Conventional in vitro fertilization maybe yields more available embryos than intracytoplasmic sperm injection for patients with no indications for ICSI

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Abstract: Many physicians suggest that performing ICSI instead of IVF for all cases just because they thought that ICSI yields more available embryos than IVF. However, we found that IVF results in better fertilization per retrieved oocyte (72.12 ± 19.60% versus 59.54 ± 21.38%, P < 0.01) and day 3 available embryo per retrieved oocyte rates (54.89 ± 23.53% versus 50.54 ± 22.68%, P < 0.05) than ICSI after analysis of 218 cycles using sibling oocytes in combined IVF/ICSI for patients with no indications for ICSI. We also found a positive correlation between the degeneration rate after ICSI, oocyte immaturity rate, and rate of 2 pn per retrieved oocyte obtained from IVF compared to ICSI, as well as the day 3 available embryo rate between IVF and ICSI. It is possible that outcome may be due to more in vitro-matured oocytes achieved in IVF fertilization compared with ICSI fertilization, and a considerable portion of the mature oocytes were degenerated after ICSI. Therefore, it is suggested that ICSI should not be performed in all cases of in vitro conception. IVF is preferable to ICSI for cases in which a relatively low possible fertilization failure occurs in conventional IVF.

Keywords: Sibling oocytes, combined IVF/ICSI, oocyte immaturity rate, oocyte degeneration rate, available embryos

Introduction

Since the first report of human pregnancies achieved by intracytoplasmic sperm injection (ICSI) [1], 20 years of improvements in the ICSI technique have been implemented. ICSI is being mainly used for three indications: severe or moderate male-factor infertility, low fertilization or fertilization failure in Conventional in vitro fertilization (conventional IVF) previously and unexplained infertility. ICSI performed by the indications is to improve the fertilization rate and decrease the fertilization failure rate because these cases maybe a high fertilization failure or low fertilization occurs after performing IVF [2].

But, some physicians have suggested that ICSI should be performed instead of IVF because they thought that ICSI should yield more available embryos (AE) than IVF in all cases, even though there are no indications for ICSI. However, Taylor et al. [3] reported that conventional IVF results in a higher FR, more AE were obtained from IVF than ICSI in patients with increased oocyte immaturity. Taylor et al. reasoned that during IVF the more immature oocytes undergo maturation and have an opportunity to fertilize spermatozoa in an incubator overnight, thus more AE were obtained by IVF than ICSI. In addition, ICSI is more invasive than IVF. Oocyte degeneration after ICSI reduces the number of mature oocytes for fertilization and can result in a lower FR, thereby decreasing the number of AE. Is it probable that the above differences in procedure between ICSI and IVF may affect the fertilization outcome, and finally affect the number of AE obtained?
In our infertility centre, ICSI were being mainly used for “above three indications” (male factor infertility, previous failed or low fertilization IVF cycle and unexplained infertility). But, combined IVF/ICSI would be performed due to the patient’s own desire to ICSI. These patients showed normal sperm and were well-defined female infertility (tubal, endometriosis or ovulatory dysfunction), and any of above indications were not fitted for them. And they desired to ICSI just because of fears of fertilization failure or want to obtain more embryos. So, dose ICSI yield more available embryos than IVF for these patients? To address the issue, we performed a retrospective analysis of 218 cycles using sibling oocytes in combined IVF/ICSI (SOI/I) for patient with no indications to ICSI (January 2011-December 2013).

Materials and methods

Patients

Between January 2011 and December 2013, 218 SOI/I cycles for patient with no indications to ICSI were considered for the study in the Infertility Centre. The patients ranged in age from 22 to 44 years, with a mean (± SD) of 32.9 ± 4.1 years. The primary causes of infertility were tubal (n = 135), endometriosis (n = 34), ovulatory dysfunction (n = 49).

The study was approved by the Ethics Committee of Peking University Third Hospital and all patients signed written informed consent.

Ovarian stimulation

Patients were down-regulated with gonadotropin-releasing hormone (GnRH) agonist using long or short protocols and stimulated with follicle-stimulating hormone (FSH) and human menopausal gonadotropins (hMG). Ovarian activity was monitored by regular ultrasound scans and serum sex hormone assays. A dose of 10,000 U of human chorionic gonadotrophin (hCG) was administered when the leading cohort follicle reached a diameter of 18-20 mm. Oocyte retrieval was performed through vaginal puncture under ultrasound guidance 36-38 hours after hCG administration [4].

