Early cumulus cell removal reduces available embryo rate while having no negative effect on live-birth and malformation rate in IVF: a propensity score-matched cohort study

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Research

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Abstract

Background Previous studies of the effect of early cumulus cell removal (ECCR) on clinical outcomes remain controversial. Some studies indicated that ECCR combined early rescue ICSI contributed to avoid total fertilization failure, while the other studies demonstrated that ECCR may be detrimental to early embryo development. The aim of this study is to investigate the efficacy and safety of early cumulus cell removal (ECCR) during human IVF.

Methods A retrospective analysis was performed between January 2011 and December 2016. The study enrolled 655 couples who underwent IVF treatments with ECCR. After propensity score matching at a 1:2 ratio, 1310 couples who underwent overnight coincubation of gametes were selected. All data were obtained from the Shanghai First Maternity and Infant Hospital IVF patient database. The main outcome measure was the live birth rate and the secondary outcome measures were the normal fertilization rate, polyspermy rate, available embryo rate, clinical pregnancy rate, miscarriage rate and malformation rate.

Results No significant differences were found in the live birth rate (28.55% vs 28.4%; RR of 1.008; 95% CI: 0.869-1.170; p=0.916), clinical pregnancy rate (48.28% vs 45.16%; RR of 1.069; 95% CI: 0.951-1.202; p=0.268), implantation rate (32.67% vs 33%; p=0.896), miscarriage rate (13.33% vs 9.32%; RR of 1.43; 95% CI: 0.916-2.232; p=0.115), neonatal congenital anomalies rate (1.32% vs 1.01%; RR of 1.306; 95% CI: 0.315-5.417; p=0.713) or birthweight between the two groups. The study showed that ECCR was associated with a significantly lower fertilization rate (73.86% vs 80.12%; p=0.000), normal fertilization rate (2PN)(62.76% vs 69%, p=0.000) and available embryo rate (59.62% vs 62.29%, p=0.001). There were no significant differences in the polyspermy rate (11.10% vs 11.11%, p=0.982) and cleavage rate (93.93% vs 93.50%, p=0.279) between the ECCR group and traditional insemination group.

Conclusions ECCR tended to confer increased risk of a lower available embryo rate but had no negative effect on the live birth rate or the neonatal malformation rate.

Background

Total fertilization failure (TFF) in in vitro fertilization (IVF) is a desperate event for patients. It is reported that the incidence of TFF after traditional IVF is 4%-16% [1, 2]. When TFF happens, medical teams have limited options. The first option is to cancel the current IVF cycle and to change to intracytoplasmic sperm injection (ICSI) in the subsequent cycle, which may bring a heavy financial and emotional burden. The second choice is to perform ICSI in the present cycle. Because of low efficacy [3], late rescue ICSI is not the best option.

Short coincubation of gametes and early removal of cumulus cells combined with early rescue ICSI has been demonstrated to avoid TFF [4] and has been offered to populations at high risk of TFF. It has been indicated that early rescue ICSI not only contributes to avoidance of TFF or lower fertilization [5, 6] but also may be beneficial by reducing potential detrimental exposure to free oxygen radicals (ROS) and
other metabolic products which come from spermatozoa [7, 8]. This protocol was adopted by many IVF centers in China.

However, the efficacy and safety of early removal of cumulus cells remains debatable. The early removal of cumulus cells may be detrimental to embryo development [9] due to breakage of the important cross-talk between oocyte and cumulus cells during early embryos development [10]. In our IVF center, less than 20% patients who underwent early removal of cumulus cells were offered early rescue ICSI (unpublished), and most patients’ oocytes were instead continued in culture after removal of the cumulus cells in which two poly bodies were seen, indicating normal fertilizing capabilities of the oocyte and sperm. To avoid TFF, most patients who did not need to perform early rescue ICSI were offered early cumulus cell removal. Whether early cumulus cell removal has negative effects on embryo developmental potential and live birth rate are the main questions we sought to answer in this study, in which we will classify the clinical applicability, effectiveness and safety of early removal of cumulus cells.

