Effects of early-cleavage embryo transfer on in vitro fertilization-embryo transfer pregnancy outcomes

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Summary

Purpose: To observe the effects of early-cleavage embryo transfer (ET) on pregnancy outcomes in vitro fertilization-embryo transfer (IVF-ET). *Materials and Methods:* The data of 6,548 two pro-nucleate (2PN) embryos and 968 patients who underwent IVF or intracytoplasmic sperm injection (ICSI) were analyzed. Of the 968 cycles, early-cleavage embryos were used in 432 cycles (early-cleavage group), late-cleavage embryos were used in 246 cycles (late-cleavage group), and both early and late-cleavage embryos were used in 290 cycles (mixed group). *Results:* High-quality embryo rate was significantly higher in early-cleavage group than in late-cleavage group (82.74% vs 59.83%; $p < 0.01$). Both clinical pregnancy and implantation rates in IVF or ICSI were significantly higher in early-cleavage group than in late-cleavage group (all $p < 0.01$). In ICSI, both clinical pregnancy and implantation rates were significantly higher in mixed group than in late-cleavage group (all $p < 0.05$). *Conclusion:* Early-cleavage ET can improve pregnancy outcomes in IVF or ICSI..

Key words: Clinical pregnancy rate; Early cleavage; High-quality embryo; Implantation rate.

Introduction

How to choose a high-quality embryo with development potential to improve clinical pregnancy rate has become a focal point in vitro fertilization-embryo transfer (IVF-ET). Embryo morphology score has occupied a leading place in the choice of embryos. In recent years, a great deal of attention has been paid to early cleavage. It has been reported that early cleavage is an indicator of embryo quality and development potential [1]. It has been described that pregnancy outcomes are strongly associated with embryo morphology and high-quality ET has similar pregnancy outcomes, but pregnancy outcomes are not significantly correlated with early cleavage [2]. It has been believed that early cleavage has predictive value for the pregnancy outcomes in intracytoplasmic sperm injection (ICSI), but has no effects on the pregnancy outcomes of IVF [3]. At present, most studies are about that the effects of early cleavage on pregnancy outcomes are evaluated in day two or day three ET, or different stimulation protocols. Little research has been done regarding the effects of early cleavage on pregnancy outcomes in ICSI or IVF. The purpose of this study was to observe the effects of early cleavage on pregnancy outcomes in ICSI or IVF, and further confirm that early cleavage possesses better embryo development potential. This study has certain significance for improvement in IVF-ET pregnancy outcomes.

Materials and Methods

All study methods were approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All the subjects enrolled into the study gave written formal consent to participate.

Early cleavage was observed in all IVF and ICSI cycles performed in the present Center between March and August 2011. The data of 968 cycles were retrospectively analyzed. Inclusion criteria included (1) patients were less than 40-years-old; (2) day three high-quality embryos were transferred; (3) IVF was mainly due to unilateral or bilateral oviduct obstruction, chronic pelvic inflammatory disease, endometriosis, polycystic ovary syndrome (PCOS), the frequency of failure of artificial insemination by husband ≥ three, and unexplained infertility; (4) ICSI was mainly due to severe oligo-astheno-teratospermia, obstructive azoospermia, and low fertilization rate after conventional IVF. Exclusion criteria were (1) donor oocytes or sperm used in IVF; (2) rescue ICSI. Of the 968 patients, 546 patients had primary infertility (56.40%), and 422 patients had secondary infertility (44.60%).

Superovulation protocol

Superovulation was performed according to long-protocol or ultralong-protocol [4]. When down-regulation reached the standard, gonadotropin (Gn) which was intramuscularly given. Gn, highly-purified recombinant follicle-stimulating hormone (r-FSH), was gonal-F or puregon (100 IU/ampoule). The dose of Gn was adjusted according to follicular development and serous endocrine. Highly-purified menotropin for injection (75 IU/ampoule) was or was not given in the late follicular phase. When the dominant follicle ≥ 18 mm, 2,000 IU of human chorionic gonadotropin (HCG) and 250 ug of ovidrel were intramuscularly given; 34 to 36 hours later, oocytes were collected by transvaginal ultrasound-guided puncture.

