

## Original Article

# Effect of simultaneous observation of early cleavage and syngamy on clinical pregnancy in IVF and ICSI cycles

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Received July 11, 2018; Accepted September 12, 2018; Epub October 15, 2019; Published October 30, 2019

**Abstract:** Objective: The goal of this study was to evaluate the effect of simultaneous observation of early cleavage (EC) and syngamy on predicting the pregnancy outcomes during *in-vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles. Methods: Clinical data of 1987 pregnant women receiving IVF, ICSI, or the combination were retrospectively analyzed. A total of 1453 cycles of IVF, 425 cycles of ICSI, and 109 cycles of combination of IVF and ICSI were performed. Two embryos were transferred for each patient. According to the observed cleavage stage of transferred embryos, all patients were divided into six groups (EC+EC, EC+syngamy, syngamy+syngamy, EC+O, syngamy+O and O+O). Clinical pregnancy rate and implantation rate were calculated and statistically compared among different groups. Results: The clinical pregnancy rates in the EC+EC (62.90%) EC+syngamy (66.76%) and syngamy+syngamy groups (68.66%) were significantly higher than those in the remaining groups (all  $P<0.05$ ). Similar outcomes were observed in terms of the live birth rate. The abortion rate significantly differed among six groups ( $P<0.01$ ). For two-group comparison test, the abortion rate in the O+O group was 13.16%, which was the highest among all six groups ( $P<0.01$ ). The abortion rate in the EC+O group was 6.67%, which was the lowest among six groups ( $P<0.05$ ). Conclusion: During IVF or ICSI, the implantation rate and the clinical pregnancy rate are the highest when both EC and syngamy are observed compared with those when merely one/none of these two events occur, which significantly reduces the frequency of embryo observation and minimize negative impact upon embryo selection.

**Keywords:** Early cleavage, syngamy, pregnant outcome, IVF, ICSI

## Introduction

Along with progression and advancement of human assisted reproduction, a variety of interventional strategies have been chosen for the embryo selection for intrauterine embryo transfer during *in-vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles [1]. The possibility of choosing sufficient embryos with high implantation potential will allow for the decline in the quantity of intrauterine transferred embryos. This will lead to a decrease in the percentage of multiple pregnancies and its complications. The scoring criteria of embryo selection are based on the dynamic observation of embryo morphology conducted on the 1<sup>st</sup> day when the fertilization and early cleavage are assessed, on the 2<sup>nd</sup> and 3<sup>rd</sup> days when the

embryo cleavage and blastomere fragmentation are monitored and combinations of these criteria can be properly evaluated on the 5<sup>th</sup> day [2-4].

Embryo selection is a challenging and demanding task on the embryo transfer day. A variety of parameters should be observed and considered to enhance the quality of the selected embryos, such as early cleavage (EC), syngamy, blastomere morphology, and blastocyst grading, etc [5].

EC refers to embryonic mitosis occurring ( $28\pm1$ ) hours after IVF and ( $26\pm1$ ) hours following ICSI. Syngamy is defined as the fusion of two gametes to form a zygote at ( $23\pm1$ ) hours after human assisted reproduction. In previous stud-

ies, physicians have performed embryo selection twice to observe the incidence of EC and syngamy, which instead exert certain negative effect upon the transferred embryos [6-8].

In this clinical trial, a retrospective analysis of clinical data of 1987 pregnant women receiving IVF, ICSI or combination was conducted. To reduce the embryo damage induced by embryo selection, the time point (25 hours after insemination) was chosen when both EC and syngamy could be observed. In addition, the implantation rate and clinical pregnancy rate were calculated and statistically compared to evaluate the feasibility of the time point for embryo observation and selection in clinical practice.

### Materials and methods

#### *Baseline data*

In this retrospective study, baseline data and medical records of 1987 pregnant women, aged 21-35 years, receiving IVF, ICSI or combination were retrospectively analyzed between January 2014 and December 2015 in Xiangya Hospital, Central South University. A total of 1453 cycles of IVF, 425 cycles of ICSI, and 109 cycles of combination of IVF and ICSI were performed. Two embryos were transferred for each participant. According to the results of embryo morphological observation, all enrolled women were assigned into six groups including EC+EC, EC+syngamy, syngamy+syngamy, EC+O, syngamy+O and O+O (O represents neither EC nor syngamy occurs).