Materials And Methods:

Patients

This study was reviewed and approved by the Institutional Reviews Boards and Ethics Committee of Shanghai First Maternity and Infant Hospital, China. This study was a retrospective cohort study of patients undergoing IVF-ET treatment between January 2011 and December 2016 at the Reproductive Medicine Center.

The inclusion criteria were as follows: first IVF treatment, female aged between 20 and 42 years and cleavage-stage embryo transfer. Patients were excluded from the study if they had congenital or acquired uterine malformations, abnormal results on parental karyotyping, uterine fibroid, adenomyosis, untreated hydrosalpinx and blastocyst-stage embryo transfer. Well controlled diabetes and hypertension were not exclusion criteria. The use of donor semen was allowed.

Couples with unexplained infertility or primary infertility with a longer infertility duration were routinely given short-time fertilization and early cumulus cell removal for a fertilization check (insemination time, 6 h). Unexplained infertility was defined as no cause for infertility identified after a complete infertility evaluation or failure to conceive after intrauterine insemination (IUI) treatment [11, 12]. The study comprised 655 patients who received short coincubation and early cumulus cell removal and 1310 patients who undergone traditional IVF with overnight coincubation of gametes after propensity score matching (PSM) based on female age and primary infertility or secondary infertility.

Measures

The primary outcome of interest was the live birth rate per starting cycle. Additional outcomes included the clinical pregnancy rate, normal fertilization rate, polyspermy fertilization rate, implantation rate, miscarriage rate and malformation rate.
Cumulus Cells Removal

In the early cumulus cell removal group, cumulus cells were mechanically removed from cumulus-oocyte complexes (COCs) after 4-6 h of coincubation. When two polar bodies were present, a zygote was considered fertilized. Zygotes with two polar bodies were transferred to another fresh microdroplet without sperm and cultured overnight. In the traditional IVF group, cumulus cells were removed after 18-20 h insemination for a fertilization assessment.

Statistical analysis

We used a PSM that was based on propensity score to construct a weighted cohort of patients who differed with respect to time of fertilization check but were similar with respect to other measured characteristics. A PSM with a 0.05 caliper width was used to match the two groups at a 1:2 ratio.

For univariate analysis of categorical variables, chi-square tests were used. Where the chi-square analysis of a 2×2 table did not meet the assumption that less than 20% of expected values were less than 5, the Fisher's exact test was used. Independent t-tests and rank-sum tests were used for continuous variables. Losses before follow-up were excluded. Statistical analysis was performed using STATA software version 12.0.

Results

A total of 655 cycles were enrolled in the early cumulus cell removal group, and after PSM, 1310 cycles were included in the traditional IVF group. The baseline characteristics of the patients between the two groups are shown in Table 1. There were no significant differences in terms of female age, BMI, basal serum level of FSH and proportion of primary infertility and nulliparity between the two groups. The early cumulus cells removal group showed a longer period of infertility compared to the traditional IVF group. The proportion of unexplained infertility in early cumulus cell removal group was significantly higher compared to the traditional IVF group. Similarly, the early cumulus cell removal group showed significantly more total doses of gonadotropin and longer durations of stimulation.
Table 1
Baseline demographics of study cohort

