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# Conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI): which is preferred for advanced age patients with five or fewer oocytes retrieved?

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## Abstract

**Purpose** To investigate the clinical outcomes of conventional IVF and ICSI in female patients aged 40 years and over with no more than five oocytes retrieved and non-male factor infertility.

**Methods** A retrospective study of a cohort of 644 patients undergoing IVF/ICSI treatment. The 534 female patients aged ≧ 40 years with no more than five oocytes retrieved and non-male factor infertility undergoing their first conventional IVF cycles were assigned in IVF group. The rest of 110 patients aged 40 years and over with no more than five oocytes retrieved and non-male factor infertility undergoing first ICSI cycles were recruited in ICSI group.

**Results** Our results showed the clinical pregnancy, live birth and miscarriage rates were similar between the IVF and ICSI groups (21.59% vs. 13.25%, $P > 0.05$; 12.16% vs. 6.02%, $P > 0.05$; 43.68% vs. 54.55%, $P > 0.05$; respectively), however, the implantation and cumulative live-birth rates were significantly higher in the IVF compared to the ICSI group (15.11% vs. 7.75%, 14.59% vs. 5.56%, $P < 0.05$), though the IVF group had a lower normal fertilization rate (61.56% vs. 76.00%, $P < 0.001$).

**Conclusions** Our study provides strong evidences that the conventional IVF exhibits advantages over the ICSI method in non-male factor infertility for advanced age patients with five or fewer oocytes retrieved.

**Keywords** Advanced-age female · In vitro fertilization · Intracytoplasmic sperm injection · Non-male factor infertility · Cumulative live birth rate

## Introduction

The nowadays society trends are increasingly focused on pursing education and careers, and therefore, women delay childbearing toward their 30s or even 40s [1]. In China, this trend has exponentially grown also due to the implementation of universal two-child policy. Generally, women refer to infertility treatment when encounter difficulties in conceiving, expecting that the assisted reproductive technology (ART) will overcome their issues; however, the clinical pregnancy rates of advanced-age women undergoing ART treatment are dissatisfied and negatively related to the maternal age. Ron et al. have reported a live-birth rate of 7, 2, 7 and 0% in women aged 41, 42, 43 and 44 undertaking regular IVF cycles [2]. In a similar trend, in another research conducted by Gray et al., clinical pregnancy rates of 7.7, 5.4, and 1.9% in women of age 42, 43 and 44 have been observed, whereas the patients over 45 have shown no pregnancy [3].

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One of the main reasons that leads to less efficiency in IVF/ICSI outcomes in advanced-age female is represented by a general decline of the quality and quantity of collected oocytes, regardless of the stimulation protocol [4–7]. The previously reported studies on IVF oocytes have highlighted that oocyte aneuploidy rate increases with age, going exponentially from 10% in women < 35 years, to 30, 40, and 100% at the ages of 40, 43, and over 45, respectively [8]. Additionally, advanced-age females are facing maternal and obstetrical complications, including prematurity, maternal, fetal and neonatal death, gestational diabetes, genomic disorders and low fetal birth weight [9, 10].

For those cases of advanced-age with a few oocytes retrieved, their oocytes are invaluable, and avoiding embryo losses is of paramount importance. Therefore, the appropriate selection of the most effective fertilization strategy to boost their chances of a successful live-birth is essential. The intracytoplasmic sperm injection (ICSI) has emerged as a promising alternative due to its high fertilization rates; the proportion of ICSI during IVF cycles has increased in the USA from 34% in 1996 to 76% in 2012 [11]. A recent report released by the International Committee for Monitoring Assisted Reproductive Technologies, the ICSI method is even conducted with 100% cycles in several countries [12]. Regardless of its increasing application prospects, there is no reliable evidence that ICSI can lead to improved clinical outcomes. Currently, numerous therapists prefer ICSI as an optimal approach to ensure high fertilization rates and obtain a sufficient embryos transfer in advanced-age women with fewer oocytes retrieved without male factor infertility. However, to the best of authors' knowledge, literature shows limited evidence that would favor this practice. Therefore, in the current study, we investigated the performance of standard IVF and ICSI in non-male factor infertility in women with age ≥ 40 years and ≤ 5 oocytes obtained,and identified IVF, not ICSI, produced a higher implantation rate and a cumulative live birth rate.