#### *Inclusion criteria*

The eligible women were aged  $\leq 35$  years; those with the level of follicle stimulating hormone (FSH)  $< 10$  IU/L on the 2<sup>nd</sup> day after menstruation; those who received IVF or ICSI for the first time; those who had received IVF/ICSI for one cycle of treatment; those who received the gonadotropin-releasing hormone (GnRH) agonist long protocol; those who underwent embryo implantation at 66-68 h (day 3) after IVF or ICSI.

#### *Ovarian stimulation*

The gonadotropin-releasing hormone (GnRH) agonist long protocol and the GnRH antagonist protocol were adopted. A dosage of 0.5 mg of leuprolide acetate (Lupron, Takeda, Germany)

was administered daily on day 21 of the previous menstrual cycle. Once serum levels of estradiol (E2)  $< 40$  pg/ml were achieved, recombinant follicle-stimulating hormone (FSH; Gonal-F; Serono, Switzerland) and human menopausal gonadotropin (hMG; Menopur; Ferring, Germany) were administered from the third day of menstruation until the day of human chorionic gonadotropin (hCG) administration. The doses were adjusted according to the ovarian response of each patient. Recombinant FSH and hMG were administered daily from the third day of the menstrual cycle. The doses were also adjusted according to individual ovarian responses. Once the dominant follicle reached 14 mm in mean diameter, 0.25 mg cetrorelix acetate (Cetrotide; Serono, Germany) was administered subcutaneously daily until the day of hCG administration. Final maturation was induced using either recombinant hCG (Ovidrel; Serono, Italy) or urinary hCG (Profasi; Serono, Switzerland) when at least 2 leading follicles reached 18 mm in diameter followed by oocyte retrieval 34-36 hours later. On day 3, the embryonic morphology was graded and embryo transfer was performed 72 hours after oocyte retrieval.

#### *IVF and ICSI*

For the male counterparts with a sperm concentration of  $< 10 \times 10^6$ /mL, motility  $< 25\%$ , or normal morphologic sperm (Kruger's strict criteria)  $< 4\%$ , the enrolled women received the ICSI technique, whereas those without male factor infertility were treated with conventional IVF therapy. Both IVF and ICSI were performed 3-5 hours after oocyte aspiration. For the IVF procedure, each oocyte was inseminated with  $10 \times 10^3$  motile spermatozoa in a single drop of 40  $\mu$ L medium (G-IVF<sup>TM</sup> Irvine Scientific). For the ICSI procedure, 1-2  $\mu$ L washed spermatozoa was placed in 10% polyvinylpyrrolidone (PVP; Irvine Scientific) and sperm were injected using standard techniques. Each embryo was cultured in a single drop of 40  $\mu$ L medium (G-1<sup>TM</sup> PLUS vitrolife) that was covered with mineral oil (SAGE IVF) in an atmosphere of 5% O<sub>2</sub>, 6% CO<sub>2</sub> and 89% N<sub>2</sub> at 37°C.

#### *Observation of embryonic cleavage and syngamy*

Normal fertilization was confirmed by the incidence of two pro-nuclei and two polar bodies at

approximately 16-20 hours (day 1) after IVF or ICSI treatment. On the same day, EC examination was performed 25-27 hours after IVF or ICSI. Embryos displaying 2 cells at inspection were considered EC embryos, whereas those that had not yet cleaved to the 2-cell stage were considered as non-EC embryos. Embryos were further examined for their quality at 66-68 hours (day 3) after IVF or ICSI. Day-3 embryos were classified according to blastomere number as follows: rapid cleavage ( $\geq$  number as follows: rapid cleavage (bryos were classified a6 cells). On the basis of quality, day-3 embryos were graded as four levels: level I as a score of 4, II as a score of 3, III as 2 and IV as 1 score, respectively [9].