| female partner | early cumulus cells removal group (n = 655) | traditional insemination group (n = 1310) | P value |
|----------------|---------------------------------------------|-------------------------------------------|---------|
| age (year)     | 31.3 ± 3.6                                  | 31.3 ± 3.6                                | 1       |
| BMI (kg/m²)    | 21.8 ± 3.2                                  | 21.6 ± 2.99                               | 0.1724  |
| basal FSH (mIU/L) | 6.705 (5.53, 7.85)   | 6.78 (5.62, 8.05)                     | 0.2494  |
| period of infertility (year) | 4 (2, 5)                                  | 3 (2, 5)                                  | 0       |
| primary infertility (%) | 512 (78.2)                               | 1024 (78.2)                               | 1       |
| nulliparity (%) | 629 (96.03)                                 | 1247 (95.19)                               | 0.423   |
| infertility factors | 0                                       | 0                                         | 0       |
| female (%)     | 270 (41.2)                                  | 804 (61.4)                                |         |
| male (%)       | 55 (8.4)                                    | 57 (4.4)                                  |         |
| both female and male (%) | 110 (6.8)                               | 300 (22.9)                                |         |
| unexplained (%) | 220 (33.6)                                 | 149 (11.4)                                |         |
| COS protocol   | 0                                           | 0                                         |         |
| long protocol (%) | 560 (85.5)                               | 993 (75.8)                                |         |
| short protocol (%) | 31 (4.7)                                 | 113 (8.6)                                 |         |
| GnRH antagonist protocol (%) | 41 (6.3)                                 | 83 (6.3)                                  |         |
| minimal stimulation (%) | 14 (2.1)                                 | 84 (6.4)                                  |         |
| ultra-long protocol (%) | 9 (1.4)                                  | 37 (2.8)                                  |         |
| COS total dose of Gn (IU) | 2025 (1650, 2700) | 1950 (1550, 2475)                     | 0.0002  |
| days of stimulation (days) | 12.12 ± 3.27                           | 11.34 ± 3.14                              | 0       |
| E2 on HCG day (pg/ml) | 2461.905(1687, 3622.69)    | 2212.13 (1204.66, 3205)                   | 0       |
| LH on HCG day (IU/L) | 0.825 (0.49, 1.53)                     | 1.125 (0.56, 2.41)                       | 0       |
The fertilization and clinical outcomes are shown in Table 2. A total of 8188 and 13757 oocytes were retrieved in the early cumulus cell removal group and traditional IVF group, respectively. There was a significantly higher mean number of oocytes retrieved in early cumulus cell removal group than in traditional IVF group. The percentages of fertilized oocytes, normal fertilized oocytes (2PN) and available embryos on day 3 were 73.86%, 62.76% and 59.62%, respectively, and were significantly lower than those in the traditional IVF group (80.12%, 69% and 62.29%, respectively, p < 0.01). The rates of polyspermy and cleavage were similar between the two groups. No significant differences were observed in the mean days of embryos transferred and implantation rate between the early cumulus cell removal group and the traditional IVF group. The clinical pregnancy rate and live birth rate per start cycle in the early cumulus cell removal group were 48.28% (RR of 1.069; 95% CI: 0.951–1.202; p = 0.268) and 28.55% (RR of 1.008; 95% CI: 0.869–1.170; p = 0.916) respectively, and there was no significant difference between two groups. The miscarriage rate was 13.33% in the early cumulus cell removal group, and 9.32% in the traditional IVF group, with a relative risk of 1.43(95% CI: 0.916–2.232, p = 0.115). In the early cumulus cell removal group, a total of 227 babies were delivered, 3 of whom were reported as neonatal anomalies (heart disease), while in the traditional insemination group, there were 494 live-born babies, 5 of whom were abnormal (heart disease, urinary tract malformation), and there was no significant difference in the neonatal congenital anomaly rate (1.32% vs 1.01%; RR of 1.306; 95% CI: 0.315–5.417; p = 0.713) between the two groups. No significant difference was identified between the two groups in terms of birthweight.
Table 2
Comparison of fertilization and clinical outcomes between the two groups

