

Original Article

Fertilization method of half-ICSI: is it worth recommending for patients with unexplained infertility?

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Received November 17, 2015; Accepted March 25, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Objectives: To investigate whether half-ICSI is worth recommending for patients with unexplained infertility. Methods: We adopt the half-ICSI method and split the sibling oocytes by conventional in-vitro fertilization (c-IVF) or intracytoplasmic sperm injection (ICSI). Based on different fertilization methods, the oocytes were divided into two groups as follows: c-IVF and ICSI group. Main outcome Measure: Fertilization, cleavage, normal fertilization, good-quality embryo and transferable embryo rate. Results: We observed no significant difference in aspects of fertilization and cleavage rate ($P>0.05$). However, the normal fertilization, good-quality embryo and transferable embryo rate were significantly lower in c-IVF group than in ICSI group ($P<0.05$). Conclusions: ICSI might be more suitable for the patients with unexplained infertility than half-ICSI.

Keywords: Half-ICSI, fertilization, high-quality embryo

Introduction

At present, the average fertilization rate of most centers has reached 60%~70%, however, in some patients with normal parameters, fertilization failure (FF) or low fertilization (<25%) in c-IVF cycles may still occur [1, 2]. FF is frustrating to patients, adding further emotional strain and increased financial costs to an already stressful treatment. FF may be associated with various factors such as hormonal environment, oocyte quality, abnormal sperm, stimulation protocol and disruption of sperm-egg interaction [3, 4]. There are also some unexplained factors which could result in FF.

For the patients with unexplained infertility, it is often unclear whether c-IVF or ICSI would be the most appropriate reproductive to apply. As well known, the main reason for fertilization failure (FF) is a lack of sperm penetration owing to the failure of the spermatozoon to bind to the zonapellucida or to successfully penetrate it [5, 6]. To some extent, ICSI might be the solution which could effectively avoid the incidence of FF, as the oocyte membrane is mechanically pierced, enabling bypassing of biological obstacles [7, 8]. Nevertheless, there is no doubt that

it inevitably caused damage to the egg membrane. A number of groups have started to use ICSI for all patients with the argument that unexpected FF can then be avoided [9]. In our view, if successful fertilization could happen in the method of c-IVF, ICSI might not be a prior consideration. So in our center, for some couples with unexplained infertility, splitting the sibling oocytes by c-IVF and ICSI was adopted to effectively minimize FF. Because two different fertilization methods were applied we should attach great importance to the embryonic development of these patients. With this in mind, the aim of this study was to compare the embryonic development of c-IVF and ICSI group in the patients whose fertilization method was half-ICSI. Through comparative analysis, we attempted to find a more reasonable fertilization method for the patients with unexplained infertility.

Materials and methods

Patients and setting

This study was a retrospective analysis of the data from our center and was approved by the Ethics Review Board of the Northwest Women's

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and Children's Hospital. This study contains 109 infertile couples from January 2013 to January 2015. All of them attempted half-ICSI treatment on the first try. 74 patients were into the cycles for the first time and half-ICSI was selected for unexplained infertility. Other 35 patients have gained an undesirable fertilization outcome in the first c-IVF cycle, so the half-ICSI treatments were attempted for them in the second cycle. We split the sibling oocytes by c-IVF and ICSI separately in the first and second cycles. Based on different fertilization methods, the oocytes were divided into two groups as follows: c-IVF and ICSI group. The fertilization, cleavage, normal fertilization, good-quality embryo and transferable embryo rate were compared between two groups. For half-ICSI patients in this study, all the semen reached c-IVF fertilization and the age of females were not less than 34 years. In all the occasions, semen volume, concentration, and motility were determined according to 5th WHO criteria (semen volume ≥ 1.5 ml, concentration $\geq 15 \times 10^6$ /mL, total count $\geq 39 \times 10^6$, total motility $\geq 40\%$ or progressive motility $\geq 32\%$).

Our inclusion criteria: (1) The first attempted half-ICSI cycle. (2) The number of retrieved oocytes was not less than 10. (3) The oocytes were randomly assigned to c-IVF and ICSI group. (4) The infertility duration of these patients with unexplained infertility was not less than 3 years.

The fertilization failure would occur in the c-IVF oocytes for part of the half-ICSI cycles and we defined these cycles as rescue-ICSI which were not concluded in previous study. We also compared female's age, sperm concentration, progressively motile sperm rate and normal sperm morphology rate between half-ICSI and rescue-ICSI group. In this study, rescue-ICSI is early rescue-ICSI (reinsemination at 4-6 h after initial insemination).

