Comparing the pregnancy outcomes of Re-ICSI and ICSI embryos in fresh ET and FET cycles

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Abstract. Early rescue intracytoplasmic sperm injection (Re-ICSI) can prevent total fertilization failure (TFF) during conventional *in vitro* fertilization (IVF). However, the implantation rate of Re-ICSI embryos is lower than that of direct ICSI during fresh embryo transfer (ET). The aim of the present study was to investigate the effect of frozen ET (FET) after Re-ICSI. In the present retrospective study, primary infertility patients that underwent the first Re-ICSI and ICSI treatment, were studied. The clinical pregnancy rate, implantation rate, ectopic pregnancy, abortion rate and live birth rate were analyzed between the Re-ICSI and ICSI groups in fresh ET and FET cycles. The average age of patients between Re-ICSI and ICSI groups in fresh ET and FET cycles was (29.0±3.2 vs. 29.1±3.1, and 29.1±3.3 vs. 28.9±3.0), respectively (P>0.05). Compared with ICSI embryos, the clinical pregnancy, implantation and live birth rates of Re-ICSI embryos were lower in fresh ET cycles. By contrast, there were no significant differences in the pregnancy, implantation and live birth rates between the Re-ICSI and ICSI embryos during the FET cycles. Re-ICSI coupled with FET may overcome the impaired outcomes in fresh ET.

Introduction

Intracytoplasmic sperm injection (ICSI) was introduced in the early 1990s to treat severe male infertility. However, there is still insufficient evidence to suggest that ICSI should be used in couples without male factor infertility (1). If conventional *in vitro* fertilization (IVF) cycles (co-culture of oocytes and cumulus cells for 18-20 h) resulted in total fertilization failure (TFF), late-ICSI would be performed. In recent years, to avoid TFF or low fertilization in conventional IVF, short co-incubation of gametes (4-6 h) combined with early-rescue ICSI (Re-ICSI) has been widely practiced in numerous IVF laboratories (1,2).

Re-ICSI was provided to those oocytes with unclear release of the second polar body, 6 h after initial insemination, since the second polar body was reportedly released in nearly 90% of fertilized oocytes by 6 h (2). It should be clearly noted that the removal of cumulus cells in ICSI procedure was presented 3-4 h after oocyte retrieval, earlier than that in Re-ICSI group (2). Oocytes used for direct ICSI were treated with hyaluronidase for dispersing the cumulus cells, whereas the cumulus granulosa cells were removed directly after 4 h of co-incubation of gametes in Re-ICSI group (1,2).

Previous studies have indicated that short insemination has no detrimental effects on clinical outcomes in human IVF and that Re-ICSI can attain acceptable pregnancy outcomes (3). However, the Re-ICSI embryos showed adverse pregnancy outcomes (significantly lower implantation rate) compared with those of the directly ICSI embryos in the fresh embryo transfer (ET), as the sperm in the Re-ICSI group were microinjected into oocytes 4-6 h later than those in the ICSI group (2).

A meta-analysis of late-ICSI performed along with frozen embryo transfer (FET) may overcome the technical and biological issues associated with the fresh transfer after late-ICSI (1). However, whether Re-ICSI embryos would be a benefit from the FET process remains unknown.

The purpose of the present study was to retrospectively investigate the clinical outcomes of embryos derived from Re-ICSI and direct ICSI insemination in fresh ET and FET cycles.

Materials and methods

Patients. The present retrospective cohort study was carried out at the Center for Reproductive Medicine and Infertility, the Fourth Hospital of Shijiazhuang, from January 2016 to

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November 2021. Patients with primary infertility that were included in the analysis were at the ages of ≤35 years, with retrieved oocytes ≥5 in their first short-term IVF + Re-ICSI cycle or directly ICSI treatment because of severe oligospermia and underwent their first fresh ET or FET.

The inclusion criteria were women undergoing their first Re-ICSI/ICSI cycle with two high quality cleavage embryos in D 3 fresh ET cycle, or single vitrified-warmed blastocyst transfer (SVBT) with D 5 high quality blastocyst in their first FET cycle (all embryos frozen) (Fig. 1).

