ORIGINAL ARTICLE

Comparison of IVF outcomes using conventional insemination and ICSI in ovarian cycles in which only one or two oocytes are obtained

Comparaison de la FIV avec insémination classique et ICSI en cycles ovariens dans laquelle une seule ou deux ovocytes sont obtenues

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KEYWORDS
IVF; ICSI; Poor ovarian response; Fertilization rate; Good-quality embryo rate; Pregnancy rate; Single oocyte retrieval

Summary
Objective. — Compare outcomes of patients in whom only one or two oocytes were retrieved who underwent conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Materials and methods. — Patients who received IVF and only one to two oocytes were obtained on the day of oocyte retrieval. Fertilization rate, good-quality embryo rate, pregnancy Demographic data and clinical characteristics were recorded and analyzed.

Results. — Of 194 patients, 118 received conventional IVF and 76 ICSI. There were no significant differences in age (mean age, 36 years in both groups), infertility parameters, and number of oocytes retrieved between the groups. ICSI patients had a higher fertilization rate (82.7% vs. 67.0%) and 2PN fertilization rate (78.8% vs. 59.7%); however, no difference in good-quality embryo rate or PR was noted. For women less than 35 years of age, there was no significant difference in these outcome parameters between the groups. For patients greater or equal to 35 years of age ICSI was associated with a higher fertilization rate (83.1% vs. 62.4%) and 2PN fertilization rate (80.3% vs. 55.0%); however, there was no difference in PR.

Conclusions. — ICSI did not improve the good-quality embryo rate or clinical PR rate compared to conventional IVF using semen with normal parameters in women with poor ovarian reserve.

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Introduction

There are an increasing number of patients with a poor ovarian response during in vitro fertilization (IVF) — embryo transfer due to the postponement of childbearing and the effects of reproductive system surgery, and the reported incidence is 9 to 24% [1]. The cycle cancellation rate is high, and fertilization and pregnancy rates (PRs) are low in patients with a poor ovarian response who undergo IVF, and the number of oocytes obtained during an ovarian cycle is only one to two in some patients [2,3]. Thus, when the number of oocytes is limited it is important to choose the most appropriate fertilization method to maximize the chances of a successful pregnancy.

Intracytoplasmic sperm injection (ICSI) is an alternative to conventional insemination for patients undergoing IVF that involves injection of a single spermatozoon into a mature oocyte. ICSI has been adopted by many centers, especially in instances of male azoospermia, unexplained infertility, as well as low oocyte retrieval [4–6]. However, conflicting data exists if PR are improved with ICSI, which is a more complex and more expensive procedure than conventional insemination, and is not without inherent risks [7].

Gozlan et al. [4] compared ICSI with conventional insemination in patients with single oocyte retrieval and found that in patients less or equal to 39 years old or greater than 39 years old with favorable semen quality fertilization rates and PRs were similar, whereas in cases of poor semen quality ICSI was associated with higher fertilization rates but no difference in PRs in the lower age women, but both higher fertilization rates and PRs in the women greater than 39 years of age. Ou et al. [6] reported a higher normal fertilization rate with ICSI than conventional IVF, but no difference in implantation rate or clinical PR. Check et al. [8], in a study of patients with unexplained infertility, found a significantly higher fertilization rate with ICSI than conventional insemination, but significantly higher clinical and live delivered PRs with conventional insemination. Other studies have reported that there is no difference in the maximum number of blastomere numbers is cycles using conventional insemination versus ICSI [9], no difference in embryo quality with ICSI versus conventional insemination [10], and that ICSI embryos may be less likely to implant [11].

Thus, the purpose of this retrospective analysis was to compare the outcomes of patients with poor ovarian reserve in whom only one or two oocytes were retrieved during a cycle who underwent conventional IVF and ICSI.

Methods

Subjects

We retrospectively analyzed the clinical data of patients who received IVF at our center from January 2007 to December 2010, and only one to two oocytes were obtained on the day of oocyte retrieval. Patients were excluded from the analysis if: male oligoasthenospermia was present, they had received pre-implantation genetic diagnosis, and a chromosomal abnormality was present in at least one person of the couple. This study was approved by the Institutional Review Board of our hospital, and all patients provided written informed consent to the treatments they received.