Short-term insemination

The retrieved oocytes were incubated for two to three hours, then placed in sperm (1.0×10^6/ml/1.2×10^6/ml). Four to six hours later, most cumulus cells were removed. After fertilization was determined according to second polar body, the embryos were incubated for 16-18 hours followed by observing pronuclei.

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ICSI insemination

The retrieved oocytes were incubated for two to three hours, then were digested with hyaluronidase to remove cumulus cells. Mature oocytes were used in ICSI, 16-18 hours later, pronuclei were observed.

Pronuclei were observed according Scott and Smith scoring system [5]. Two polar bodies or two pronuclei, or both two polar bodies and two pronuclei were regarded as normal fertilization. One pronucleus or multiple pronuclei (≥ three) were regarded as abnormal fertilization. No pronucleus and cleavage were regarded as fertilization failure.

Early cleavage was observed 27-28 hours after IVF or 24-25 hours after ICSI, respectively. Cleavage into two cells or more was regarded as early-cleavage embryo.

Embryo quality

Embryo quality was evaluated according to Peter scoring system 48 and 72 hours after ovum pick-up [6]. Embryo grading criteria were (1) grade I: uniform blastomere with intact zona pellucida and moderate refractivity; (2) grade II: slightly nonuniform blastomere and less than ten percent fragment; (3) grade III: blastomere as that in grade II with intact zona pellucida and less than 50% fragment; (4) grade IV: blastomere being viable and more than 50% fragment; (5) grade V: 2PN in day two, or delayed fertilization; (6) grade VI: inviable embryo, blastomere lysis. High-quality embryo includes grades I and II embryos.

Choice and transplantation of high-quality embryos

Grade I or II normal zygotes with six cells or more were transferred 72 hours after ovum pick-up. Two embryos or less were transferred in the patients with the first cycle, three embryos were transferred in the patients with the age ≥ 35 years or more than two cycles. Progesterone was intramuscularly injected and duphaston was orally given for luteal support. HCG in urine and blood were determined 14 days and 18 days after ET, respectively. It was diagnosed as clinical pregnancy that B-mode ultrasound showed embryo sac and fetal heart beat 35 days after ET.

Grouping

In this study, there were 968 cycles. Of the 968 cycles, early-cleavage embryo was used in 432 cycles (early-cleavage group), late-cleavage embryo was used in 246 cycles (late-cleavage group), and both early and late-cleavage embryos were used in 290 cycles (mixed group).

Statistical analysis

Statistical analysis was performed with SPSS 16.0 software. Measurement data were expressed as mean ($\bar{x} \pm s$) and were analyzed with *t* test. Numeration data were expressed as rate and were analyzed with Chi-squared (χ^2) test. Test criterion was set at alpha (α) = 0.05 and statistical significance was established at $p < 0.05$.

Results

In 968 patients with the age of 30.93 ± 4.47 years, fertility rate was 80.80% (7,819 / 9,676) and cleavage rate was 96.11% (7,515 / 7,819). High-quality embryo rate was significantly higher in early-cleavage embryos (82.74%) than in late-cleavage embryos (59.89%) ($p < 0.01$, Table 1).

Table 1. — *Relation between early cleavage and high-quality embryos.*

	Early-cleavage embryoes	Late-cleavage embryoes
No. of normal fertilized ovum (n)	3,384	3,164
No. of day 3 high-quality embryos (n)	2,800 (82.74)*	1,895 (59.89)
High-quality embryos in IVF (%)	82.37 (2,360 / 2,865)*	59.60 (1,114 / 1,869)
High-quality embryos in ICSI (%)	84.78 (440 / 519)*	60.31 (781 / 1,295)

IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; * indicates $p < 0.01$, compared with late-cleavage embryos.

Pregnancy outcomes in IVF

This study included 694 IVF cycles. There were no statistical differences in age, duration of infertility, dose of Gn, basal FSH, levels of estradiol (E2), and progesterone (P) on HCG day and endometrial thickness on the day of ET between the three groups (all $p > 0.05$). The number of retrieved oocytes was significantly more in early-cleavage group than in mixed group ($p < 0.05$). Cleavage rate was significantly higher in early-cleavage group (97.26%) than in other two groups, and high-quality embryo rate was significantly lower in late-cleavage group (52.56%) than in other two groups (all $p < 0.01$). There was no significant difference in the number of high-quality ETs between the three groups. Clinical pregnancy and implantation rates were significantly higher in early-cleavage group (61.92% and 38.47%) than in other two groups (all $p < 0.05$). Clinical pregnancy and implantation rates were similar in late-cleavage group and mixed group. There were no significant differences in high-order birth rates and spontaneous abortion rates between the three groups (Table 2).