Conventional IVF and ICSI

The oocytes retrieved from each patient were collected into the center of a 4-well dish containing medium, then divided evenly into 2 (if ≤ 10 oocytes) or 4 wells (if > 10 oocytes), and > 20 oocytes were divided two 4-well dishes, and so on. Embryos were divided into two groups by allotting the oocytes from the first well to conventional insemination or ICSI in alternating order. In IVF, oocytes were inseminated by conventional IVF 3-4 h after oocyte retrieval. Spermatozoa were collected using the swim-up technique with 100,000 motile spermatozoa per insemination dish. ICSI was performed, as previously described (Palermo et al., 1992).

The ICSI procedure was performed by seven operators and there were two kinds of micro-injection systems (oil- and air-hydraulic). In the oil-hydraulic system, the micromanipulation equipment consisted of a microinjector with an oil-hydraulic system and a microholder with an air-hydraulic system (Narishige, Japan). In the air-hydraulic system, the micromanipulation equipment consisted of a microinjector and a microholder, both with built-in air syringes (Research Instruments, England). Oocyte degeneration was identified at the time of the ICSI procedure and 16-18 h later when fertilization was assessed.

Fertilization and embryo assessment

Between 16 and 18 h after insemination, the IVF and the ICSI oocytes were examined for fertilization and groups were made for 0 pn, 1 pn, 2 pn, 3 pn, and ≥ 3 pn, and whether or not the oocyte had been degenerated after ICSI.

The cleavage of the 2 pn oocytes and the quality of the embryos were evaluated on day 3 (day 0 was the day of oocyte retrieval). Day 3 embryos were assessed and graded as AE (≥ 5 cells and ≤ 30% fragmentation, or 4 cells and ≤ 10% fragmentation). Those embryos with equal-sized 6-8 cells and ≤ 10% fragmentation were defined as good quality embryos. The embryo cleavage rate was calculated as the number of cleavage embryos divided by the number of 2 pn oocytes. The good quality embryo rate was calculated as the number of good quality embryos divided by the number of AE.

Statistical analysis

Data were analyzed using the chi-squared test and Pearson’s correlation coefficient, as appropriate. In per cycles, a rate of 2 pn rate per
Table 1. Fertilization, cleavage and embryos results after the 218 cycles using sibling oocytes in combined IVF/ICSI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IVF</th>
<th>ICSI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of oocytes</td>
<td>1,976</td>
<td>1,983</td>
<td></td>
</tr>
<tr>
<td>Total 2 PN/injected oocyte</td>
<td>71.00 (1,403/1,976)</td>
<td>72.02 (1171/1,626)</td>
<td>NS</td>
</tr>
<tr>
<td>Total 2 PN/assigned oocyte</td>
<td>71.00 (1,403/1,976)</td>
<td>59.05 (1171/1,983)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Mean fertilization rate per cycle/assigned oocyte</td>
<td>72.12 ± 19.60</td>
<td>59.54 ± 21.38</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cleavage embryos/2 pn oocytes</td>
<td>96.86 (1,359/1,403)</td>
<td>98.98 (1159/1,171)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Good-quality embryos/available embryos</td>
<td>73.54 (792/1,077)</td>
<td>74.64 (727/974)</td>
<td>NS</td>
</tr>
<tr>
<td>Total available embryos/injected oocyte</td>
<td>54.50 (1,077/1,976)</td>
<td>59.90 (974/1,626)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Total available embryos/assigned oocyte</td>
<td>54.50 (1,077/1,976)</td>
<td>49.12 (974/1,983)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Mean available embryos rate per cycle/assigned oocyte</td>
<td>54.89 ± 23.53</td>
<td>50.54 ± 22.68</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SD or %. Available embryos = embryos with ≥ 5 cells and ≤ 30% fragmentation, or 4 cells and ≤ 10% fragmentation; Good-quality embryos = embryos with equal-sized 6-8 cells and ≤ 10% fragmentation. NS: Not statistically significant.

In group ICSI, the degeneration rate was 7.32% (119/1626). The degeneration rates among the

Table 2. Oocytes degeneration rates among ICSI operators and micromanipulation system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ICSI operators</th>
<th>Micromanipulation system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>281</td>
<td>193</td>
</tr>
<tr>
<td>Degeneration rate (%)</td>
<td>8.19</td>
<td>6.21</td>
</tr>
<tr>
<td>P-value</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

system-oil = the micromanipulation equipment consisted of a microinjector with oil-hydraulic system and a microholder with air-hydraulic system (Narishige, Japan); system-air = the micromanipulation equipment consisted of a microinjector and a microholder both with built-in Air Syringes (Research Instruments, England). NS: Not statistically significant. *among ICSI operators. **micromanipulation system.

retrieved oocyte obtained from IVF (2PNR-IVF) to 2 pn rate per retrieved oocyte obtained from ICSI (2PNR-ICSI) was studied in relation to the oocyte immaturity and degeneration rates, as well as a rate of AE rate per retrieved oocyte obtained from IVF (AER-IVF) to AE rate per retrieved oocyte obtained from ICSI (AER-ICSI). All analyses were performed with the Statistical Package for Social Sciences (version 17.0; SPSS, Inc., Chicago, IL, USA). Statistical significance was defined as a P < 0.05.