Controlled ovarian hyperstimulation

Triptorelin acetate 0.1 mg/day was administered during the early stage of IVF, the mid-luteal phase, or after the start of the menstrual cycle in that ovarian cycle until human chorionic gonadotropin (HCG) was injected. Follicle-stimulating hormone (FSH) was given to promote follicle development when the serum estradiol (E2) level was less than 30 pg/mL. The dosage of FSH was 150–225 IU/day. Transvaginal B-mode ultrasound, urinary luteinizing hormone (LH), and serum
E2 were used to monitor follicle growth. When a dominant follicle had a diameter greater or equal to 18 mm or two dominant follicles had a diameter greater or equal to 17 mm, an intramuscular injection of 10,000 U HCG was given. B-mode ultrasound-guided transvaginal puncture and oocyte retrieval was carried out 34–36 h after HCG injection. Oocyte retrievals were performed by persons of similar experience.

Sperm treatment

On the day of oocyte retrieval, conventional semen analysis was carried out according to the World Health Organization (WHO) laboratory standards of human semen and sperm [12]. Normal semen was defined as having a sperm density of greater or equal to $20 \times 10^6$/mL and motility grade a greater or equal to 25%, or (grade a + grade b) greater or equal to 50%. Oligoasthenospermia was defined as a sperm density of less than $20 \times 10^6$/mL and motility grade a less than 25%, or (grade a + grade b) less than 50%. Motile sperm were collected using gradient centrifugation and the swim up method.

Determination of fertilization and successful fertilization

Insemination was carried out 4 h after oocyte retrieval. The conventional IVF insemination density was 10,000 live sperms/egg. For patients who received ICSI, hyaluronidase was used to remove granular cells before ICSI was performed. ICSI was only performed for oocytes in M II phase. After fertilization, samples were placed in an incubator at 37 °C in a 5–6% CO$_2$ atmosphere. The cells were examined after 16 to 20 h to determine whether a pronucleus was present in order to determine whether fertilization occurred. Appearance of two pronuclei indicated successful fertilization. ICSI was performed by persons of similar experience.

Embryo observation and transfer

Ovum cleavage was observed, and the embryo score was assessed 48 h (D2) and 72 h (D3) after oocyte retrieval. The criterion for a good-quality embryo on D2 was greater than four cells, and on D3 was greater or equal to six embryonic blastomeres and that the embryonic blastomeres were uniform in size with less 20% fragments. Embryos with more than four cells and less than 20% fragments on the third day after oocyte retrieval were chosen for embryo transfer.

Observation of clinical pregnancy

Urine and blood HCG was measured 14 days after embryo transfer, and positive results indicated biochemical pregnancy. Clinical pregnancy was indicated by identification of an intrauterine gestational sac and fetal heart beat by
Conventional insemination vs. ICSI

Table 2  Outcomes of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).
Les résultats d’IVF et ICSI.

<table>
<thead>
<tr>
<th></th>
<th>IVF (n = 118)</th>
<th>ICSI (n = 76)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsuccessful cycles(^{a,b})</td>
<td>1 (0, 2)</td>
<td>1 (0, 2)</td>
<td>0.052</td>
</tr>
<tr>
<td>Cycles cancelled(^{c,d})</td>
<td>38 (32.2)</td>
<td>22 (28.9)</td>
<td>0.632</td>
</tr>
<tr>
<td>Pregnancy rate(^{c,d})</td>
<td>16 (13.6)</td>
<td>8 (10.5)</td>
<td>0.531</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>176</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate(^{c,d})</td>
<td>118 (67.0)</td>
<td>86 (82.7)</td>
<td>0.004(^e)</td>
</tr>
<tr>
<td>2PN fertilization rate(^{c,d})</td>
<td>105 (59.7)</td>
<td>82 (78.8)</td>
<td>0.001(^e)</td>
</tr>
<tr>
<td>2PN embryo cleavage rate(^{c,d})</td>
<td>100 (95.2)</td>
<td>79 (96.3)</td>
<td>0.711</td>
</tr>
<tr>
<td>Good-quality embryo rate(^{c,d})</td>
<td>70 (70.0)</td>
<td>48 (60.8)</td>
<td>0.195</td>
</tr>
</tbody>
</table>

Pregnancy rate is expressed as pregnancies per transfers.  
\(^a\) P-values are based on Mann-Whitney U test.  
\(^b\) Data are presented as median and interquartile range.  
\(^c\) P-values are based on Chi\(^2\) test.  
\(^d\) Data are presented as number (percentage).  
\(^e\) Significant difference between the two groups, \(P < 0.05\).

B-mode ultrasound 5 weeks after embryo transfer. PR was described as clinical pregnancies per transfers.