The exclusion criteria included, donor eggs/sperm, patients with oocyte maturation disorder, chromosomal abnormalities, hyperprolactinemia, thyroid dysfunction, women with congenital or secondary uterine abnormalities such as unicornuate uterus, septate uterus or uterine didelphys, adenomyosis, uterine submucosal fibroids, intrauterine adhesions and endometriosis, or other endometrial diseases, or endometrial thickness <7 mm on the D of embryo transfer.

Data were collected by searching electronic medical records and entering the inclusion and exclusion criteria. A total of 293 Re-ICSI cycles and 326 ICSI cycles were enrolled in the present study, in which 313 were obtained from fresh ET cycles (151 Re-ICSI and 162 ICSI) and 306 from SVBT cycles (142 Re-ICSI and 164 ICSI).

The procedures that were conducted in the present study adhered to the tenets of the Declaration of Helsinki. The present study was approved (approval no. 20220049) by the Research Ethics Committee of the Fourth Hospital of Shijiazhuang (Shijiazhuang, China).

Stimulation, oocyte retrieval, fertilization, embryo culture and scoring. The process of ovarian stimulation and oocyte retrieval has been previously described by Jiang *et al* (4). Sperm was performed using standard IVF/ICSI insemination procedure by density gradient centrifugation. Insemination was performed after 38~40 h of trigger.

IVF Short co-incubation and Re-ICSI. Each oocyte is incubated with ~20,000 sperm cells, and the cumulus granulosa cells were removed after 4 h co-incubation of gametes. In patients with a missing second polar body in any of the retrieved oocytes or with a low fertilization rate (<30%), the MII oocytes were rescued and underwent the same ICSI method (Re-ICSI insemination) at ~6 h after fertilization (4).

ICSI. Oocytes used for directly ICSI were treated with bovine hyaluronidase (Sigma-Aldrich; Merck KGaA) for dispersing the cumulus cells after 39~40 h of trigger. And the MII oocytes undergo the ICSI insemination.

Fresh ET. 'High quality embryos' should have 7-9 cells with no more 20% fragments on D3, but may be a little uneven in appearance. On D 3 two high quality embryos were selected for embryo transfer, based on endometrial factors, the occurrence of ovarian hyperstimulation syndrome or personal reasons.

Other embryos were transferred into G-2 culture medium in group culture (Vitrolife). In the morning of D 5 or D 6, blastocysts were scored by two experienced embryologists using the system of Gardner and Schoolcraft (5). 'High quality blastocysts' were ≥3BB blastocysts.

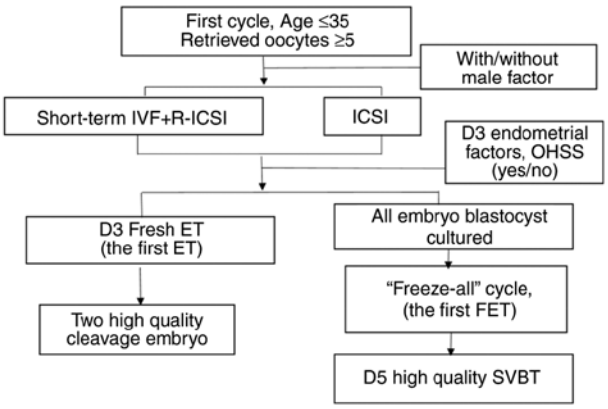

Figure 1. D3 embryo and D5 SVBT from Re-ICSI and ICSI groups in fresh ET and FET cycles. OHSS, ovarian hyperstimulation syndrome; ET, embryo transfer; FET, frozen embryo transfer; SVBT, single vitrified-warmed blastocyst transfer.