Results

There were 218 SOI/I cycles in the current study; 1976 and 1, 983 oocyte complexes were randomly subjected to IVF and ICSI, respectively.

In IVF, 2 pn was observed in 1,403 oocytes on day 1 after oocyte retrieval. On day 3 after oocyte retrieval, there were 1,359 cleavage zygotes, 1,077 AE, and 792 good quality embryos (Table 1).

In ICSI there were 1,626 MII (injected by ICSI). The next day, 2 pn was observed in 1171 oocytes after ICSI, and a total of 119 degenerated oocytes were found. On day 3 after oocyte retrieval, there were 1159 cleavage zygotes, 974 AE, and 727 good quality embryos (Table 1).

On the basis of the number of oocytes assigned, the (mean) 2 pn rate was significantly higher in IVF than ICSI (71.00% [72.12 ± 19.60] vs. 59.05% [59.54 ± 21.38]). The cleavage rate was significantly higher in ICSI than IVF insemination (98.98% vs. 96.86%). There was no difference in the rate of good quality embryos between ICSI and IVF (74.64% vs. 73.54%). Finally, on the basis of the number of oocytes assigned, the mean AE rate was significantly higher in IVF than ICSI (54.50% [54.89 ± 23.53%] vs. 49.12% [50.54 ± 22.68%]; Table 1).

In group ICSI, the degeneration rate was 7.32% (119/1626). The degeneration rates among the
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Table 3. Pearson’s correlation coefficient between 2PNR-IVF/2PNR-ICSI, AER-IVF/AER-ICSI and oocyte immaturity rate, degeneration rate in 218 cycles using sibling oocytes in combined IVF/ICSI

<table>
<thead>
<tr>
<th>Cycle (n = 218)</th>
<th>Oocyte immaturity rate</th>
<th>Degeneration rate</th>
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<tbody>
<tr>
<td>2PNR-IVF/2PNR-ICSI</td>
<td>0.371a</td>
<td>0.513a</td>
</tr>
<tr>
<td>AER-IVF/AER-ICSI</td>
<td>0.254a</td>
<td>0.429a</td>
</tr>
</tbody>
</table>

2PNR-IVF = 2 PN rate per retrieved oocyte obtained from IVF, 2PNR-ICSI = 2 PN rate per retrieved oocyte obtained from ICSI; AER-IVF = available embryos rate per retrieved oocyte obtained from IVF, AER-ICSI = available embryos rate per retrieved oocyte obtained from ICSI. *P < 0.05.

seven different ICSI operators were not significantly different, as well as between the two micromanipulation systems (Table 2).

In this study, the rate of 2PNR-IVF to 2PNR-ICSI and AER-IVF to AER-ICSI were positively correlated with the degeneration and oocyte immaturity rates (Table 3).

Discussion

Many physicians suggest that performing ICSI instead of IVF for couples with no male factor infertility is justified because ICSI should yield more AE than IVF. However, our study showed that IVF results in better fertilization per retrieved oocyte and more formation of day 3 AE per retrieved oocyte than ICSI in SOI/I cycles for patient with no indications to ICSI. The outcome may be due to different procedures between ICSI and IVF techniques.

It is well-known that during ICSI only mature oocytes are injected, while after ICSI immature oocytes are discarded in most infertility centres. During conventional IVF oocytes are incubated in the presence of the spermatozoa overnight (16-18 h). Thus, the increased time in the incubator during IVF allows some immature oocytes to undergo maturation and have an opportunity to fertilize (undergo normal fertilization) with spermatozoa. As a result, conventional IVF can yield more fertilized oocytes and more AE for transfer or freezing.

