

Effect of ICSI on Subsequent Blastocyst Development and Pregnancy Rates¹

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Purpose: To investigate whether ICSI (intracytoplasmic sperm injection) results in decreased blastocyst formation and pregnancy compared to IVF (in vitro fertilization).

Methods: We performed a retrospective analysis of blastocyst transfer (BT) offered routinely to patients under age 40 with \geq three 8-cell embryos on day 3 and compared IVF to ICSI cycles. Sequential media were used with P1 until day 3, then Blastocyst Medium until day 5/6.

Results: There were 131 IVF and 75 ICSI cycles. There was no difference in age, number of oocytes, zygotes, 8-cell embryos, blastocysts on days 5 and 6, or embryos transferred. Progression to blastocyst was similar (78% for IVF and 73% for ICSI) as was the viable pregnancy rate (51.4% for IVF and 55% for ICSI). No cycles failed to form blastocysts.

Conclusions: The progression to blastocyst and the likelihood of conceiving a viable pregnancy were unaltered by ICSI. Thus it seems appropriate for programs to offer BT to patients undergoing ICSI using the same inclusion criteria applied to their IVF patients.

KEY WORDS: Blastocyst; ICSI; IVF; male factor infertility; pregnancy rate.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) has become a valuable procedure commonly used in assisted reproductive technologies. In the most recent report summarizing U.S. data from 1998, 37% (23876/64210) of all in vitro fertilization (IVF) cycles used ICSI (1). Since women undergoing ICSI to treat male factor infertility often have an otherwise good prognosis for conception, the risk of multiple gestations is a relevant issue in this patient population. Blastocyst transfer (BT) has been reported to be a valuable tool for helping control the risk of high-order multiples by reducing the number of embryos transferred (2–7). However, several IVF programs have been reluctant to offer BT to their ICSI patients based on reports

that ICSI may contribute to a reduced capacity for blastocyst formation compared to conventional IVF. In our program we have routinely offered BT to good prognosis IVF patients irrespective of whether they had conventional insemination or ICSI. The present study was undertaken to investigate whether ICSI results in reduced blastocyst formation and pregnancy rates compared to IVF.

MATERIALS AND METHODS

Since January 1998, all patients under age 40 with three 8-cell embryos on day 3 were offered BT. The decision to use ICSI was based upon abnormal semen parameters and/or previous low fertilization rates with IVF. Two hundred and six patient cycles met criteria and underwent BT with IVF or ICSI. A retrospective analysis of these cycles was performed.

A regimen of sequential media was utilized starting with P1 + 10% SSS (Irvine Scientific, Santa Ana, CA) until day 3, then moving to Blastocyst Medium with 10% SSS (Irvine Scientific, Santa Ana, CA) until

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day 5 or 6. A viable pregnancy was defined as fetal cardiac activity seen on ultrasound by 7 weeks of gestation.

Statistical analysis was performed with the Student's *t*-test and chi-squared analysis. Significance was set at 0.05.

All patients signed consent forms for their treatment and IRB approval was obtained for review of laboratory and clinical data.

RESULTS

One hundred thirty-one patient cycles used conventional IVF and 75 cycles used ICSI. The groups did not differ in age (34.3 ± 3.3 year vs. 34.7 ± 3.1 year), mean number of oocytes (15.5 ± 6.9 vs. 17.5 ± 6.8), number of zygotes (10.1 ± 3.8 vs. 11.4 ± 4.4), number of 8-cell embryos (6.6 ± 2.8 vs. 6.2 ± 3.0), number of blastocysts on day 5 (3.8 ± 2.7 vs. 3.3 ± 1.9), or total number of blastocysts by day 6 (5.2 ± 3.2 vs. 4.5 ± 2.4). The mean number of blastocysts transferred was 2.26 ± 0.6 for IVF and 2.27 ± 0.5 for the ICSI group. Progression to blastocyst from 8-cell embryos was 78% in the conventional IVF group and 73% in the ICSI group. No cycles failed to form blastocysts. Viable pregnancy rates for the IVF group were 51.4% compared to 55% in the ICSI group. All *p* values were greater than 0.05 (Table I).

DISCUSSION

The use of BT for embryos fertilized by ICSI has been brought into question by several reports in the literature that suggest that ICSI embryos fare worse in extended culture. The first study to suggest this was a cohort study by Shoukir *et al.* (8). By culturing

168 supernumerary embryos resulting from ICSI and comparing them to 423 supernumerary embryos from IVF, a significantly lower rate of blastocyst formation from day 2–3 embryos was seen in coculture (26.8% with ICSI vs. 47.3% with IVF). Patients with higher forward motility had improved blastocyst development, suggesting a paternal influence on blastulation rate.

