Arch Obstet Gynecol. 2023;4(2):50-55.

Archives of Obstetrics and Gynaecology Research Article

# Early Cumulus Cell Removal Increases Cumulative Live Birth Rate while Having No Negative Effect on the Malformation Rate in *In vitro* Fertilization: A Propensity Score-Matched Cohort Study

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**Received date:** April 24, 2023, **Accepted date:** April 27, 2023

**Citation:** Sun LJ, Liang SS, Liu MH, Pan JP, Huang MY, Teng XM, et al. Early Cumulus Cell Removal Increases Cumulative Live Birth Rate while Having No Negative Effect on the Malformation Rate in *In vitro* Fertilization: A Propensity Score-Matched Cohort Study. Arch Obstet Gynecol. 2023;4(2):50-55.



## Abstract

**Objective:** The aim of this study was to investigate the efficacy and safety of early cumulus cell removal (ECCR) during human *in vitro* fertilization (IVF).

**Methods:** A retrospective analysis was performed between January 2011 and December 2019. The study enrolled 1,131 couples who underwent IVF treatment with ECCR. After propensity score matching at a 1:1 ratio, 1,131 couples who underwent overnight coincubation of gametes were selected. The main outcome measure was the cumulative live birth rate. Secondary outcome measures included the cumulative pregnancy rate, polyspermy rate, available embryo rate, miscarriage rate, malformation rate, time to live birth, and oocyte-to-baby rate.

**Results:** There were no significant differences found between the two groups in the polyspermy rate, available embryo rate, miscarriage rate, time to live birth, oocyte-to-baby rate, and neonatal congenital anomalies rate. The results of the study showed that ECCR was associated with a significantly higher cumulative live birth rate and cumulative pregnancy rate, along with a significantly lower fertilization rate.

**Conclusion:** ECCR tended to confer increased cumulative live birth rate and had no negative effect on the neonatal malformation rate.

**Keywords:** Early cumulus cell removal, *In vitro* fertilization, Cumulative live birth rate, Malformation rate, Cumulative pregnancy rate

## Introduction

Total fertilization failure (TFF) in *in vitro* fertilization (IVF) is a discouraging event for patients. Reportedly, the incidence of TFF after IVF is 4-16% [1,2]. Following TFF, medical teams have limited options. The primary option is to cancel the current IVF cycle and change to intracytoplasmic sperm injection (ICSI) in the subsequent cycle, which may bring a heavy financial and emotional burden to the patient. An alternative choice is to perform ICSI in the current cycle. However, due to its low effectiveness [3], late rescue ICSI is not considered a favorable option.

Short co-incubation of gametes combined with early rescue ICSI has been demonstrated to prevent TFF [4]. This approach has been offered to populations at high risk of TFF. It has been indicated that early rescue ICSI contributes to avoidance of TFF [5,6] or lower fertilization rate [7] .

It may be beneficial by reducing potential detrimental

exposure to free oxygen radicals (reactive oxygen species [ROS]) and other metabolic products derived from spermatozoa [8,9]. This protocol was adopted by numerous IVF centers in China.

However, the efficacy and safety of ECCR remains debatable. ECCR may be detrimental to embryo development [10,11] due to breakage of the important cross-talk between oocyte and cumulus cells during the early embryonic developmental stages [12]. Although one retrospective study showed that ECCR was not associated with adverse pregnancy and neonatal outcomes [13], due to detriment on early embryonic development, whether ECCR was associated with birth defect, that is, the safety of ECCR needs further studies. In our IVF center, less than 20% patients whose oocytes were performed early rescue ICSI after short co-incubation (unpublished data). However, the oocytes from most patients were not performed early rescue ICSI, in which two poly bodies were observed after removal of the cumulus cells, indicating normal fertilizing capabilities of the oocyte and sperm. To avoid TFF, most patients who did not need to undergo early rescue ICSI were offered ECCR. Hence, the aim of this study was to investigate whether ECCR exerts an effect on the developmental potential of the embryo and cumulative live birth rate (CLBR), as well as clarify the effectiveness and safety (birth defects) of ECCR.

## Patients and Methods

### Patients

The protocol of this study was reviewed and approved by the Institutional Reviews Boards and Ethics Committee of Shanghai First Maternity and Infant Hospital, China on August 15, 2019 (reference number: 2019-57). This was a retrospective cohort study of patients undergoing IVF-embryo transfer treatment between January 2011 and December 2019 at the Reproductive Medicine Center of Shanghai First Maternity and Infant Hospital.