Pregnancy outcomes in ICSI

This study included 274 ICSI cycles. There were no statistical differences in general status between the three groups (all $p > 0.05$). Fertility rate was significantly higher in late-cleavage group (76.11%) than in mixed group ($p < 0.05$), but cleavage rate was significantly lower in late-cleavage group (93.5%) than in other two groups (all $p < 0.01$), and high-quality embryo rate was lower in late-cleavage group compared with other groups but without significant difference. Clinical pregnancy and implantation rates were significantly higher in early-cleavage group (73.13% and 45.21%) and mixed group (65.43% and 39.76%) than late-cleavage group (all $p < 0.05$). Clinical pregnancy rate (73.13%) and implantation rate (45.21%) in early-cleavage group were higher compared with mixed group but without statistical significance (all $p > 0.05$). There were no significant

Table 2. — *Comparison of general status and pregnant outcomes in IVF between the three groups.*

	Early-cleavage group	Late-cleavage group	Mixed group
No. of IVF cycles (n)	365	120	209
Age (years)	30.8 ± 4.0	31.6 ± 4.0	30.9 ± 3.9
Duration of infertility (years)	4.1 ± 2.8	4.3 ± 3.0	4.1 ± 3.1
bFSH (mIU/ml)	7.6 ± 2.0	7.8 ± 2.4	7.3 ± 2.2
Dose of Gn (IU)	2,299.4 ± 837.2	2,337.7 ± 809.3	2,277.0 ± 838.0
E2 on HCG day (pg/ml)	4,422.7 ± 2,156.6	4,409.5 ± 2,218.8	4,043.8 ± 2,085.2
P on HCG day (ng/ml)	0.7 ± 0.4	0.7 ± 0.4	0.8 ± 0.8
Endometrial thickness on ET day (mm)	12.4 ± 2.6	12.3 ± 2.3	12.5 ± 2.6
No. of retrieved oocytes (n)	10.5 ± 4.9#	9.9 ± 5.1	9.5 ± 4.8
Fertility rate (%)	84.78 (3,243/3,825)	83.04 (989/1191)	83.14 (1,657/1,993)
Cleavage rate (%)	97.26 (3,154/3,243)**	92.92 (919/989)	94.93 (1,573/1,657)
High-quality embryo rate (%)	63.82 (2,013/3,154)	52.56 (483/919)*	62.17 (978/1,573)
No. of transferred embryoes (n)	2.0 ± 0.4	2.0 ± 0.4	2.1 ± 0.4
Clinical pregnancy rate (%)	61.92 (226/365) **	40.83 (49/120)	44.98 (94/209)
Implantation rate (%)	38.47 (287/746) **	28.57 (70/245)	31.85 (143/449)
Abortion rate (%)	9.29 (21/226)	14.29 (7/49)	7.45 (7/94)
High-order birth rate (%)	32.74 (74/226)	38.78 (19/49)	41.49 (39/94)

IVF: in vitro fertilization; bFSH: basal follicle-stimulating hormone; Gn: gonadotropin; HCG: human chorionic gonadotropin; ET: embryo transfer; ** indicates $p < 0.05$, compared with late-cleavage group and mixed group; * indicates $p < 0.05$, compared with early-cleavage group and mixed group and # indicates $p < 0.05$, compared with mixed group.

Table 3. — *Comparison of general status and pregnant outcomes in ICSI between the three groups.*