Statistical analysis

Comparability between the IVF and ICSI groups was tested using independent two-sample \(t\) test for continuous variables and Chi\(^2\) test/Fisher’s exact test for categorical variables. Continuous variables were represented as mean ± standard deviation (SD) and categorical data were represented by number (n) and percentage (%). The number of unsuccessful cycles between the IVF and ICSI groups was compared using the Mann-Whitney U test. Logistic regression analysis was performed to assess outcomes adjusting for confounding factors. All statistical assessments were two-sided and evaluated at the 0.05 level of significance. Statistical analyses were performed using SPSS 15.0 statistics software (SPSS Inc., Chicago, IL, USA).

Results

The study group consisted of 194 infertile patients undergoing assisted reproduction treatment between the years 2007 and 2010 at our hospital. All male partners had normal sperm characteristics as defined by the WHO criteria (12). Of the 194 patients, 118 patients (60.8%) received conventional IVF and 76 patients (39.2%) received ICSI. The mean age of the females in the IVF group was 36.14 ± 4.57 years.

Table 3  Outcomes of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) stratified by patient age.  
Les résultats d’IVF et ICSI par l’âge.

<table>
<thead>
<tr>
<th></th>
<th>Age &lt; 35 years (n = 66)</th>
<th>Age ≥ 35 years (n = 128)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF (n = 45)</td>
<td>ICSI (n = 21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsuccessful cycles(^{a,b})</td>
<td>0 (0, 1)</td>
<td>1 (0, 2)</td>
<td>0.317</td>
</tr>
<tr>
<td>Cycles cancelled(^{c,d})</td>
<td>10 (22.2)</td>
<td>4 (19.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Pregnancy rate(^{c,d})</td>
<td>6 (13.3)</td>
<td>4 (19.0)</td>
<td>0.714</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>67</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate(^{c,d})</td>
<td>50 (74.6)</td>
<td>27 (81.8)</td>
<td>0.422</td>
</tr>
<tr>
<td>2PN fertilization rate(^{c,d})</td>
<td>45 (67.2)</td>
<td>25 (75.8)</td>
<td>0.378</td>
</tr>
<tr>
<td>2PN embryo cleavage rate(^{c,d})</td>
<td>44 (97.8)</td>
<td>24 (96.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Good-quality embryo rate(^{c,d})</td>
<td>26 (59.1)</td>
<td>14 (58.3)</td>
<td>0.952</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>IVF (n = 73)</td>
<td>ICSI (n = 55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsuccessful cycles(^{a,b})</td>
<td>1 (0, 2)</td>
<td>1 (0, 2)</td>
<td>0.121</td>
</tr>
<tr>
<td>Cycles cancelled(^{c,d})</td>
<td>28 (38.4)</td>
<td>18 (32.7)</td>
<td>0.511</td>
</tr>
<tr>
<td>Pregnancy rate(^{c,d})</td>
<td>10 (13.7)</td>
<td>4 (7.3)</td>
<td>0.249</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>109</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate(^{c,d})</td>
<td>68 (62.4)</td>
<td>59 (83.1)</td>
<td>0.003(^e)</td>
</tr>
<tr>
<td>2PN fertilization rate(^{c,d})</td>
<td>60 (55.0)</td>
<td>57 (80.3)</td>
<td>0.001(^e)</td>
</tr>
<tr>
<td>2PN embryo cleavage rate(^{c,d})</td>
<td>56 (93.3)</td>
<td>55 (96.5)</td>
<td>0.680</td>
</tr>
<tr>
<td>Good-quality embryo rate(^{c,d})</td>
<td>44 (78.6)</td>
<td>34 (61.8)</td>
<td>0.054</td>
</tr>
</tbody>
</table>

Pregnancy rate is expressed as pregnancies per transfers.  
\(^a\) P-values are based on Mann-Whitney U test.  
\(^b\) Data are presented as median and interquartile range.  
\(^c\) P-values are based on Chi\(^2\) test.  
\(^d\) Data are presented as number (percentage).  
\(^e\) Significant difference between the two groups, \(P < 0.05\).
Table 4  Comparison of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in patients with single oocyte retrieval.