## Statistical analysis

SPSS software version 19.0 for Windows was adopted for statistical analysis (SPSS Inc., Chicago, IL, U.S.). The differences in mean between two variables were statistically analyzed by using the Mann-Whitney U test and unpaired *t*-test. The differences in the rates of implantation, clinical pregnancy, abortion, live birth, and EC were calculated using the chi-square test. A *P* value of less than 0.05 was considered statistically significant.

## Results

### Baseline data

Clinical data of 1987 pregnant women receiving IVF, ICSI or combination were retrospectively analyzed. A total of 1453 cycles of IVF, 425 cycles of ICSI, and 109 cycles of combination IVF and ICSI were performed. Two embryos were transferred for each patient. Among all groups, the mean age, body mass index (BMI) and years of infertility did not significantly differ (all  $P>0.05$ ). The proportion of secondary infertility ranged from 51.61% to 53.69% with no statistical significance among six groups ( $P>0.05$ ). Baseline levels of FSH and LH and the number of antral follicles did not significantly differ among different groups (all  $P>0.05$ ). In addition, no statistical significance was observed in the percentage of ICSI treatment among six groups (all  $P>0.05$ ), as illustrated in **Table 1**.

### Laboratory parameters

Serum levels of estrogen detected on the day of HCG administration ranged from (3412.15±

510.23) IU/L to (3735.10±410.78) IU/L, and no statistical significance was documented in terms of the estrogen levels among six groups ( $P>0.05$ ). In the EC+syngamy group, endometrial thickness was measured as (11.11±2.03) mm, which was slightly larger compared with those in the remaining groups with no statistical significance ( $P>0.05$ ). The mean number of retrieved oocytes was quantitatively measured from 10.41±2.16 to 11.22±3.25, and no statistical significance was noted among different groups ( $P>0.05$ ). In all groups, two embryos were transferred for each enrolled female patient, as demonstrated in **Table 2**.

### Clinical pregnancy rate

The clinical pregnancy rate significantly differed among six groups ( $P<0.01$ ). For two-group comparison test, the pregnancy rate (30.24%) in the O+O group was significantly lower than that in each of the remaining five groups (all  $P<0.05$ ). The clinical pregnancy rate in the EC+syngamy group was 66.76%, which was significantly higher compared with 56.17% in the syngamy+O group ( $P<0.05$ ). Moreover, statistical significance was observed between the syngamy+syngamy group and the syngamy+O group (68.66% v.s. 56.17%;  $P<0.01$ ), as shown in **Table 2**.

### Live birth rate

Chi-square test demonstrated that the live birth rate significantly differed among six groups ( $P<0.01$ ). For two-group comparison test, the live birth rate in the O+O group was calculated as 28.11%, which was significantly lower than that in each of the remaining five groups (all  $P<0.05$ ). The live birth rates in the EC+syngamy group (64.77%), EC+EC group (60.32%) and syngamy+syngamy group (63.75%) were significantly higher compared with 56.13% in the EC+O group and 51.54% in the syngamy+O group (all  $P<0.05$ ). However, no statistical significance was observed between any two groups among the EC+syngamy group (64.77%), EC+EC group (60.32%) and syngamy+syngamy group ( $P>0.05$ ), as demonstrated in **Table 2**.

### Abortion rate

The abortion rate significantly differed among six groups ( $P<0.01$ ). For two-group comparison test, the abortion rate in the O+O group was 13.16%, which was the highest among all six groups ( $P<0.01$ ). The abortion rate in the EC+O