|                                      | early cumulus cells removal group (n = 655) | traditional insemination group (n = 1310) | relative risk no. (%) (95%CI) | P value |
|--------------------------------------|--------------------------------------------|------------------------------------------|-----------------------------|---------|
| No. of retrieved oocytes (n)         | 11 (8.16)                                  | 10 (6.14)                                | NA                          | 0       |
| fertilization rate % (n)             | 73.86 (6048/8188)                          | 80.12 (11022/13757)                     | NA                          | 0       |
| normal fertilization rate % (n)      | 62.76 (5139/8188)                          | 69 (9493/13757)                          | NA                          | 0       |
| polyspermy rate % (n)               | 11.10 (909/8188)                           | 11.11 (1529/13757)                      | NA                          | 0.982   |
| cleavage rate % (n)                  | 93.93 (5681/6048)                          | 93.50 (10306/13757)                     | NA                          | 0.279   |
| available embryos rate (n)           | 59.62 (3387/5681)                          | 62.29 (6420/10306)                      | NA                          | 0.001   |
| days of transferred embryos (day)    | 2.70 ± 0.49                                | 2.66 ± 0.53                             | NA                          | 0.1717  |
| No. of transferred embryos (n)       | 1.93 ± 0.33                                | 1.89 ± 0.36                             | NA                          | 0.0437  |
| clinical pregnancy rate % (n)        | 48.28 (225/466)                            | 45.16 (429/950)                         | 1.069 (0.951–1.202)         | 0.2677  |
| implantation rate % (n)              | 32.67 (294/900)                            | 33 (594/1800)                           | NA                          | 0.896   |
| miscarriage rate % (n)               | 13.33 (30/225)                             | 9.32 (40/429)                           | 1.43 (0.916–2.232)          | 0.115   |
| live-birth rate per start cycle % (n)| 28.55 (187/655)                            | 28.32 (371/1310)                        | 1.008 (0.869–1.170)         | 0.916   |
| malformation rate % (n)*             | 1.32 (3/227)                               | 1.01 (5/494)                            | 1.306 (0.315–5.417)         | 0.713   |
| birth weight (g)                     |                                            |                                          |                             |         |
| singletons                           | 3356.65 ± 507.92                           | 3295.41 ± 446.55                        | NA                          | 0.2117  |
| twins                                | 2515.44 ± 465.64                           | 2454.48 ± 406.52                        | NA                          | 0.2622  |

* malformation among live-born fetuses, early cumulus removal group: 147 singletons and 40 twins; traditional insemination group: 248 singletons and 123 twins
| early cumulus cells removal group (n = 655) | traditional insemination group (n = 1310) | relative risk no. (%) (95% CI) | P value |
|------------------------------------------|------------------------------------------|--------------------------------|---------|

* malformation among live-born fetuses, early cumulus removal group: 147 singletons and 40 twins; traditional insemination group: 248 singletons and 123 twins

**Discussion**

TFF is a frustrating experience for patients that carries heavy financial and emotional burdens. The occurrence of TFF is still hard to predict, but many IVF centers in China have adopted short coincubation of gametes and early cumulus cell removal combined with early Rescue ICSI (R-ICSI) to avoid TFF.

In our IVF center, we found that only a small portion of patients were offered R-ICSI and that most patients’ oocytes were only cleared of cumulus cells after a short time of coincubation of gametes and there was no need to perform R-ICSI (unpublished data). Although brief coincubation of gametes decreases the level of ROS, early removal of cumulus cells may disturb the crosstalk between oocytes and the cumulus-oocyte complex (COC), which plays an important role in early development of embryos [13], which begs the question of whether early removal of cumulus cells affects IVF outcomes. There are controversial results regarding this question. A prospective randomized sibling-oocytes study showed that early cumulus removal after 3 h of insemination had no effect on the normal fertilization rate but was associated with a higher polyspermy rate [14]. Another retrospective study showed that early removal of COC after 6 h of coincubation with gametes has a significantly lower polyspermy rate [15]. Further, a prospective cohort study found that removal of COC after 2–4 h insemination had an effect on polyspermy rate [5]. Although there were conflicting results regarding polyspermy rate, all of these papers demonstrated that early cumulus cell removal did not affect normal fertilization [5, 14, 15]. Our study showed that early cumulus cell removal was associated with lower normal fertilization but had no effect on polyspermy. These controversial results may be due to different times of COC removal. At early times after insemination, the oocytes are more vulnerable [9], and the earlier the removal of COC is, the greater susceptibility to damage. Different times of COC removal after insemination maybe pose different effects on fertilization procedures, which needs further study.

