergo a day 3 embryo transfer versus those that had a day 5 embryo transfer.

MATERIALS AND METHODS

Patients/Embryo Culture

A retrospective analysis of IVF cycles performed from January 2001 through June 2002 was undertaken at the Toll Center for Reproductive Sciences of

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Abington Memorial Hospital. Institutional review board approval was not obtained because of the retrospective nature of this study. Three hundred and thirty-one patients were noted in our computer database who underwent IVF with or without intracytoplasmic sperm injection (ICSI) and had a day 3 or day 5 embryo transfer. There were 282 patients with embryos transferred on day 3 and 49 patients with embryos transferred on day 5. All patients were counseled prior to their IVF cycle that they would be candidates for a day 5 embryo transfer if they had at least three 8-cell embryos of grade 1 or 2 quality. Controlled ovarian stimulation protocols were determined on the basis of patient's age, day 3 FSH levels, and/or clomiphene challenge test results. Follicular maturation was monitored by daily E₂ levels and transvaginal ultrasounds. Human chorionic gonadotropin (5000–10,000 IU) was administered when at least two follicles reached a mean diameter of 17 mm, and transvaginal oocyte retrieval was performed 36 h later. Patients underwent conventional in vitro fertilization or intracytoplasmic sperm injection on the basis of appropriate indications (9).

After 12–18 h of incubation, fertilization was confirmed by the identification of two pronuclei. The zygotes then were removed from the insemination droplet and placed in P-1 Media (Irvine Scientific, Santa Ana, CA) or IVC-ONE (InVitroCare, Inc., San Diego, CA). All embryos were maintained at 37°C in 5% CO₂ air atmosphere. Cleavage rates and morphological appearance were assessed daily. Selective assisted hatching was performed as previously described (10). On day 3, patients with three or more 8-cell embryos of grades 1–2 were given the option of having a day 3 transfer or extended culture to day 5 with blastocyst transfer. If extended culture was desired, the embryos were transferred into IVC-THREE sequential media (InVitroCare, Inc., San Diego, CA) or Irvine Blast media (Irvine Scientific, Santa Anna, CA) as appropriate in regard to their initial culture media and observed daily for blastocyst development. Both day 3 and day 5 transfers were performed with a Wallace catheter (Marlow Technologies, Willoughby, OH).

Clinical pregnancy was defined as the presence of a fetal cardiac activity on ultrasound. Pregnancy rates were the number of clinical pregnancies per transfer. Implantation rate included the number of intrauterine sacs per the number of embryos transferred.

Statistical Analysis

Comparisons between groups were made by using Fisher's exact test, chi-square analysis, or Student's *t* test. $P \leq 0.05$ was considered significant.

RESULTS

A total of 331 patients ≤ 40 years old underwent IVF from January 2001 to June 2002. Two hundred and eighty-two patients had day 3 transfers, and 49 patients elected to have a day 5. Of the 282 patients who had a day 3 embryo transfer, 108 patients met the criteria for a blastocyst transfer but declined. The average patient age was 34 ± 4 for both groups, and there were no statistically significant differences in the indications for IVF (see Table I).

The number of embryos transferred was significantly higher in the day 3 versus day 5 transfers ($P < 0.0001$). The subset of patients who met the criteria for a blastocyst transfer by having greater than or equal to three 8-cell embryos who declined day 5 transfer did not have any statistically significant difference in pregnancy, implantation, and multiple gestation rates compared to those patients electing to have day 5 transfers (see Table II). The pregnancy, implantation, and multiple gestation rates between day 3 and day 5 transfers were similar overall (see Table III). Cancellation rates did not differ (7% vs. 4%).

DISCUSSION

In the United States, the current IVF standard is to transfer multiple day 3 cleaved embryos. Unfortunately, overall take-home baby rates remains relatively low with a high risk of multiple gestations (2).