SVBT procedures. The procedure was always performed using one blastocyst for each straw. An artificial shrinkage (AS), using a laser pulse was performed before vitrification. The blastocyst was then moved at room temperature (22-25˚C) to Kitazato, Corp. along with equilibration solution (ES). After 6-8 min, the blastocyst was quickly washed in vitrification solution (VS) for 45-60 sec and transferred onto the straw (Kitazato) using a micropipette and immersed vertically into liquid nitrogen (6).

A Thaw Kit (Kitazato) was used for warming. The carrier containing the embryo was removed from the straw and placed quickly into the dish containing the thawing medium (thawing solution) preheated at 37˚C. The blastocysts immediately fell from the device and could be easily identified in the medium. After 1 min, blastocysts were transferred to the DS medium (dilution solution) for 3 min at room temperature 22-25˚C. In the last two steps, blastocysts were placed for 5 min, in the WS1 medium and WS2 (washing solution). The embryo was then returned to G-2 medium for culture until transfer. At this stage, an assessment was performed on an inverted microscope to establish if the embryo survived based on morphological integrity of the inner cell mass and trophectoderm.

FET of SVBT. Hormone therapy cycles were used as the endometrial preparation for the FET. After 1 or 2 h of culture the embryo was reassessed and often the re-expansion of the blastocyst was reported; this indicated that the embryo physiologically survived the warming procedure. Embryo transfer was normally performed within 2 or 3 h (6).

Clinical outcome. B ultrasound was used to observe the gestational sac and fetal heart at 35 Ds after implantation was diagnosed as clinical pregnancy. Implantation rate was defined as the ratio of the number of gestational sacs and fetal heart observed under B ultrasound and the number of transferred embryos (6). The clinical pregnancy rate, implantation rate, ectopic pregnancy, abortion rate and live birth rate were analyzed.

Data analysis. Statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc.). The results are presented as the mean ± standard deviation (SD). The mean values of

Table I. Comparing outcomes of D3 embryo between ICSI and Re-ICSI groups in fresh embryo transfer cycles.

	Re-ICSI	ICSI	χ^2/t	P-value
ET 2 cleavage embryo cycle (n)	151	162		
Patient age, years	29.0±3.2	29.1±3.1	1.410	0.160
Infertility duration, years	3.6±2.7	3.5±2.5	1.551	0.122
Body mass index, kg/m^2	23.9±4.2	23.7±4.2	0.148	0.882
Clinical pregnancy rate, % (n)	52.3 (79/151)	64.2 (104/162)	4.542	0.033
Implantation rate, % (n)	34.4 (104/302)	42.3 (137/324)	4.065	0.044
Ectopic pregnancy rate, % (n)	0 (0/90)	1.9 (2/104)	1.749	0.186
Abortion rate, % (n)	10.0 (8/79)	11.5 (12/104)	0.092	0.762
Sex ratio (male/female)	1.23 (49/40)	1.09 (61/56)	0.173	0.677
Live birth rate, % (n)	58.9 (89/151)	69.4 (117/162)	6.128	0.013

ICSI, intracytoplasmic sperm injection; Re-ICSI, early-rescue ICSI.

Table II. Comparing outcomes of D5 SVBT between ICSI and Re-ICSI groups in frozen embryo transfer cycles.

	Re-ICSI	ICSI	χ^2/t	P-value
SVBT cycle	142	164		
Patient age, years	29.1±3.3	28.9±3.0	1.490	0.138
infertility duration, years	3.5±2.7	3.4±2.4	1.141	0.255
Body Mass Index (kg/m^2)	23.4±3.9	23.2±3.8	1.774	0.078
Clinical pregnancy rate, % (n)	68.3 (97/142)	68.3 (112/164)	0.000	1.000
Implantation rate, % (n)	70.4 (100/142)	70.1 (115/164)	0.003	0.954
ectopic pregnancy, % (n)	1.0 (1/97)	0 (0/112)	1.160	0.281
Abortion rate, % (n)	17.5 (17/97)	17.9 (20/112)	0.004	0.950
Sex ratio (male/female)	1.45 (48/33)	1.19 (51/43)	0.443	0.505
Live birth rate, % (n)	57.0 (81/142)	57.3 (94/164)	0.002	0.961

SVBT, single vitrified-warmed blastocyst transfer; ICSI, intracytoplasmic sperm injection; Re-ICSI, early-rescue ICSI.

two groups were compared using the independent samples unpaired t-test. The Kolmogorov-Smirnov normality test was performed before t-test. Percentages were compared using the χ^2 test and $P<0.05$ was considered to indicate a statistically significant difference.