<table>
<thead>
<tr>
<th></th>
<th>IVF (n = 60)</th>
<th>ICSI (n = 34)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsuccessful cycles³</td>
<td>1 (0, 2)</td>
<td>1 (1, 3)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Cycles cancelled⁴</td>
<td>29 (48.3)</td>
<td>12 (35.3)</td>
<td>0.221</td>
</tr>
<tr>
<td>Pregnancy rate⁵</td>
<td>6 (10.0)</td>
<td>2 (5.9)</td>
<td>0.706</td>
</tr>
<tr>
<td>Fertilization rate⁶</td>
<td>40 (66.7)</td>
<td>29 (85.3)</td>
<td>0.050</td>
</tr>
<tr>
<td>2PN fertilization rate⁷</td>
<td>33 (55.0)</td>
<td>29 (85.3)</td>
<td>0.003*</td>
</tr>
<tr>
<td>2PN embryo cleavage rate⁸</td>
<td>31 (93.9)</td>
<td>27 (93.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Good-quality embryo rate⁹</td>
<td>20 (64.5)</td>
<td>16 (59.3)</td>
<td>0.614</td>
</tr>
</tbody>
</table>

Pregnancy rate is expressed as pregnancies per transfers.
³ P-values are based on Mann-Whitney U test.
⁴ Data are presented as median and interquartile range.
⁵ P-values are based on Chi² test/Fisher’s exact test.
⁶ Data are presented as number (percentage).
⁷ Significant difference between the two groups, P < 0.05

and in the ICSI group was 36.64 ± 4.66 years. Demographic data and clinical characteristics of the patients in the two groups are presented in Table 1. There were no significant differences in age, FSH level, primary or secondary infertility, duration of infertility, cycles, and number of oocytes retrieved between the two groups (all, P > 0.05). In the IVF group, oviduct abnormalities accounted for 63.6% of the reasons for infertility, as compared with 40.8% in the ICSI group (P = 0.002), and unknown reasons for infertility accounted for 9.3% of the cases in the IVF-ET group as compared to 26.3% in the ICSI group (P = 0.002). The two groups were similar with respect to pelvic adhesions and endometriosis as the reason for infertility. The clinical outcomes between the IVF-ET and ICSI groups are shown in Table 2. There were significant differences in fertilization rates and two pronuclear (2PN) fertilization rates between the two group (P < 0.005). Patients who underwent ICSI had a higher fertilization rate (82.7% vs. 67.0%, respectively, P = 0.004) and a 2PN fertilization rate (78.8% vs. 59.7%, respectively, P = 0.001) than those who underwent IVF. No difference, however, was noted in good-quality embryo rate, PR, or ongoing delivery rate between the two groups. The miscarriage rates in the IVF and ICSI groups were 1/16 and 1/8, respectively.

Of 194 patients, 66 were less than 35 years of age and 128 were greater or equal to 35 years of age. The clinical outcomes of IVF and ICSI stratified by patient age are shown in Table 3. For women less than 35 years of age, there was no significant difference in number of unsuccessful cycles, cycles cancelled, PR, fertilization rate, 2PN fertilization rate, 2PN embryo cleavage rate, and good-quality embryo rate between the IVF and ICSI groups (all, P > 0.005). However, for patients greater or equal to 35 years of age those who underwent ICSI had a higher fertilization rate (83.1% vs. 62.4%, respectively, P = 0.003) and 2PN fertilization rate (80.3% vs. 55.0%, respectively, P = 0.001) than those who underwent IVF. However, there was no difference in PR.

Of the 194 women, 94 (48.5%) had single oocyte retrieval. In patients with single oocyte retrieval (n = 94; 48.5%), those who underwent ICSI had a higher number of unsuccessful cycles (P = 0.012) and 2PN fertilization rate (85.3% vs. 55.0%, respectively, P = 0.003) than those who underwent IVF (Table 4). No significant difference in cycles cancelled, PR, fertilization rate, 2PN embryo cleavage rate, and good-quality embryo rate between the two groups were found (P > 0.05). Logistic regression analysis was performed to assess outcomes adjusting for confounding factors, and no differences were found (Table 5).