## Materials and methods

### Study design

The study was performed at the Center for Reproductive Medicine, Shandong University. A total of 534 subjects from IVF group and 110 subjects from ICSI group were collected from June 2011 to May 2016. The data selection process is detailed in Fig. 1. The study groups met the following inclusion criteria: (1) female of age 40–43 years, (2) first ART cycle for IVF or ICSI, (4) short protocol used in ovarian hyperstimulation, (5) the number of oocytes retrieved to be no more than five, (6) male partners with normal spermatozoa, according to the criteria provided by the WHO fifth edition sperm parameter reference values. An informed consent has been obtained from all subjects and the study was approved by the Institutional Review Board of Reproductive Medicine, Shandong University.

### Ovarian stimulation

The short protocol used for all patients under investigation included the administration of a dose (0.1 mg) of triptorelin acetate (Ferring AG, Switzerland) on day 3 of menstrual cycle. One or 2 days later, rFSH (Gonal-F, Serono, Switzerland) was daily administered until the hCG trigger day. This was followed by oocyte retrieval 36 h after hCG administration.

### Insemination and embryo transfer

IVF insemination was performed in 4-well plates containing approximately 100,000 motile spermatozoa for each oocyte. ICSI was carried out under an inverted microscope (Nikon, Japan), and all procedures employed sequential culture media supplied by Vitrolife (G-IVF, G1 and G2; Scandinavian IVF Science, Sweden); embryo transfers were performed using a Wallace catheter under ultrasound guidance. According to Pussant's criteria, if there were more than one good-quality embryos on day 3, embryo transfer were conducted on day 3, or all embryos were incubated up to the blastocyst stage [13]. The blastocysts were transferred and evaluated on day 5 over 4BC grade in accordance to Garden and Lane's criteria [14]. For frozen embryo transfer (FET) cycles, the vitrified blastocysts were thawed on the morning of the transfer day, and received subsequent assisted hatching 30 min after thawing. The embryos were cultured for 4–5 h at 37 °C, 6% $CO_2$ was introduced before the transfer, and only the expanded blastocysts were transferred.

### Diagnosis of pregnancy

The success of the clinical pregnancy was confirmed by monitoring the cardiac activity 7 weeks after the embryo transfer. Miscarriage was defined as a pregnancy loss before 28 weeks.

### Statistical analysis

The software SPSS version 16.0 was used for statistical analysis. The Student's *t*-distribution and $X^2$ test or Fisher's exact test were used to obtain group comparisons. A $P$ value < 0.05 was considered statistically significant. Clinical outcomes were compared with logistic regression analysis, adjusting for primary infertility (%). We also controlled for female age, number of oocytes retrieved and endometrial