## Early cleavage and syngamy on pregnant outcomes

**Table 1.** Comparison of baseline data among six groups

Parameter	EC+EC	EC+syngamy	EC+0	Syngamy+syngamy	Syngamy+0	0+0	P value
Number of cases (n)	310	352	155	469	324	377	-
Age (year)	29.72±2.38	29.97±2.35	29.91±2.62	29.89±2.14	30.05±3.11	30.02±1.79	0.13
BMI	21.92±1.44	22.19±2.03	22.20±1.25	23.04±2.25	21.89±2.01	21.62±1.34	0.15
Infertility duration (year)	4.28±1.12	4.38±2.33	4.52±1.02	4.18±0.96	4.57±2.35	4.66±0.88	0.67
Percentage of secondary infertility (%)	52.16	53.69	51.61	52.67	51.73	51.99	0.44
Baseline FSH (IU/L)	6.23±0.25	5.97±1.02	5.98±2.11	6.15±2.44	5.99±3.11	6.22±1.25	0.27
Baseline LH (IU/L)	5.13±2.01	5.26±1.02	5.96±1.58	5.27±2.02	5.32±2.15	5.01±1.22	0.51
Percentage of ICSI (%)	28.67	25.28	22.58	18.98	20.28	29.44	0.36
Number of antral follicles (n)	6.92	6.50	6.49	6.17	6.33	6.37	0.17

Note: 0 represents neither EC nor syngamy occurs for one single transferred embryo.

**Table 2.** Comparison of laboratory parameters and pregnancy outcomes among six groups

Parameters	EC+EC	EC+syngamy	EC+0	Syngamy+syngamy	Syngamy+0	0+0	P value
Estrogen level on HCG administration day (IU/L)	3566.46±425.22	3593.09±523.12	3437.06±336.21	3735.10±410.78	3412.15±510.23	3467.55±502.48	0.52
Endometrial thickness (mm)	10.81±1.25	11.11±2.03	10.81±1.77	11.09±2.25	10.70±2.17	10.88±1.44	0.18
Mean number of retrieved oocyte (n)	11.04±3.66	11.22±3.25	10.41±2.16	11.65±3.45	10.65±4.10	10.94±2.16	0.28
Number of transferred embryos (n)	2	2	2	2	2	2	-
Clinical pregnancy rate (%)	195/310 (62.90)	235/352 (66.76)	90/155 (58.06)	322/469 (68.66)	182/324 (56.17)	114/377 (30.24)	<0.01
Live birth rate (%)	187/310 (60.32)	228/352 (64.77)	87/155 (56.13)	299/469 (63.75)	167/324 (51.54)	106/377 (28.11)	<0.01
Abortion rate (%)	22/195 (11.28)	21/235 (8.94)	6/90 (6.67)	37/322 (11.49)	13/182 (7.14)	15/114 (13.16)	<0.01

group was 6.67%, which was the lowest among six groups ( $P<0.05$ ), followed by 7.14% in the syngamy+0 group and 8.94% in the EC+syngamy group. Abortion rates were almost equal between the EC+EC group (11.28%) and the syngamy+syngamy group (11.49%) with no statistical significance ( $P>0.05$ ), as illustrated in **Table 2**.

## Discussion

Embryo selection is a challenging task performed on the day of embryo transfer day. A variety of clinical and laboratory parameters including pronuclear morphology, EC, syngamy, blastomere morphology, and blastocyst grading can be utilized to enhance the quality of the transferred embryos [10]. Clinical outcomes of EC embryos should be evaluated when using the elective single embryo transfer (eSET) and DET approaches. For both IVF and ICSI cycles, the effect of EC on embryo transfer using mixed embryos could not be accurately evaluated. Multiple factors have been reported to affect the outcomes and prognosis of IVF and ICSI treatment [11]. Nevertheless, embryo morphology is the most common and widely adopted parameter to choose the best-quality embryos for subsequent embryo transfer. In recent years, double embryo transfer (DET) procedures have been highly recommended, it is of great significance to select the high-quality embryos to enhance the rates of implantation, pregnancy, and live birth and lower the abortion rate [12]. The use of EC identification to select embryos in humans has been first reported by Edwards et al. [13]. Previous studies have demonstrated that EC is an effective indicator for viable embryo selection in humans [6-9]. Moreover, application of the DET method with EC embryos results in higher implantation and pregnancy rates compared with those obtained by using the DET method with non-EC embryos [10-12]. The influence of EC on the live birth rate has been controversial. Emiliani et al. [3] have claimed that assessment of EC is not significantly associated with the delivery rate of single embryo transfer, whereas Lundin et al. [11] have proposed that EC serves as an independent predictor for the evaluation of live birth rate during the ICSI cycles.