Results

The pregnancy outcomes of two quality embryos between Re-ICSI and ICSI groups in fresh D 3 ET cycles. There were 151 Re-ICSI cycles and 162 ICSI cycles. The average age and body mass index (BMI) of patients with primary infertility in the Re-ICSI and ICSI groups were 29.0±3.2 vs. 29.1±3.1, and 23.9±4.2 vs. 23.7±4.2, respectively ($P>0.05$). The rates of clinical pregnancy, implantation and live birth in Re-ICSI group was lower than ICSI group in fresh ET cycles ($P<0.05$). No significant differences were observed in the ectopic pregnancy rate, abortion rate and sex ratio between the two groups (Table I).

The pregnancy outcomes of SVBT between Re-ICSI and ICSI groups in D 5 FET cycles. In 306 FET cycles, Re-ICSI was 142 cycles and ICSI was 164 cycles. The survival rate of D 5 high quality blastocyst was 100% in SVBT cycles. The average age and BMI of patients in two groups were 29.1±3.3 vs. 28.9±3.0, and 23.4±3.9 vs. 23.2±3.8, respectively ($P>0.05$). There were no significant differences in rates of clinical pregnancy, implantation, ectopic pregnancy, abortion, sex ratio and live birth between the two groups in SVBT cycle (Table II).

Discussion

Previous pregnancy history, duration of infertility, forward-moving sperm counts, and abnormal sperm, serve as a precursor in predicting fertilization failure; thus, whether a patient should conduct short-time insemination should be advised based on the aforementioned indexes (7). During the procedures of short co-incubation, patients with primary infertility, presented <30% fertilization rate and were obliged to undergo Re-ICSI treatment. Re-ICSI was carried out to reduce the occurrence of TFF and near-total FF (NFF) (1). The incidence of NFF was at 6.49% (864/13,317), whereas TFF was at 4.21% (561/13,317) (2).

The important question needed to be asked in the present study is when exactly is the right time, for the RE-ICSI to be performed to successfully achieve fertilization (2). For the second polar body performing Re-ICSI after 6 h of co-incubation can salvage cases of IVF fertilization failure. Higher fertilization rate after Re-ICSI indicated that all oocytes without signs of fertilization after 6 h of co-incubation should undergo Re-ICSI (8).

However, the effectiveness of Re-ICSI remains controversial. In a previous study of long-term (20 h) and short-term (4 h) insemination combined with Re-ICSI of sibling oocytes, there were 11 cycles in which Re-ICSI was performed because of TFF occurring in the oocytes with the short-term insemination. In six of these cycles, fertilization occurred in patients of the 20 h insemination group (9).

Early cumulus cell removal alone (4 h) had similar pregnancy outcomes compared with 20 h after insemination (10). However, the pregnancy outcomes of Re-ICSI embryos in fresh cycles remained controversial. Zeng *et al* (2) showed that Re-ICSI embryos had lower implantation rates than ICSI embryos in fresh ET. Jiang *et al* (11) showed that the rates of implantation, clinical pregnancy, and live births were similar between the Re-ICSI and ICSI groups; however, it was not emphasized if all the transferred embryos came from Re-ICSI, and that only seven cycles were included. Pregnancy outcomes of Re-ICSI and ICSI embryos during FET cycles are less reported.

In the present study, primary infertility patients were selected with ≤35 years old and ≥5 oocytes in their first Re-ICSI/ICSI cycle to minimize the influence of age and oocytes factors. In the Re-ICSI cycles, especially part of oocytes IVF and part of oocytes Re-ICSI cycles, the embryos came from short-term insemination embryos and Re-ICSI embryos together (2). In the present study, the embryos transferred all from Re-ICSI fertilization.