The inclusion criteria were: first IVF cycle with cleavage stage embryo transfer; and females aged 20-42 years. The exclusion criteria were: blastocyst transfer, congenital or acquired uterine malformations; abnormal results on parental karyotyping; presence of uterine fibroids; adenomyosis; and untreated hydrosalpinx. Well-controlled diabetes and hypertension were not exclusion criteria. The use of donor semen was allowed 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 treatment [14,15]. The study included 1,131 patients who received short coincubation and ECCR, and 1,131 patients who underwent traditional IVF with overnight coincubation of gametes after propensity score matching (PSM) based on age, body mass index, basal serum follicle-stimulating hormone, primary infertility or secondary infertility, and the number of retrieved oocytes.

### Measures

The primary outcome of interest was the CLBR (defined as one live birth from one initiated ART cycle, including all cycles in which fresh and/or frozen embryos are transferred. The delivery of twins is registered as one delivery). Additional outcomes included the cumulative clinical pregnancy rate defined as clinical pregnancy from one initiated ART cycle, polyspermy fertilization rate (number of fertilized oocytes with >2PN divided by number of COC inseminated), miscarriage rate (number of miscarriage before 28 gestation weeks divided by number of clinical pregnancy), malformation rate (number of newborns with birth defect divided by number of newborns), time to live birth (duration between the date of oocyte collection and the date of live birth), and oocyte-to-baby rate (number of newborns divided by number of achieved oocytes). The neonatal malformation was classified and coded according to the International Classification of Diseases, 10$^{th}$ Revision (ICD-10).

### Cumulus cell removal

In the ECCR group, cumulus cells were mechanically removed from cumulus-oocyte complexes (COCs) following 6 h of coincubation. Presence of two polar bodies indicated successful fertilization of a zygote. Fertilized zygotes were transferred to another fresh microdroplet without sperm and cultured overnight. In the traditional IVF group, cumulus cells were removed after 18-20 h of insemination for fertilization assessment.

### Statistical analysis

The PSM method using nearest neighbor matching at a proportion of 1:1 was utilized to adjust for factors that influence the probability of receiving different time of fertilization check.

Chi-squared tests were used for the univariate analysis of categorical variables. In cases in which the chi-squared analysis of a 2×2 table did not meet the assumption (i.e., <20% of expected values were <5), the Fisher's exact test was used. Independent t-tests and rank-sum tests were used for the analysis of continuous variables. Losses prior to follow-up were excluded. Statistical analysis was performed using the R Version 4·0·5.

## Results

A total of 1,131 patients were enrolled in the ECCR group; following PSM, 1,131 patients were included in the traditional IVF group. The baseline characteristics of the patients are shown in **Table 1**. There were no significant differences between the two groups in terms of age, body mass index, basal serum level of follicle-stimulating hormone, and nulliparity. The

**Table 1. Baseline demographics of the study cohort.**

| Female partner | Early cumulus cell removal group (n=1,131) | Traditional insemination group (n=1,131) | P-value |
|---|---|---|---|
| **Age *(years)** | 31.1 ± 3.53 | 30.9 ± 3.89 | 0.3595 |
| **BMI * (kg/m²)** | 21.97 ± 3.29 | 21.86 ± 3.18 | 0.405 |
| **Basal FSH #(mIU/L)** | 6.4 (5.3, 7.6) | 6.5 (5.4, 7.6) | 0.2502 |
| **Period of infertility # (years)** | 4 (2,5) | 3 (2,4) | 0 |
| **Primary infertility (%)** | 866 (76.6) | 797 (70.5) | 0.001 |
| **Nulliparity (%)** | 1,091 (96.5) | 1,085 (95.9) | 0.5825 |
| **Infertility factors** | | | 0 |
| **Female (%)** | 705 (62.3) | 796 (70.4) | |
| **Male (%)** | 184 (16.3) | 89 (7.9) | |
| **Both female and male (%)** | 98 (8.6) | 164 (14.5) | |
| **Unexplained (%)** | 144 (12.7) | 82 (7.3) | |
| **COS protocol** | | | 0 |
| **Long protocol (%)** | 853 (75.4) | 799 (70.6) | |
| **Short protocol (%)** | 29 (2.6) | 28 (2.4) | |
| **GnRH antagonist protocol (%)** | 197 (17.4) | 200 (17.6) | |
| **Minimal stimulation (%)** | 33 (2.9) | 82 (7.3) | |
| **Ultra-long protocol (%)** | 10 (0.9) | 13 (1.1) | |
| **COS** | | | |
| **Total dose of Gn # (IU)** | 1,950 (1,500, 2,584) | 1,800 (1,425, 2,334) | 0 |
| **Days of stimulation # (days)** | 11 (10,13) | 10 (9, 12) | 0 |
| **E2 on HCG day # (pg/mL)** | 2,563 (1,758, 3,689) | 2,516 (1,586, 3,641) | 0.09517 |
| **LH on HCG day # (IU/L)** | 0.99 (0.53, 1.88) | 1.36 (0.7, 2.58) | 0 |
| **P on HCG day # (ng/mL)** | 0.85 (0.61, 1.16) | 0.834 (0.61, 1.13) | 0.60 |