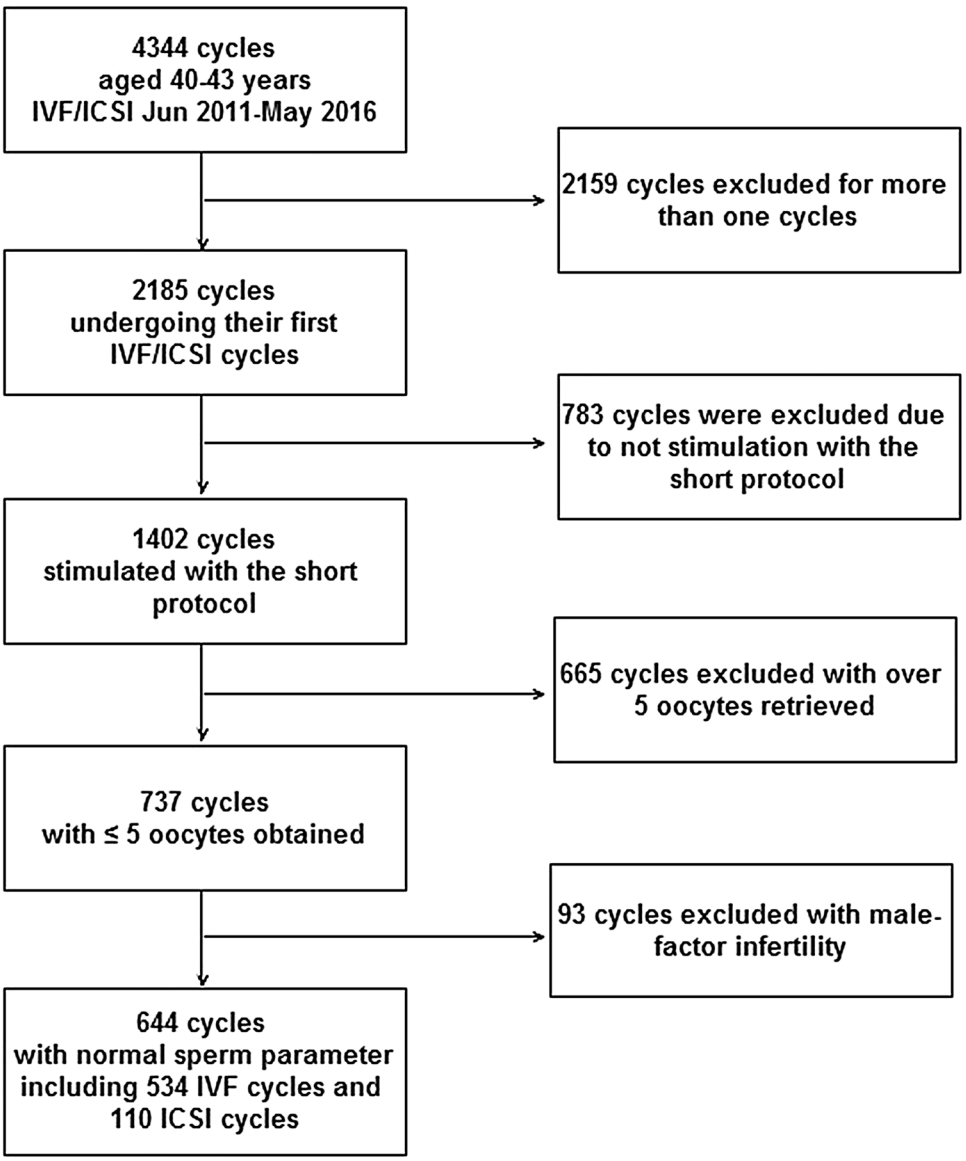


**Fig. 1** Flowchart of patients selection

thickness due to its potential significance although the three parameters were similar between the groups.

## Results

### Baseline characteristics

The baseline characteristics were compared between the IVF group with 534 subjects and the ICSI group involving 110 subjects. As shown in Table 1, except for the percentage of primary infertility which was lower in the IVF group (11.99% vs. 31.82%, $P < 0.001$), there were no other significant differences on the baseline characteristics between the IVF group and the ICSI group.

### IVF/ICSI embryonic outcomes

As listed in Table 2, there were no statistical significance in the fertilization, good quality embryo and cancelation rates between the two groups. As expected, the normal fertilization rate of IVF group was lower compared to the ICSI group (61.56% vs. 76.00%, $P < 0.001$). In addition, the mean number of embryos transferred, the percentage of day 3 embryo transfer and day 5 embryo transfer were similar between the two groups.

### IVF/ICSI clinical outcomes

As shown in Table 3, the clinical pregnancy and live birth rates in the IVF group were higher than those in the ICSI group (21.59% vs. 13.25%, $P > 0.05$; 12.16% vs. 6.02%,

**Table 1** Clinical characteristics of IVF/ICSI subjects

| | IVF ($n = 534$) | ICSI (n = 110) | $P$ value |
|---|---|---|---|
| Female age (years) (± SD) | 41.34 ± 1.08 | 41.32 ± 1.01 | 0.86[a] |
| BMI (kg/m$^2$) | 24.33 ± 3.27 | 24.27 ± 3.06 | 0.85[a] |
| Years of infertility | 5.73 ± 4.98 | 6.80 ± 5.52 | 0.06[a] |
| Primary infertility (%) | 11.99% (64/534) | 31.82% (35/110) | < 0.001[b] |
| Basal serum FSH (IU/l) | 9.10 ± 3.77 | 8.76 ± 2.97 | 0.38[a] |
| E2 on hCG trigger day (pg/ml) | 1577.30 ± 761.04 | 1701.52 ± 688.31 | 0.11[a] |
| Total dose of Gn administrated (IU) | 2506.30 ± 1253.36 | 2417.70 ± 1339.66 | 0.51[a] |
| Endometrial thickness (cm) | 1.01 ± 0.20 | 0.99 ± 0.22 | 0.50[a] |