Table I. Comparison of Day 3 Versus Day 5 Embryo Transfers Cause of Infertility

	Day 3	Day 5	Significance
Endometriosis	70	7	ns ^a
Idiopathic	4	1	ns ^a
Male factor	87	15	ns ^a
Ovarian dysfunction	22	3	ns ^a
Tubal factor	50	13	ns ^a
Ovarian failure	1	0	ns ^a
Diminished ovarian reserve	50	10	ns ^a

Note. ns, not significant.

^a χ^2 Test

Table II. Comparison of Day 3 Embryos (with 3 Eight Cells) to Day 5 Transfers

	Day 3 (3 eight cells)	Day 5	Significance
No. clinical pregnancies	56	19	—
No. multiples	22	7	—
Twins	19	7	$P = 0.96$ (ns) ^a
Triplets	3	0	$P = 1.0$ (ns) ^b
Implantation rate	21%	23%	$P = 0.15$ (ns) ^a
Pregnancy rate	52%	39%	$P = 0.18$ (ns) ^a
Multiple gestation/clinical pregnancy	39%	37%	$P = 0.97$ (ns) ^a

Note. ns, not significant.

^a Two-tailed *t* Test; χ^2 test.

^b Fisher's exact test.

Reasons proposed for the relatively poor implantation rates of day 3 embryos include asynchrony between the embryo and the endometrium as well as aneuploidy rates in human embryos. With the current advances in the in vitro culture conditions, a greater number of embryos are now able to reach the blastocyst stage of development (4). A number of studies have noted improved implantation rates, similar or improved pregnancy rates, and a decrease in the number of multiple gestations with blastocyst transfers (3–6). Other studies have not supported these findings (7,8).

Our study noted similar pregnancy, implantation, and multiple gestation rates between day 3 and day 5 transfers. Because of the retrospective nature of our study and relatively small sample size, we cannot rule out a type 2 error. A post hoc power analysis demonstrated that to achieve an 80% power we needed a sample size of 376 patients in each group to detect a 10% difference in pregnancy rates. These findings

are comparable to a randomized controlled trial by Coskun *et al.* that reported similar implantation rates (21 and 24%), pregnancy rates (39%), and multiple gestation rates (33 and 38%) (7).

We attempted to examine the group of patients who had greater than or equal to 8-cell embryos who chose a day 3 or day 5 transfer in more detail. This comparison is more valid than the overall comparison, and still did not reveal any significantly different outcomes.

Although the multiple gestation rates were similar in both the groups, there was a trend toward a decrease in higher order multiples in the blastocyst group (8 vs. 0). This is consistent with other reports (3,4,6). Gardner *et al.* reported that transferring fewer blastocysts could decrease higher order multiple gestations (3). With the increased implantation rate of blastocysts, good prognosis patients could undergo a single embryo transfer and maintain a reasonable pregnancy rate with a reduced chance of multiple pregnancies (3,5).

In conclusion, we were unable to detect any significant differences in outcome between day 3 and blastocyst embryo transfers even in a comparison of patients eligible for day 5 transfer who elected to have day 3 transfer of embryos. This is an area of great interest and debate in our literature. It is important that additional research continues in order to identify which population of patients may benefit from prolonged embryo culture.

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Table III. Comparison of Day 3 Versus Day 5 Embryo Transfers

	Day 3	Day 5	Significance
Number of transfers	282	49	—
Total no. embryos transferred	935	114	—
Average no. embryos transferred	3.5	2.3	$P \leq 0.0001$ ^a
No. clinical pregnancies	97	19	—
No. multiples	44	7	—
Twins	36	7	$P = 0.81$ (ns) ^b
Triplets	8	0	$P = 0.35$ (ns) ^c
Implantation rate	17%	23%	$P = 0.13$ (ns) ^b
Pregnancy rate	34%	39%	$P = 0.67$ (ns) ^b
Multiple gestation/clinical pregnancy	45%	37%	$P = 0.67$ (ns) ^b

Note. ns, not significant.

^a Two-tailed *t* Test.

^b χ^2 test.

^c Fisher's exact test.

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