Regarding the effect of early cumulus cell removal on the developmental potential of embryos, current data shows similarly controversial results. Some studies have found that early removal of COC was associated with higher available embryos rate [16, 17], other studies have shown that early removal of COC did not affect embryonic development [5, 14], while Wei D et al. found that early removal of cumulus cells may impact embryonic development [9]. Our study showed available embryos on Day 3 in early removal of COC group was significantly lower than traditional IVF group. These conflicting results may be due to different times of COC removal after insemination and different patients recruited. Further RCTs are required to classify the effect of early removal of COC on the developmental potential of embryos.
A meta-analysis published in 2013 showed that a brief coincubation was associated with a higher ongoing pregnancy rate and clinical pregnancy rate [18]. Current studies demonstrated that early removal of COC has no significant difference in clinical pregnancy rate and live birth rate compared to traditional IVF. Our study also showed that early cumulus cell removal had no negative effect on the clinical pregnancy rate and live birth rate and that early removal of COC did not increase the miscarriage rate. Similarly, our study did not indicate that early removal of COC elevated the rate of malformations.

Our study has some limitations that should be considered. First, although this study adopted propensity score-match to weigh patients’ basal characteristics, our study was a retrospective cohort study. Second, the study was conducted at a single IVF center, so our results may not be applicable to other IVF centers.

Conclusions

In conclusion, our retrospective PSM study showed that early cumulus cell removal results in a lower available embryos rate but has no negative effect on the live birth rate. Further studies are required to confirm our findings. There is a real need for noninvasive biomarkers to predict TFF in order to decrease the percentage of patients who had early cumulus cell removal performed on their oocytes to avoid TFF.

Abbreviations

| abbreviations                           |            |
|----------------------------------------|------------|
| early cumulus cell removal             | ECCR       |
| Total fertilization failure            | TFF        |
| In vitro fertilization                 | IVF        |
| intracytoplasmic sperm injection       | ICSI       |
| free oxygen radicals                   | ROS        |
| intrauterine insemination              | IUI        |
| propensity score matching              | PSM        |
| cumulus-oocyte complexes               | COCs       |
| early Rescue ICSI                      | R-ICSI     |
Declarations

Ethics approval and consent to participate

This study received ethics approval from Ethics Committee of Shanghai First Maternity and Infant Hospital on 15th August, 2019, Reference No.: 2019-57

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests
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Authors' contributions

Data acquisition and analysis: Min Hao Liu, Li Juan Sun and Mei Yuan Huang. Data interpretation: Jia Ping pan and Shan Shan Liang. Conception of the study and drifting: Hai Xia Wu

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**Tables**

Table 1 Baseline demographics of study cohort
Table 2 Comparison of fertilization and clinical outcomes between the two groups