Values are presented as number (%) or mean ± SD

[a] $t$ test

[b] $\chi^2$ test or fisher's exact test

**Table 2** IVF/ICSI outcomes of advanced age patients undergoing their first IVF/ICSI cycles

| | IVF ($n = 534$) | ICSI ($n = 110$) | $P$ value |
|---|---|---|---|
| Number of oocytes retrieved | 3.25 ± 1.35 | 3.39 ± 1.24 | 0.31[a] |
| Fertilization rate | 83.63% (1451/1735) | 83.69% (272/325) | 0.98[b] |
| Normal fertilization rate | 61.56% (1068/1735) | 76.00% (247/325) | < 0.001[b] |
| Good quality embryo rate | 63.01% (673/1068) | 62.75% (155/247) | 0.94[b] |
| Good quality embryo rate per oocyte | 38.79% (673/1735) | 41.55% (155/373) | 0.32[b] |
| Cancelation rate | 18.54% (99/534) | 16.36% (18/110) | 0.59[b] |
| Number of embryos transferred | 1.66 ± 0.64 | 1.67 ± 0.61 | 0.92[a] |
| Day 3 embryo transfer (%) | 94.04% (379/403) | 91.57% (76/83) | 0.40[b] |
| Day 5 embryo transfer (%) | 5.96%(24/403) | 8.43% (7/83) | 0.40[b] |

Values are presented as number (%) or mean ± SD

[a] $t$ test

[b] $\chi^2$ test or fisher's exact test

**Table 3** IVF/ICSI clinical outcomes of advanced age patients undergoing their first IVF/ICSI cycles

| | IVF ($n = 534$) | ICSI ($n = 110$) | $P$ | Adjusted $P$ | Adjusted OR |
|---|---|---|---|---|---|
| Implantation rate | 15.11% (105/695) | 7.75% (11/142) | 0.02 | – | – |
| Clinical pregnancy rate | 21.59% (87/403) | 13.25% (11/83) | 0.08 | 0.17[a] | 1.63 (0.81–3.27)[b] |
| Miscarriage rate | 43.68% (38/87) | 54.55% (6/11) | 0.49 | 0.71[a] | 0.76 (0.18–3.30)[b] |
| Live birth rate | 12.16% (49/403) | 6.02% (5/83) | 0.11 | 0.08[a] | 2.62 (0.89–7.66)[b] |
| Cumulative live birth rate per cycle | 14.59%(62/425) | 5.56% (5/90) | 0.02 | 0.02[a] | 3.58 (1.24–10.36)[b] |

[a] $P$ value after logistic regression analysis controlling for primary infertility (%), female age, number of oocytes retrieved and endometrial thickness

[b] OR with 95% CI. Logistic regression analysis was performed by adjusting for primary infertility (%), female age, number of oocytes retrieved and endometrial thickness

$P > 0.05$) and the miscarriage rate was lower in the IVF group than that in the ICSI group (43.68% vs. 54.55%, $P > 0.05$), although the difference was not significant. However, the implantation rate in the IVF group was markedly higher than that in the ICSI group (15.11% vs. 7.75%, $P < 0.05$). After logistic regression analysis adjusting for primary infertility, female age, number of oocytes retrieved and endometrial thickness, the cumulative live-birth rate in the IVF group have been statistically higher in comparison with the ICSI one (14.59% vs. 5.56%, $P < 0.05$; OR 3.58; 95% CI. 1.24–10.36). Furthermore, we matched the patients according to female age (40, 41, 42 and 43) and number of oocytes retrieved (1, 2, 3, 4 and 5), respectively. As expected, the cumulative live birth rates in the IVF subgroups were higher than those in the ICSI subgroups,

although there was no significant difference between them (Supplementary Table 1 and Table 2).

## Discussion

The present study provides evidence that the conventional IVF produced a higher implantation rate and a cumulative live-birth rate over ICSI for advanced age patients with five or fewer eggs retrieved and non-male factor infertility.

In normal responder patients, ICSI has been proven no advantage to conventional IVF and is a more invasive and costly technique [15, 16]. While for poor responders, the result has been inconsistent. In a study involving 84 poor ovarian-responded cases, Ou et al. revealed ICSI has a beneficial trend in clinical pregnancy rate, implantation rate and live birth rate [17]. However, Fang et al., stated the ICSI method has not produced better clinical rates compared to the conventional IVF for the patients having one or two oocytes, which is in good agreement with our study to some extent [18]. Therefore, we recommend that IVF is the preferred ART strategy for all responder patients without male factor infertility.