Subsequently, Dumoulin *et al.* (9) evaluated supernumerary embryos, in a large retrospective study with 1628 consecutive cycles. They showed a significant decrease in development to blastocyst of surplus embryos from ICSI (23% vs. 31.8% for IVF). Griffiths *et al.* (10) published results from two prospective series, the first of which examined embryos from conventional IVF for tubal infertility and compared them to ICSI embryos in patients with male factor infertility. Significantly fewer embryos formed blastocysts after ICSI (8.9%) than after IVF (23.5%). The second series of embryos analyzed consisted of eight cases where sibling oocytes were nonselectively inseminated either by conventional IVF or by ICSI with spermatazoa from the same semen specimen. ICSI embryos formed blastocysts only 20% of the time compared to a 50% blastulation rate for IVF embryos. Menezo and Barak (11) also published results of a prospective comparison of supernumerary embryos resulting from short insemination IVF compared to ICSI. They reported a significant decrease in blastocyst formation from ICSI (34.2% with ICSI vs. 43.8% with IVF), although it must be noted that significantly fewer embryos were present on day 2 in the ICSI group.

Poorer quality supernumerary embryos may be a suboptimal way to evaluate blastocyst development with ICSI. Other studies that have not used surplus embryos have shown a decreased blastulation rate with ICSI. Patton *et al.* (3), Coskun *et al.* (12), Gardner *et al.* (13), Miller and Smith (14), and Zollner *et al.* (15) in cohort retrospective studies found blastulation rates from 26 to 48% with ICSI compared to 41 to 57% with IVF. Nevertheless, the pregnancy and implantation rates with BT were similar with conventional IVF and ICSI in these studies (3,12,13,15).

However, there are studies that do not support a decreased blastulation rate. Three studies, by Marek *et al.* (16), Wilson *et al.* (17), and Karaki *et al.* (7), have shown no difference in blastocyst formation rates and have confirmed the previous findings of no difference in pregnancy and implantation rates with BT using IVF or ICSI.

Table I. Characteristics of IVF and ICSI Cycles

	IVF	ICSI
Number of cycles	131	75
Average age ^a	34.3 ± 3.3	34.7 ± 3.1
Mean number oocytes ^a	15.5 ± 6.9	17.5 ± 6.8
Mean number of zygotes ^a	10.1 ± 3.8	11.4 ± 4.4
Mean number of 8-cell embryos ^a	6.6 ± 2.8	6.2 ± 3.0
Mean number of blastocysts on day 5 ^a	3.8 ± 2.7	3.3 ± 1.9
Mean number of blastocysts on day 6 ^a	5.2 ± 3.2	4.5 ± 2.4
Mean number blastocyst transferred ^a	2.26 ± 0.6	2.27 ± 0.5
Progression to blastocyst from 8-cell embryo ^a	78%	73%
Viable pregnancy rate	51%	55%

Note. *P* > 0.05 for all categories.

^a ± Standard deviation.

A perhaps more relevant way of assessing whether blastocyst culture is detrimental in ICSI cycles is to compare day 5 to day 3 embryo transfer results. Patton *et al.* (3) found similar pregnancy rates in ICSI cycles (43% with day 5 and 21% with day 3), but the implantation rate was significantly higher with day 5 (36%) compared to day 3 (11%). Coskun *et al.* (12), in a prospective randomized study, found equal pregnancy rates (44% with day 5 and 45% with day 3) and implantation rates (24% with day 5 and 27% with day 3). Most recently, Wilson *et al.* (17) showed a significantly higher pregnancy rate with blastocyst transfer compared to day 3 transfer in ICSI cycles (51% with day 5 vs. 37% with day 3).

Several authors have proposed that the manipulation of the oocyte that occurs with ICSI may make the resultant embryo more susceptible to damage in prolonged culture. Dumoulin *et al.* (18) found that the type of membrane breakage, the volume of aspirated cytoplasm, and the technician performing ICSI significantly influenced development to the blastocyst stage of surplus embryos. Depending upon the technician performing the procedure, they report a blastulation rate ranging from 14 to 28%.

Some studies suggest that the lower blastulation rate of ICSI embryos may be a reflection of a negative paternal influence, which remains hidden in the first 3 days and seen only after genomic activation at the 4–8-cell stage (19). A study by Balaban *et al.* (20) showed that the source of spermatozoa, usually indicative of the severity of the male infertility, affected the blastocyst formation rate and implantation rate. Spermatozoa from nonobstructive azoospermic patients resulted in the lowest fertilization and blastocyst rates. Miller and Smith (14) found that decreased progressive motility and sperm morphology were significantly correlated with diminished blastocyst development and quality. Gianaroli *et al.* (21), in a study performing PGD on embryos resulting from severe male factor infertility, found an increase in the rate of de novo abnormalities. In patients with round spermatid-derived embryos, Urman *et al.* (22) reported a very low blastulation rate (7.6%), and no pregnancies in 58 couples.

CONCLUSIONS

Although there have been reports in the literature suggesting a decreased in vitro blastulation rate after ICSI, our findings show that the developmental competence of ICSI embryos does not appear to be

compromised when extended culture is offered to patients with good quality cleavage stage embryos. The likelihood of conceiving a viable pregnancy was also unaltered. A definite assessment of blastocyst culture in ICSI cycles can only be obtained by a large randomized prospective study requiring multicenter cooperation. Meanwhile, the data from this study suggest that it is not inappropriate for programs to offer BT to patients undergoing ICSI using the same inclusion criteria that they apply to their IVF patients.

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