Ovarian stimulation

Most patients used the standard long and short protocols with GnRH agonist (GnRH-a, Decapeptyl Germany) and recombinant FSH (GONAL-f, Merck Serono Italy; Puregon, Organon Netherlands) for controlled ovarian hyperstimulation (COH). Other protocols with or without human menopausal gonadotrophin (HMG, Li Zhu, China) were also adopted in COH accord-

ing to the patients' response to stimulation. 10 000 units of human chorionic gonadotrophin (hCG) was administered when >3 follicles were >18 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasonography-guided aspiration [10].

Conventional IVF fertilization and ICSI treatment

Semen samples were collected by masturbation for semen analysis after an abstinence of 3-7 d. IVF fertilization is performed 2-2.5 h after oocyte retrieval by conventional IVF. Each oocyte is incubated with approximately 40,000 sperm and fertilization is allowed to occur naturally. Short-term fertilization was adopted and the cumulus granule cells were peeled off 4-4.5 h after fertilization.

Three skilled ICSI operators, each having at least 6 years of experience, injected the metaphase II oocytes by the direct penetration technique. Oocytes were placed individually into 5 μ l droplets of IVF solution covered under warm mineral oil. Sperm were placed in a central 5 μ l droplet of polyvinylpyrrolidone (PVP) solution, and the procedure was performed on the heated stage of an inverted microscope.

Evaluation and selection for embryo transfer

A morphologic score was given for day-3 embryo according to the number of blastomeres, homogeneous degree of blastomeres and degree of cytoplasmic fragmentation: grade I (8-10 blastomeres, even homogeneous blastomeres $<10\%$ cytoplasmic fragmentation), grade II (6-7 or >10 blastomeres with even homogeneous blastomeres of no cytoplasmic fragmentation, 8-10 blastomeres, even homogeneous blastomeres with 10%-20% cytoplasmic fragmentation), grade III (uneven and non-homogeneous blastomeres with 20-50% cytoplasmic fragmentation), and grade IV (uneven and non-homogeneous blastomeres with $>50\%$ cytoplasmic fragmentation). The good-quality embryo contains grade I and grade II embryo. The transferable embryo contains grade I, grade II and grade III embryo [11].

Statistical analysis

Data were analyzed using the SPSS 17.0 for Windows. When comparing means between different groups, the paired Student's t-test was

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Table 1. Comparison of embryonic outcomes between c-IVF and ICSI group in the first half-ICSI cycles

Parameter	c-IVF group	ICSI group	P value
No. of patients (n)	75	75	/
No. of oocytes (n)	606	608	/
Fertilization (% , n)	72.94 (442/606)	72.37 (440/608)	0.824
Cleavage (% , n)	98.19 (434/442)	97.73 (430/440)	0.627
Normal Fertilization (% , n)	55.78 (338/606)	70.07 (426/608)	<0.001
High-quality embryo (% , n)	38.71 (168/434)	47.91 (260/430)	<0.001
Transferrable embryo (% , n)	60.37 (262/434)	72.88 (322/430)	<0.001

Table 2. Comparison of embryonic outcomes between c-IVF and ICSI group in the second half-ICSI cycles

Parameter	c-IVF group	ICSI group	P value
No. of patients (n)	35	35	/
No. of oocytes (n)	255	236	/
Fertilization (% , n)	63.53 (162/255)	71.19 (168/236)	0.071
Cleavage (% , n)	96.91 (157/162)	93.45 (157/168)	0.113
Normal Fertilization (% , n)	39.22 (100/255)	66.95 (158/236)	<0.001
High-quality embryo (% , n)	23.57 (37/157)	38.22 (60/157)	0.004
Transferrable embryo (% , n)	47.13 (74/157)	78.98 (124/157)	<0.001

Table 3. Clinical data of half-ICSI and rescue-ICSI group for the first cycle in this study

Parameter	Half-ICSI group	Rescue-ICSI group	P value
No. of patients (n)	74	23	/
Female's age (y)	31.81±4.30	31.26±4.68	0.423
Sperm concentration (10 ⁶ /ml)	50.89±26.09	47.33±29.04	0.525
Progressively motile sperm (%)	58.11±22.70	50.24±24.22	0.194
Normal sperm morphology (%)	2.61±1.79	2.39±1.63	0.371

applied. The χ^2 -tests test was used for group comparison of rate. Differences were considered statistically significant at $P<0.05$.

Results

For the first oocytes-retrieved cycles, half-ICSI fertilization was selected by 74 patients. 606 oocytes were fertilized by the method of c-IVF and 608 oocytes were fertilized by ICSI. Our data demonstrated no significant difference in aspects of fertilization and cleavage rate ($P>0.05$). The normal fertilization, good-quality embryo and transferable embryo rate were significantly lower in c-IVF group than in ICSI group ($P<0.05$) (Table 1).