Currently, the evaluation of transferred embryos is mainly conducted according to embryo morphology. However, any of scoring method

has certain limitations. EC and syngamy are both effective indicators for the transferred embryo evaluation [14]. Syngamy observation is generally performed at  $23\pm 1$  hours after fertilization, and early cleavage observation is constantly performed at  $28\pm 1$  hours after IVF and  $26\pm 1$  hours following ICSI [15]. Since the embryo physiology generally endures for a period of time, the observed time span ( $\pm 1$  hour) is relatively large. In order to reduce the damage to the transferred embryos, the frequency of embryo observations should be reduced. Consequently, an intermediate time point for embryo observation was chosen when both EC and syngamy could be possibly observed simultaneously. The time point of 25 hours after fertilization was chosen in current clinical trial.

In our study, all enrolled participants were specifically assigned into six groups according to the outcomes of embryo observation. In addition, three groups can be roughly classified including either EC or syngamy for both embryos, either EC or syngamy for one of the two embryos and neither EC nor syngamy observed for both embryos. Then, both baseline data, laboratory parameters and pregnancy-related parameters were recorded and statistically compared among different groups. Age, BMI, infertility duration in years, proportion of secondary infertility, baseline FSH and LH, and the number of antral follicles did not significantly differ among all six groups. Consequently, potential effects of these baseline parameters upon the pregnancy outcomes were minimized to a large extent.

Regarding laboratory parameters, the serum level of estrogen detected on the day of HCG administration ranged from  $(3412.15\pm 510.23)$  IU/L to  $(3735.10\pm 410.78)$  IU/L, and no statistical significance was documented among six groups. In the EC+syngamy group, the endometrial thickness was measured as  $(11.11\pm 2.03)$  mm, which was slightly larger compared with those in the remaining groups with no statistical significance. The mean number of retrieved oocytes was quantitatively measured from  $10.41\pm 2.16$  to  $11.22\pm 3.25$ , and no statistical significance was noted among different groups.

The clinical pregnancy rate significantly differed among six groups. For two-group comparison test, the pregnancy rate (30.24%) in the 0+0 group was significantly lower than that in each



of the remaining five groups, suggesting both EC and syngamy are indicators for predicting pregnancy outcomes. The clinical pregnancy rate in the EC+syngamy group was significantly higher compared with that in the syngamy+O group. Moreover, statistical significance was observed between the syngamy+syngamy group and the syngamy+O group (68.66% v.s. 56.17%;  $P<0.01$ ), indicating that simultaneous observation of both EC and syngamy yields higher pregnancy rate compared with observation of one single parameter.

Furthermore, the live birth rate significantly differed among six groups. For two-group comparison test, the live birth rate in the O+O group was the lowest, indicating that both EC and syngamy are associated with live birth rate. The live birth rates in the EC+syngamy group, EC+EC group and syngamy+syngamy group were significantly higher compared with those in the EC+O group and syngamy+O group. These results prompted simultaneous observation of both EC and syngamy yields which were higher in live birth rate compared with the observation of one single parameter.

The abortion rate in the O+O group was 13.16%, which was the highest among all six groups. The abortion rate in the EC+O group was 6.67%, which was the lowest among six groups. The abortion rate outcomes indicate no significant correlation between simultaneous observation of both EC and syngamy and the abortion rate.

## Conclusion

Taken together, this retrospective analysis of clinical data of 1987 pregnant women receiving IVF, ICSI or combination demonstrates that simultaneous observation of both EC and syngamy at 25 hours after fertilization is significantly positively correlated with the clinical pregnancy rate and live birth rate. Nevertheless, it is not intimately associated with the abortion rate. The abortion is subject to a variety of factors, which remains to be further elucidated.

## Disclosure of conflict of interest

None.

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