Intracytoplasmic Sperm Injection Increases Embryo Fragmentation Without Affecting Clinical Outcome

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Purpose: To examine the effect of intracytoplasmic sperm injection (ICSI) on embryo fragmentation and implantation rates in those embryos chosen for transfer compared to conventional in vitro fertilization (IVF).

Methods: We compared 253 infertility patients (71 ICSI and 182 IVF) with respect to age, semen analysis, number of embryos transferred, embryo fragmentation, implantation rate, and pregnancy rate. Embryo fragmentation was determined by one observer at the same laboratory over the entire study period.

Results: A statistically significant difference was observed in mean embryo grade between IVF (2.2 ± 0.84) and ICSI (2.5 ± 0.77), P = 0.01. Additionally, the IVF patients had significantly more nonfragmented (grade I) embryos compared to the ICSI group, P < 0.01.

Conclusions: These data suggest that ICSI, irrespective of semen parameters, may increase embryo fragmentation and produce fewer nonfragmented grade I embryos while maintaining implantation and pregnancy rates similar to conventional IVF.

KEY WORDS: embryo fragmentation; in vitro fertilization; intracytoplasmic sperm injection; pregnancy rate; sperm parameters.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) offers couples with the diagnosis of male factor infertility an effective treatment option (1). Palermo et al. (2) popularized this complex process in humans. As the technique evolved, oocyte number and quality were found to be the main determinants of success in treating male infertility (3,4). However, successful implantation and pregnancy with in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) also are strongly associated with embryo morphology, speed of embryo development, and age of the female patient.

Prior to the clinical implementation of ICSI, teratozoospermia was associated with morphological abnormalities in embryos of couples undergoing microsurgical fertilization (5,6). More recently for both IVF and ICSI, poor seminal parameters have been proposed to influence embryo morphology, developmental capacity, and implantation rates (7–10). Since success in assisted reproductive technology cycles is universally accepted to be dependent on embryo quality, an important and unresolved question is whether the mechanical disruption of the ooplasm adversely affects embryo morphology.

Evidence that microinjection of the oocyte may adversely affect embryo morphology is inconclusive. The quality of embryos produced by ICSI, when compared to embryos produced by conventional IVF, has been reported to be comparable, inferior, or superior (2,11–13). In a case–control study, Bar-Hava et al. (13) reported that IVF embryos were superior to ICSI in both morphology and clinical pregnancy rates. In contrast, Yang et al. (11) evaluated 13 couples with non-male factor infertility and randomized the oocytes to ICSI or conventional insemination, they concluded that fertilization rates similar to IVF rates could be achieved with ICSI in non-male factor infertility patients and that ICSI produced more “grade A” embryos than conventional IVF. Given the uncertainty regarding the possible effect of ICSI on embryo quality, the goal of this study was to examine the question of whether the process of oocyte micromanipulation is
associated with a more fragmented embryo at the time of embryo transfer.

**MATERIALS AND METHODS**

The study population consisted of 556 patients undergoing IVF at the Walter Reed Assisted Reproductive Technology Center from July 1996 to December 1997. All diagnoses of infertility were included. Inclusion criteria included those patients undergoing IVF or ICSI who were 37 years of age or less, had embryo grading by our primary embryologist, had an embryo transfer occurring no less than 72 hr after retrieval, and received no embryo manipulation including assisted hatching and fragment removal. Cycles in which oocytes were divided between conventional IVF and ICSI were omitted. All patients undergoing microsurgical epididymal sperm aspiration (MESA) or testicular sperm aspiration (TESA) were excluded. In couples undergoing repetitive assisted reproductive technique (ART) cycles, only their first cycle was analyzed. Additionally, no frozen embryo cycles or donor gamete cycles were included. In total, 253 patients were eligible and included in the study. Institutional Review Board approval was obtained.