In 35 couples, their first c-IVF cycles had a weak fertilization outcome. So in the second

cycles, the method of half-ICSI was selected. We compared some parameter and also observed no significant difference in the fertilization and cleavage rate ($P>0.05$). The normal fertilization, good-quality embryo and transferable embryo rate were significantly lower in c-IVF group than in ICSI group ($P<0.05$) (Table 2).

Simultaneously, in all the half-ICSI cycles, there were 23 cycles in which all c-IVF oocytes were not successfully fertilized. We observed no significance in female age, sperm concentration, progressively motile sperm rate and normal sperm morphology rate between half-ICSI and rescue-ICSI group (Table 3).

Discussion

Fertilization failure (FF) in assisted reproductive techniques (ART) is a frustrating experience for patients, with a heavy financial and emotional burden [12]. However, FF is still unavoidable, and it has been reported that, in c-IVF cycles, the proportion of FF is 5-10% [13, 14]. Although the

incidence of rescue-ICSI might improve clinical outcomes of these patients, it involves mechanical operation of stripping granulosa cells from oocytes and delayed several hours insemination compared to conventional ICSI. The effects of these procedures might make a negative effect on the subsequent neonatal outcome. So for the patients with unexplained infertility it was fairly difficult to select a more reasonable fertilization method. In our center, with regard to most of patients with unexplained infertility for many years, we selected half-ICSI fertilization to avoid the incidence of total fertilization failure.

There are two kinds of patients who selected half-ICSI fertilization method included in our study. One is that who have suffered years of infertility with unexplained reasons, and the

other is that who have lower fertilization in the first retrieved cycle, so in the second cycle half-ICSI was selected. For the first reason of half-ICSI selected, our data demonstrated that the normal fertilization, good-quality embryo and transferable embryo rate were significantly lower in c-IVF group than ICSI group ($P < 0.05$). It suggested that ICSI might be more proper for these patients. There is no doubt that the normal fertilization rate was lower in c-IVF group than ICSI group in which the immature oocytes were excluded and the abnormal fertilization rate was reduced. With increased good-quality embryo and transferable embryo rate in ICSI group, it provides the embryologists more choices for embryo transfer. There were some couples with a lower fertilization rate in their first retrieved cycle, so in the second cycle, half-ICSI was selected. And in these patients we also observed that the normal fertilization, good-quality embryo and transferable embryo rate were significantly lower in c-IVF group than ICSI group ($P < 0.05$) which was consistent with the findings of above results in the first cycles. In the aspects of blastocyst formation rate, we also found that it was higher in c-IVF group than in ICSI group. Although there was no significant difference, eventually, more high-quality embryos from ICSI group had been selected for transfer or freeze than c-IVF group which reduced the number of formative blastocysts from ICSI group. It also suggested that easier blastocyst formation would happen in ICSI group among the half-ICSI patients in this study.

The results of this study suggest that although the oocyte membrane is mechanically pierced, ICSI make no effect on embryonic development. As well known, the implantation rate was even more important than other parameter. However, there were some limitations in our study. On the one hand, these half-ICSI data constitute only a small, retrospective study from our own IVF center. This may be due that a majority of infertile couples have indefinite factors for ART treatment. On the other hand, if one embryo from c-IVF and one from ICSI transferred, it cannot be determined that which one has a successful implantation.

In the half-ICSI cycles, the fertilization failure or low fertilization in c-IVF cycles may still occur, under the circumstances rescue-ICSI will be performed for these oocytes. In our 97 half-ICSI first cycles, total fertilization failure occurred in

23 cycles. We compared the female's age, sperm concentration, progressively motile sperm rate and normal sperm morphology rate and observed that there was no significance between half-ICSI and rescue-ICSI group. It suggested that the male sperm parameter is not the main cause of fertilization failure which might be more connected with the quality of eggs. However, early rescue-ICSI involves mechanical operation of stripping granulosa cells from oocytes and delayed several hours insemination compared to conventional ICSI which might carry a higher risk for chromosomally abnormal embryos [15, 16]. So for these patients, a better clinical outcome might be obtained if ICSI selected not half-ICSI. Indeed some reports have shown that rescue-ICSI might not make a negative effect on clinical outcomes compared with conventional-ICSI and our study obtained the similar results although the numbers of half-ICSI patients were still low. It suggests that it doesn't matter even if fertilization failure occurs in c-IVF oocytes because R-ICSI could achieve equivalent effect with conventional ICSI.

In conclusion, our study suggests that ICSI might be more suitable for the patients with unexplained infertility than half-ICSI. However, the limited cases of half-ICSI patients could not show a comparison of implantation and clinical pregnancy rate between c-IVF and ICSI group. So it is more important to accumulate clinic data for a more persuasive conclusion.

Disclosure of conflict of interest

None.

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