In contrast, ICSI is more invasive than IVF and oocyte degeneration is a common phenomenon after performing ICSI. A total oocyte degeneration rate of 7-16% has been reported [5-8]. In the current study, the degeneration rate was 7.32% (119/1626), which is in agreement with the findings in previous studies. Clearly, oocyte degeneration after ICSI would reduce the number of mature oocytes to fertilization and could result in a lower FR, thereby decreasing the number of AE obtained. In the current study, the degeneration rates among ICSI operators and the two micromanipulation systems were not significantly different (Table 2), which further showed that oocyte degeneration is a common and inevitable phenomenon when using the current ICSI technique, regardless of operators or equipment. Furthermore, we found a positive correlation between the degeneration, oocyte immaturity, 2PNR-IVF to 2PNR-ICSI, and AER-IVF to AER-ICSI rates. These results strongly support the above inference that more in vitro-matured oocytes achieved normal fertilization in IVF compared with ICSI, and that a considerable portion of the oocytes were degenerated after ICSI, which would make less oocytes used by ICSI than IVF and more fertilized oocytes and AE obtained by IVF.

Although ICSI resulted in a lower FR and less AE in the current study, the good quality embryo rate did not differ between IVF and ICSI. This finding confirms the assumptions raised by others [3, 9-13]. Furthermore, embryo morphology is probably influenced by intrinsic gamete factors and not by the mode of fertilization [13].

It is difficult to study the developmental potential of in vitro-matured oocytes during IVF. However, there have been some studies on in vitro-matured oocytes in ICSI cycles, which may be illustrative [14-17]. Balakier et al. found that the cleavage rate was lower in in vitro-matured oocytes compared with MII oocytes at denudation. Based on the above results, it is possible that more in vitro-matured oocytes are obtained by NORMAL FERTILIZATION during IVF than ICSI, thus resulting in a lower cleavage rate during IVF than ICSI. The dates of this study agree with the above assumption; specifically, we demonstrated that there was a lower cleavage rate in IVF compared with ICSI. In addition, ICSI is more invasive than IVF and some oocytes were damaged after ICSI. As previously reported, oocytes with a fragile oolemma are considered to be an indicator of degeneration [6, 18], which is demonstrated by lack of resistance upon needle entry or sudden breakage. Those oocytes with oolemma that undergo sudden
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breakage, which would be degeneration after ICSI, are “rescued” by performing IVF. What is the final fate for those oocytes during IVF? In the Palermo study, it was shown that oocytes with membranes that undergo sudden breakage were lower in number of cleaved embryos compared to the other two membrane patterns (successful injection and difficult breakage), which showed a similar cleavage ability. Therefore, it is possible that the oocytes susceptible to degeneration may have a lower cleavage rate. In the current study the embryo cleavage rate was lower in IVF than ICSI, and it is likely, in part, because more oocytes, which would degenerate after ICSI, were “temporarily rescued” by performing IVF. All in all, IVF had a lower cleavage rate compared with ICSI, most likely due to the mode of fertilization.

Safety issues regarding ICSI have been a continuous concern since the report of the first infant born after ICSI. Some studies have suggested no increase in the incidence of congenital anomalies in infants born after ICSI compared with IVF or with spontaneous conception [19-22]. However, other authors have demonstrated that infants born after ICSI might indeed have an excess of major birth defects [23-25] and an increased risk of chromosomal abnormalities [19, 26]. Furthermore, the most recent studies have shown that pubertal ICSI girls were less advanced in breast development [27] and ICSI adolescents with advanced pubertal stages showed more peripheral adiposity [28]. These results are possibly due to factors related to paternal infertility and the use of suboptimal male gametes or the technique itself. Given the relatively brief history of ICSI, and its potential risk to offspring, it would seem prudent to avoid over-application of this technology.

The AE obtained mainly depend on patient clinical characteristics and individual controlled ovarian stimulation. We all know the differences between conventional IVF and ICSI in relation with immaturity, degeneration. But few realized that the differences might affect the AE obtained. The current study showed that performing IVF would result in more AE compared with ICSI for patient with no indications to ICSI in combined SOI/I cycles. The outcome may be a result of more in vitro-matured oocytes achieved during normal fertilization in IVF compared with ICSI, and a considerable part of the mature oocytes were degenerated after ICSI. In fact, the question is not whether IVF is superior to ICSI, but whether or not ICSI should yield more AE than IVF in all cases. The necessary indications for ICSI are required. It is well-known that ICSI bypasses multiple natural mechanisms and the long-term outcome of the ICSI procedure in terms of development and transfer of genetic disorders is still undefined. Thus, it is suggested that ICSI should not be performed in all cases involving in vitro conception. IVF is preferable to ICSI if cases of a very low probability of failure or low fertilization occur after performing IVF.

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Disclosure of conflict of interest

None.

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