Abbreviations: BMI: Body Mass Index; COS: Controlled Ovarian Stimulation; E2: Estradiol; FSH: Follicle-Stimulating Hormone; Gn: Gonadotropin; GnRH: Gonadotrophin-Releasing Hormone; HCG: Human Chorionic Gonadotropin; LH: Luteinizing Hormone; P: Progesterone

* mean ± SD; # median (p25, p75)

early cumulus cell removal group showed a longer period of infertility compared with the traditional IVF group. The proportions of primary infertility and unexplained infertility in the ECCR group were significantly higher compared with those of the traditional IVF group. Similarly, the ECCR group required significantly higher total doses of gonadotropin and longer durations of stimulation.

The fertilization and clinical outcomes are shown in **Table 2**. A total of 13,938 and 13,087 oocytes were retrieved from the ECCR and traditional IVF groups, respectively. There was a significantly higher mean number of oocytes retrieved from the ECCR group versus the traditional IVF group. The rates of polyspermy and cleavage were similar between the two groups. There were no significant differences observed in the percentage of available embryos between the ECCR and traditional IVF groups. The cumulative clinical pregnancy rate and CLBR in the ECCR group were 76% and 60.2%, respectively; the rates were significantly higher than those recorded in the traditional IVF group (66.3% and 51.5%, respectively; P<0.01). The miscarriage rate was 14.7% and 16.8% in the ECCR and traditional IVF groups, respectively; however, this difference was not statistically significant. Moreover, there were no significant differences observed in the mean time-to-live birth and oocyte-to-baby rate between the two groups. In the ECCR group, a total of 836 babies were delivered. Neonatal anomalies (e.g., heart disease, urinary tract malformation; **Supplementary Table 1**) were diagnosed in 25 of those

**Table 2. Comparison of fertilization and clinical outcomes between the two groups.**

| | Early cumulus cell removal group (n=1131) | Traditional insemination group (n=1131) | P-value |
|---|---|---|---|
| **Number of retrieved oocytes** | 12.32 ± 5.98 | 11.57 ± 6.81 | 0.005265 |
| **Fertilization rate, % (n)** | 72.3 (10,078/13,938) | 77.9 (10,194/13,087) | 0 |
| **Polyspermy rate, % (n)** | 7.1 (992/13,938) | 7.0 (912/13,087) | 0.6506 |
| **Cleavage rate, % (n)** | 95.2 (9,595/10,078) | 95.6 (9,748/10,194) | 0.1652 |
| **Available cleavage stage embryos rate, % (n)** | 54.3 (5,212/9,595) | 54 (5,260/9,748) | 0.6254 |
| **Cumulative pregnancy rate, % (n)** | 76 (859/1,131) | 66.3 (750/1,131) | 0 |
| **Cumulative live birth, % (n)** | 60.2 (681/1,131) | 51.5 (582/1,131) | 0 |
| **Miscarriage rate, % (n)** | 14.7 (126/859) | 16.8 (126/750) | 0.2692 |
| **Ectopic pregnancy, % (n)** | 4.0 (34/859) | 4.0 (30/750) | 1.0 |
| **Time to live birth (days)** | 321 ± 114 | 329 ± 138 | 0.133 |
| **Oocyte-to-baby rate, % (n)** | 6.0 (836/13,938) | 5.7 (751/13,087) | 0.3784 |
| **Malformation rate*, %(n)** | 3.0 (25/836) | 3.8 (29/751) | 0.336 |

* Malformation among live-born fetuses; early cumulus removal group: 526 singletons and 155 twins; traditional insemination group: 413 singletons and 169 twins

babies. In the traditional insemination group, 751 babies; of those, 29 exhibited abnormalities (**Supplementary Table 1**). There was no significant difference in the neonatal congenital anomaly rate between the two groups (3.0% vs. 3.8%, respectively; P=0.336).

## Discussion

TFF is a frustrating experience for patients, resulting in heavy financial and emotional burden. Prediction of the occurrence of TFF remains challenging; however, numerous IVF centers in China have adopted short coincubation of gametes and ECCR combined with early rescue ICSI as strategies for avoiding TFF.