| Source                              | early cumulus cells removal group(n=655) | traditional insemination group(n=1310) | P value |
|-------------------------------------|------------------------------------------|----------------------------------------|---------|
| age (year)                          | 31.3±3.6                                 | 31.3±3.6                               | 1       |
| BMI (kg/m²)                         | 21.8±3.2                                 | 21.6±2.99                              | 0.1724  |
| basal FSH (mIU/L)                   | 6.705 (5.53, 7.85)                       | 6.78 (5.62, 8.05)                      | 0.2494  |
| period of infertility (year)        | 4 (2, 5)                                 | 3 (2, 5)                               | 0       |
| primary infertility (%)             | 512 (78.2)                               | 1024 (78.2)                            | 1       |
| nulliparity (%)                     | 629 (96.03)                              | 1247 (95.19)                           | 0.423   |
| infertility factors                 |                                          |                                        | 0       |
| female (%)                          | 270 (41.2)                               | 804 (61.4)                             |         |
| male (%)                            | 55 (8.4)                                 | 57 (4.4)                               |         |
| both female and male (%)            | 110 (6.8)                                | 300 (22.9)                             |         |
| unexplained (%)                     | 220 (33.6)                               | 149 (11.4)                             |         |
| COS protocol                        |                                          |                                        | 0       |
| long protocol (%)                   | 560 (85.5)                               | 993 (75.8)                             |         |
| short protocol (%)                  | 31 (4.7)                                 | 113 (8.6)                              |         |
| GnRH antagonist protocol (%)        | 41 (6.3)                                 | 83 (6.3)                               |         |
| minimal stimulation (%)             | 14 (2.1)                                 | 84 (6.4)                               |         |
| ultra-long protocol (%)             | 9 (1.4)                                  | 37 (2.8)                               |         |
| COS                                |                                          |                                        |         |
| total dose of Gn (IU)               | 2025 (1650, 2700)                        | 1950 (1550, 2475)                      | 0.0002  |
| days of stimulation (days)          | 12.12±3.23                               | 11.34±3.14                             | 0       |
| E2 on HCG day (pg/ml)               | 2461.905 (1687, 3622.69)                 | 2212.13 (1204.66, 3205)                | 0       |
| LH on HCG day (IU/L)                | 0.825 (0.49, 1.53)                       | 1.125 (0.56, 2.41)                     | 0       |
| P on HCG day (ng/ml)                | 0.87 (0.61, 1.15)                        | 0.83 (0.58, 1.13)                      | 0.144   |
| No. of retrieved oocytes (n) | early cumulus cells removal group (n=655) | traditional insemination group (n=1310) | relative risk no.(%) (95%CI) | P value |
|----------------------------|------------------------------------------|------------------------------------------|-----------------------------|---------|
| fertilization rate (%)     | 73.86 (6048/8188)                        | 80.12 (11022/13757)                     | NA                          | 0       |
| normal fertilization rate (%) | 62.76 (5139/8188)                   | 69 (9493/13757)                          | NA                          | 0       |
| polyspermy rate (%)        | 11.10 (909/8188)                        | 11.11 (1529/13757)                      | NA                          | 0.982   |
| cleavage rate (%)          | 93.93 (5681/6048)                       | 93.50 (10306/13757)                     | NA                          | 0.279   |
| available embryos rate (%) | 59.62 (3387/5681)                       | 62.29 (6420/10306)                      | NA                          | 0.001   |
| days of transferred embryos (day) | 2.70±0.49               | 2.66±0.53                               | NA                          | 0.1717  |
| No. of transferred embryos (n) | 1.93±0.33                     | 1.89±0.36                               | NA                          | 0.0437  |
| clinical pregnancy rate (%) | 48.28 (225/466)                   | 45.16 (429/950)                         | 1.069 (0.951-1.202)        | 0.2677  |
| implantation rate (%)      | 32.67 (294/900)                      | 33 (594/1800)                           | NA                          | 0.896   |
| miscarriage rate (%)       | 13.33 (30/225)                       | 9.32 (40/429)                           | 1.43 (0.916-2.232)         | 0.115   |
| live-birth rate per start cycle (%) | 28.55 (187/655)        | 28.32 (371/1310)                        | 1.008 (0.869-1.170)        | 0.916   |
| malformation rate (%)      | 1.32 (3/227)                        | 1.01 (5/494)                             | 1.306 (0.315-5.417)        | 0.713   |
| birth weight (g)           |                                          |                                          |                             |         |
| singletons                 | 3356.65±507.92                      | 3295.41±446.55                          | NA                          | 0.2117  |
| twins                      | 2515.44±465.64                      | 2454.48±406.52                          | NA                          | 0.2622  |

* malformation among live-born fetuses, early cumulus removal group: 147 singletons and 40 twins; traditional insemination group: 248 singletons and 123 twins

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