A total of two D3 high quality embryos were selected in the first fresh cleavage-stage ET cycles, or SVBT (all embryos frozen) with D5 high quality blastocyst in their first FET cycle to reduce the effects of embryo quality and ET frequency. Another reason to choose high quality D5 blastocysts is that they have a 100% survival rate during the SVBT cycle.

In the present study, it was revealed that the clinical pregnancy rate, implantation rate and live birth rate of Re-ICSI embryos were lower than the ICSI embryos in fresh ET cycles. Whereas there was no significant difference between Re-ICSI and ICSI embryos in the FET cycles.

There were two differences between the Re-ICSI and ICSI groups, the cumulus cell removal method and fertilization time. In the Re-ICSI group, sperms were microinjected into oocytes 4-6 h later than in the ICSI group. The differences in Re-ICSI/ICSI pregnancy outcomes may be related to oocyte aging and subsequent embryonic development (2).

The results of the present study indicated that Re-ICSI/ICSI embryos present similar pregnancy outcomes during the FET cycle, which proves that Re-ICSI/ICSI embryos have similar developmental abilities. The pregnancy outcome of Re-ICSI embryos in the fresh ET cycle was lower, possibly because the fertilization time was 4-6 h later than that of the ICSI embryos, resulting in an asynchronous endometrium.

Late-ICSI (18-20 h) leads to poor clinical pregnancy outcome resulting from oocyte aging (12) and asynchronization between endometrial growth and embryo development (13). However, frozen embryo transfer appeared to improve with pregnancy rates and implantation rate in late-ICSI patients (1). The strategy of cryopreservation could overcome the loss of synchronization between endometrial growth and late-ICSI embryo development (1). In Re-ICSI cycles, unfertilized oocytes are fertilized by ICSI only 6 h later instead of nearly 1-day-old in late-ICSI. Therefore, the correlation of oocyte aging and asynchronization between endometrial growth and embryo development was minor compared with late-ICSI. Nevertheless, the results of the present study about embryos from Re-ICSI were similar with late-ICSI.

Fresh ET cycles can negatively affect clinical outcomes after Re-ICSI. In the Re-ICSI cycles, if a patient had both IVF and Re-ICSI embryos, IVF embryos should be preferred transfer in fresh ET, whereas Re-ICSI embryos should be preferred cryopreservation with the similar embryo score.

The limitations to the present study were that, the analysis was centered around the D3 embryo in fresh ET and D5 SVBT in FET cycles. There were also some patients who transferred blastocyst in fresh cycle and cleavage embryo in FET cycle. Analysis on these cases was not implemented because the number of these patients was relatively small. Another limitation is that frozen and fresh Re-ICSI were not compared in the present study, as differences between embryo development Ds (D3 vs. D5) and number (two cleavage embryos and one blastocyst) lacked comparability between the groups.

ICSI was used as golden standard procedure. Re-ICSI had lower pregnancy outcomes than ICSI in fresh ET cycles whereas Re-ICSI and ICSI had similar outcomes in FET cycles. The results clearly showed that the transfer of FET Re-ICSI embryos may overcome the impaired outcomes in fresh ET. Cryopreservation can overcome the technical and biological issues associated with the loss of synchronization between endometrial growth and embryonic development resulting from Re-ICSI. The favorable FET pregnancy outcomes may explain the discrepancy between the results derived from fresh ET and FET cycles.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YJ, XHW and JCY made substantial contributions to conception and design and were involved in drafting the manuscript or revising it critically for important intellectual content.. GS,

XHZ and SBM made substantial contributions to acquisition of data, analysis and interpretation of data. All authors reviewed the manuscript and given final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content. YJ and XHW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved (approval no. 20220049) by The Fourth Hospital of Shijiazhuang Ethics Committee (Shijiazhuang, China). The procedures used adhered to the tenets of the Declaration of Helsinki. All experiments were performed in accordance with relevant guidelines and regulations.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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