In our IVF center, we found that only a small portion of patients were offered early rescue ICSI. In addition, the oocytes of most patients were only cleared of cumulus cells after a short time of coincubation of gametes and did not need to perform early rescue ICSI (unpublished data). Although brief coincubation of gametes decreases the levels of ROS, ECCR may disrupt the crosstalk between oocytes and the COC which plays an important role in the early development of embryos [11,16]. This fact raises questions regarding the potential effects of ECCR on IVF outcomes. Thus far, studies addressing this topic have yielded controversial results. 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 [17]. Another retrospective study showed that early removal of COC after 6 h of coincubation with gametes significantly reduces the polyspermy rate [18]. Furthermore, a prospective cohort study found that removal of COC 5 h after insemination had no effect on the polyspermy rate [5]. Despite the conflicting results regarding the polyspermy rate, all these studies demonstrated that ECCR did not affect normal fertilization [5,17,18]. Recent one prospective study [19] found that ECCR at 6 h had no significant difference in fertilization, polyspermy, embryo development compared to late removal. Our study showed that ECCR was associated with a lower fertilization rate, but had no effect on polyspermy rate. Liu et al. [20] found that the timing of ECCR had a significant effect on the polyspermy rate and an earlier timing to remove the cumulus cells was associated with higher polyspermy rates. These controversial results were due to the different times of COC removal. At early times after insemination, the oocytes are more vulnerable [10]; early removal of COC is linked to greater susceptibility to damage. Different times of COC removal after insemination may result in different effects on fertilization procedures. Further study is warranted to determine these effects.

The currently available data regarding the effect of ECCR on the developmental potential of embryos are controversial. Some studies have found that early removal of COC was associated with a higher number of available embryos [7,21]; other studies have shown that early removal of COC did not affect embryonic development [5,17], whereas Wei et al. reported that ECCR may impact embryonic development [10]. Our study showed that the number of available embryos in the early removal of COC group and traditional IVF group

were similar. A retrospective study [20] demonstrated that earlier timing to remove cumulus cells was associated with higher grade 1-2 embryo rate at Day 3. These conflicting results may be due to the different times of COC removal after insemination and different patients recruited in studies. Additional randomized controlled trials are required to clarify the effect of different timing of ECCR on the early embryonic development potential.

In 2013, a meta-analysis showed that a brief coincubation was associated with a higher ongoing pregnancy rate and clinical pregnancy rate [22]. A retrospective study [13] found that ECCR had similar clinical pregnancy and live birth rate compared to late removal. Our randomized controlled trial found that brief incubation of gametes did not improve live birth rate compared to standard incubation [23]. All these papers focused on the clinical pregnancy and live birth rate. Few studied compared the cumulative pregnancy and live birth rate difference between ECCR and traditional IVF. We adopted PSM to adjust basal characteristics of patients, our present results demonstrated that early removal of COC increases the cumulative clinical pregnancy rate and CLBR compared to traditional IVF. Recent studies demonstrated that ECCR was not associated with adverse pregnancy and neonatal outcomes [13,19]. This study also showed that ECCR had no negative effect on the miscarriage rate, and that early removal of COC did not increase the rate of malformations, which was similar to the results of recent studies [13]. It has been reported that ROS derived from spermatozoa *in vitro* adversely affect oocytes and zygotes, thus influencing embryo quality [24,25]. Our study showed that ECCR exerted beneficial effects on the cumulative live birth rate. These effects may be attributed to the lower levels of ROS in culture media after short coincubation of gametes, thereby reducing the harmful influence of ROS on embryo quality.

Some limitations of this study should be considered. Firstly, although this investigation adopted PSM to weigh the baseline characteristics of patients, it was a retrospective cohort study. Secondly, the study was conducted at a single IVF center, thus, the results may not be applicable to other settings.

In conclusion, the results of the present retrospective PSM study showed that ECCR increases the CLBR, but has no negative effect on the neonatal malformation rate. Further randomized controlled studies are warranted to confirm our findings.

## Funding

This work was supported by the Shanghai Public Health Bureau under Grant number 201440005.

## Disclosure Statement

The authors report no conflict of interest.

## Author Contributions

Data collection and analysis: Min Hao Liu, Li Juan Sun, and Mei Yuan Huang.

Data analysis and interpretation: Jia Ping pan and Shan Shan Liang.

Study design and writing: Xiao Ming Teng and Hai Xia Wu.

## Acknowledgements

The authors thank staffs at the Department of Reproductive Medicine (Shanghai First Maternity and Infant Hospital) for their scientific advice and encouragement. And thanks Shanghai Public Health Bureau for the financial support.

## Data Sharing

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

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