Our present results would support a study by Samer et al., showing similar clinical pregnancy and live birth rates in advanced age women with non-male factor infertility between IVF and ICSI groups [19]. Different from their work, we have calculated the implantation and cumulative live birth rates and found the two rates were significantly higher in the IVF group. There were two possible explanations for this phenomenon. The most relevant reason for this behavior accounts on the several IVF cycles previously undertaken by the subjects forming the ICSI group, implying that ICSI has been conducted in patients with poorer prognosis compared to ours. The second assumption is based on the fact that the number of oocytes retrieved in their study has exceeded 5, indicating the patients have been relatively normal responders, and this may narrow the difference between the two sub-groups. To the best of the authors' knowledge, this study is the first paper that compared conventional IVF and ICSI in advanced age women by using the cumulative live birth rate, a key index evaluating IVF/ICSI success rate.

In this study, we have recorded a relatively lower normal fertilization rate for the IVF group compared to the ICSI one. The first reason might be due to the different denominators observed for the normal fertilization rate of each group: the denominator of the IVF group rate has corresponded to the total number of oocytes including mature and immature GV and MI oocytes, which could not be distinguished before fertilization; whereas the one assigned to the ICSI group has been the number of only MII phase oocytes. The second reason is based on the widely known fact that ICSI could increase the normal fertilization rates with one sperm injection and lower the multiple pronuclear rates in women with young or advanced-age.

IVF is a process of natural selection whereas ICSI manipulation is invasive. Oocytes of advanced-age patients might tolerate less mechanical damage caused by ICSI. Even the oocytes could survive ICSI, their developmental potential might decline rapidly due to several irreversible damages. According to our experimental observations, we consider that the intrinsic developmental potential of oocyte determine its embryo development potential, and manipulation, either by conventional IVF or ICSI, does not improve too much. Without natural competition and selection, oocyte with poor quality can get a normal fertilization by ICSI but still cannot progress toward good-quality embryo and a live fetus. This might explain although the ICSI group had a higher normal fertilization rate, they still, could not obtain better implantation, and cumulative live-birth rates compared to conventional IVF group.

Our results further demonstrated that the clinical outcome was not improved by ICSI compared to conventional IVF for advanced age patients with a few oocytes retrieved. ICSI could be applied for cases with male factor infertility, instead of for all patients. Providing that ICSI has recently emerged as an alternative, and has been used for no more than three decades, its long term potential risks remain unknown [20]. According to a study reported by Davies et al., ICSI is related to an increased risk of defects [21]. Other studies have highlighted that ICSI could triggers risks of chromosomal aneuploidy [22–25]. Therefore, IVF still should be the first line choice for aged women with no matter more or fewer oocytes obtained in non-male factor infertility.

The current study also has some limitations. The first one is the relatively small sample size of the ICSI group; the second one is the nonuniform guidelines on the use of ICSI. With the lack of clinical guidelines regarding the optimal insemination strategy in advanced females, it is the physicians' decision of which insemination method to choose. Some clinicians in our center are inclined to perform ICSI to 'ensure' maximal fertilization for advanced age patients with a few oocytes retrieved. This circumstance reflects the practice dilemma physicians confront. The last one is the nature of retrospective study. Prospective studies are needed to better illustrate the advantages of IVF or ICSI for poor responders with advanced age.

In conclusion, this study provides strong evidences that the conventional IVF exhibits advantages over the ICSI method in non-male factor infertility for female patients aged 40 years and over with no more than 5 oocytes retrieved.

**Author contributions** LH: data management/analysis, manuscript writing/editing, ZHB: data management/analysis, manuscript writing/editing, YGL: data collection, LM: manuscript editing, MSY: manuscript

editing, ZHZ: data collection, WKL: study design, manuscript writing/editing.

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## Compliance with ethical standards

**Conflict of interest** All the authors declare no conflict of interest with respect to the authorship and/or publication of this article.

**Ethical approval** All procedures performed in this study involving human participants were in accordance with the ethical standards of the Reproductive Medicine, Shandong University Research Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in this study.

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