A screening semen analysis was obtained on all patients. Patients with an abnormal semen analysis underwent a second analysis. Markedly abnormal semen analyses were evaluated with a swim-up procedure (Table I). ICSI was performed whenever the couple fulfilled one or more of the following criteria: (a) poor semen characteristics with less than 3 million total motile sperm or <10% normal morphology in ejaculate before processing; (b) prior poor fertilization (<30%) in a previous IVF cycle; and (c) excessive sperm agglutination noted on semen analysis.

Ovarian stimulation methods were similar in the IVF and ICSI cycles. The patients underwent stimulation with a variety of stimulation protocols including gonadotropin-releasing hormone (GnRH) analogue (GnRH-a) (leuprolide acetate, Lupron: TAP Pharmaceuticals; Chicago, IL) or oral contraceptive pill down-regulation followed by stimulation with exogenous gonadotropins or concomitant initiation of GnRH-a and exogenous gonadotropins (“flare” cycles). These methods have been compared in our program and found to be equivalent in clinical outcomes (14). Follicular development was monitored with serial serum estradiol levels and pelvic ultrasounds. Follicles were aspirated under transvaginal ultrasound guidance 34–36 hr after injection with 10,000 units of human chorionic gonadotropin (hCG). Luteal phase support, in the form of 50 mg progesterone in oil intramuscularly (IM) daily, was given starting on the day of oocyte retrieval and continuing until a negative pregnancy test or 8 weeks gestation.

Using transvaginal ultrasound guidance, oocytes were aspirated into a Dulbecco’s phosphate-buffered saline (PBS) solution. The oocytes were rinsed and transferred into human tubal fluid (HTF) with 10% maternal serum and maintained at 37°C in a 5% CO₂ and air incubator. For ICSI, the cumulus and corona cells were stripped from the oocyte using a hyaluronidase rinse and mechanical manipulation with a Drummond pipette. Thereafter, the oocytes were rinsed with PBS and placed in HTF embryo culture medium until ICSI was performed or until semen was added for standard IVF.

Each semen specimen was collected in a sterile disposable container after 48 to 72 hr of abstinence on the day of oocyte retrieval. The sample was allowed to liquefy at room temperature for 30 min. Afterward, sperm concentration and percent motility were determined using a Makler chamber according to World Health Organization guidelines (15). The semen specimen then underwent a standardized method for fractionation of semen on a discontinuous density gradient

**Table I. Comparison of Sperm Parameters in the IVF and ICSI Patients**

<table>
<thead>
<tr>
<th>Sperm parameters</th>
<th>IVFᵃ (n = 182)</th>
<th>ICSIᵇ (n = 71)</th>
<th>ICSI swim-upᵇ (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)ᵃ</td>
<td>3.3 ± 1.7</td>
<td>2.8 ± 1.9</td>
<td>—</td>
</tr>
<tr>
<td>Concentration (10⁶/ml)ᵃ</td>
<td>55.8 ± 25.5</td>
<td>22.3 ± 14.9</td>
<td>6.5 ± 6.1</td>
</tr>
<tr>
<td>Total count (10⁶)ᵃ</td>
<td>184 ± 91.7</td>
<td>59.1 ± 53.8</td>
<td>7.32 ± 10.6</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>47 ± 19.6</td>
<td>34.9 ± 20.4</td>
<td>75.9 ± 20.5</td>
</tr>
<tr>
<td>Total motility count (10⁶)ᵃ</td>
<td>86.5 ± 21.9</td>
<td>21.8 ± 17.3</td>
<td>4.5 ± 4.1</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>43 ± 17.4</td>
<td>28.4 ± 21.3</td>
<td>59.5 ± 37.3</td>
</tr>
<tr>
<td>Total morphology count (10⁶)ᵃ</td>
<td>79.1 ± 20.2</td>
<td>16.8 ± 8.1</td>
<td>4.3 ± 2.7</td>
</tr>
</tbody>
</table>

ᵃ Using a one-way ANOVA all values between the three groups are statistically significant (P ≤ 0.05).
b All values are means ± standard deviation for each patient population.