Discussion

This retrospective analysis revealed that the fertilization rate of ICSI was significantly higher than that of the IVF, yet there were no significant differences in duration of infertility, cleavage rate, good-quality embryo rate, or clinical PR between the two groups, suggesting that ICSI can only improve the fertilization rate without improving the good-quality embryo rate or clinical PR, or ongoing delivery rate. For patients with poor ovarian response, the number of mature eggs obtained in each cycle is low, and the eggs are particularly valuable for cycles in which only one or two mature eggs are obtained. Causes and the exact mechanism of poor ovarian response are still unclear, but age is an important factor. With increasing age, the number of oocytes reserved in the ovary decreases significantly, and poor ovarian response is closely related to ovarian reserve capacity. This reduction process is accelerated after 35 years of age [13,14]. In our analysis, the fertilization rate and the percentage of cycles without any fertilized oocytes were significantly different between the ICSI and IVF groups in patients greater or equal to 35 years of age. The fertilization rate after ICSI was higher than after IVF, and the percent-age of cycles without any fertilized oocytes after ICSI was lower than after IVF. In patients less than 35 years of age, the fertilization rate, cleavage rate, good-quality embryo rate, embryo transfer cancellation rate, and clinical PR were not significantly different between the IVF and ICSI groups. These data suggest that for older women in whom only one to two oocytes can be obtained, ICSI can improve the fertilization rate but not the pregnancy outcome. For cycles in
which only one oocyte was obtained, ICSI only improved the fertilization rate.

Some authors believe that all patients with poor ovarian response in whom only one to three eggs are obtained should receive ICSI [6,15]. An opposite view is that pregnancy outcome is not related to insemination method in patients with poor ovarian response, and that semen parameters on the day of oocyte retrieval are the most important factor affecting the decision to perform ICSI in patients with poor ovarian response. Gozlan et al. [14] reported that the outcomes of IVF and ICSI are similar when the sperm density is greater or equal to $20 \times 10^6$/mL and the motility rate is greater or equal to 35% and with these conditions IVF is recommended. Check et al. [8] reported a higher fertilization rate with ICSI in patients with unexplained infertility, but higher clinical and live delivered PR with conventional IVF.

If the semen quality is poor, better pregnancy outcomes can be obtained with ICSI [4]. Under natural conditions, sperms with motility grade a + grade b greater or equal to 30% and greater or equal to 50% will exhibit the same fertilization capacity, and the pregnancy outcome will not be affected. When the motility grade a + grade b is less than 30%, fertilization capacity may be affected to a certain extent resulting in a decreased PR [16]. Hershlag et al. [15] conducted a prospective cohort study and found that the fertilization rate after ICSI and IVF (58.1% and 56.7%, respectively) was not significantly different when the male semen parameters were at the cut off value. These studies suggest that conventional IVF should be recommended when semen parameters are within the normal range.

Our results showed that ICSI could achieve a better fertilization rate in patients in whom only 1 to 2 eggs were obtained in a cycle, but the clinical PR was not improved. A possible reason is that the quality of eggs is poor in patients with poor ovarian response, and for ICSI oocytes in M II phase are chosen and some non-M II oocytes are abandoned, thereby increasing the fertilization rate after ICSI. However, it has been shown that ICSI does not improve embryo quality [10,17], and our analysis supports this view. The good-quality embryo rate in the ICSI group in our study was not higher than in the conventional IVF group, and the reduction in the good-quality embryo rate may have decreased the clinical PR.

Criteria for treatment selection should not only consider the efficiency of the treatment methods, but also consider the corresponding risks and safety issues. During ICSI, mechanical damage to oocytes may decrease the quality of embryos and the clinical PR, and abnormal oocytes that should have been eliminated through natural selection of sperms may also be fertilized [7]. Some studies have indicated that ICSI may increase the risk of chromosomal aneuploidy [7,18,19], though others have indicated that any increase is not related to the ICSI procedure, but to semen alterations [18]. Though the evidence is inconclusive, this is a factor that should be considered when considering this alternative and one which may account for a higher fertilization rate with ICSI but no difference in PR found in some studies.

There are limitations of this study that should be considered. First are its retrospective nature and the relatively small number of patients. Second, there were differences between the two groups with respect to the causes of infertility, and it is not certain if this may have affected the findings. Lastly, we choose a threshold age of 35 years for subgroup analysis as that is the age considered “elderly” for pregnancy; results may have been different if a different age had been used.

In summary, in cycles in which one to two oocytes were obtained in women above and below the age of 35 years, ICSI did not improve the good-quality embryo rate or clinical PR compared to conventional IVF using semen with normal parameters. Based on these results, ICSI should not be recommended for all cycles in which only one to two oocytes are obtained when semen parameters are normal; conventional IVF should be performed.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

*Contribution to authorship*: Cong Fang carried out the study design, experimental studies and manuscript preparation. Jie Tang carried out the experimental studies. Rui Huang and Xiao-yan Liang carried out the clinical studies.
Li-lin Li carried out the literature research. Min-fang Zhang carried out the data acquisition.

Details of ethics approval: This study was approved by the Institutional Review Board of our hospital, and all patients provided written informed consent to the treatments they received.

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References