	Early-cleavage group	Late-cleavage group	Mixed group
No. of ICSI cycles (n)	67	126	81
Age (years)	29.8 ± 4.5	30.2 ± 4.8	30.2 ± 5.2
Duration of infertility (years)	3.9 ± 2.5	4.7 ± 3.1	4.6 ± 3.1
bFSH (mIU/ml)	7.3 ± 1.7	7.7 ± 3.4	7.6 ± 2.2
Dose of Gn (IU)	2,064.9 ± 673.5	2,117.3 ± 794.1	2,137.1 ± 736.1
E2 on HCG day (pg/ml)	4,750.3 ± 2,194.4	4,058.1 ± 2,060.3	4,269.1 ± 2,037.7
P on HCG day (ng/ml)	0.7 ± 0.3	0.6 ± 0.5	0.7 ± 0.4
Endometrial thickness on ET day (mm)	12.8 ± 2.1	12.8 ± 2.6	12.5 ± 2.2
No. of retrieved oocytes (n)	11.0 ± 4.5	10.4 ± 4.7	10.3 ± 3.1
Fertility rate (%)	73.59 (496/674)	76.11 (892/1172)#	70.94 (542/764)
Cleavage rate (%)	99.19 (492/496)	93.50 (834/892)*	99.45 (539/542)
High-quality embryo rate (%)	69.11 (340/492)	63.31 (528/834)	65.49 (353/539)
No. of transferred embryos (n)	2.2 ± 0.4	2.2 ± 0.5	2.2 ± 0.4
Clinical pregnancy rate (%)	73.13 (49/67)	46.03 (58/126) *	65.43 (53/81)
Implantation rate (%)	45.21 (66/146)	29.82 (82/275) *	39.76 (66/166)
Abortion rate (%)	6.12 (3/49)	8.62 (5/58)	7.55 (4/53)
High-order birth rate (%)	32.65 (16/49)	43.10 (25/58)	24.53 (13/53)

ICSI: intracytoplasmic sperm injection; bFSH: basal follicle-stimulating hormone; Gn: gonadotropin; HCG: human chorionic gonadotropin; ET: embryo transfer; * indicates $p < 0.01$, compared with early-cleavage group and mixed group; * indicates $p < 0.05$, compared with mixed group.

differences in high-order birth rates and spontaneous abortion rates between the three groups (Table 3).

Discussion

The final goal of assisted-reproductive technology is to improve clinical pregnancy rate and reduce high-order birth rate, so the choice of high-quality embryos with development potential is crucial to ET. At present, there are two kinds of selection criteria for cleavage-stage embryos. One kind is pronuclear morphology scoring system. The evaluation of cleavage-stage embryos by observing the size, number, and arrangement of nucleoli is readily affected by subjective factors of observers because pronucleus development has some characteristics, such as time sequence and three-dimensional spatial distribution, and the developmental outcomes of the embryos with high pronuclear morphology scoring are not necessarily good. Another kind is based on the number, size, and uniformity coefficient of blastomeres, and the proportion of fragmentation, which is most closely related to embryo quality and is one of the most important evaluation methods. However, blastomere morphology scoring system is not ideal for the evaluation of embryo development potential. In order to explore the best method for evaluation of embryo development potential, a great deal of attention has been paid to early-cleavage embryos.

Fertilized eggs may divide into two-cell embryos 20 hours after ICSI or 24 hours after IVF, which is the first mitosis of fertilized eggs and is called early cleavage. The time to the first zygotic cleavage varies by eight hours in humans (22-30 hours) [7]. Lundin *et al.* [3] observed early cleavage in mature oocytes 25-27 hours after insemination, followed by day two ET, and found that embryo quality, pregnancy rate, implantation rate, and live birth rate were significantly improved in early cleavage embryos; therefore it might serve as an independent predictor of live birth rate in ICSI. If the time to observe early cleavage after insemination is extended, early cleavage may be a parameter to select ET in IVF. Fenwick *et al.* [8] observed the development potential of early-cleavage embryos from the blastocyst stage and found that blastocyst formation and implantation rates were high in early-cleavage embryos, which further confirms that early cleavage is an important biological marker to predict embryo development potential. Since March 2011, the authors have observed early-cleavage 24 hours after ICSI and 27-28 hours after IVF, and found an early-cleavage rate of 51.67%. They also found that high-quality embryo rate was significantly higher in early-cleavage embryos than in late-cleavage embryos, which is consistent with the results from the literature [9]. This may be related to that early-cleavage embryos derive from the oocytes with higher maturity which have strong metabolic adaptation, also may be related to the calcium oscillations caused by sperm entry into oocytes because sperm quality is one of important factors to affect early cleavage. In brief, early cleavage reflects embryo development potential, but whether it affects pregnancy outcomes remains to be determined by large-sample retrospective analysis.

It is reported that early cleavage is only related to embryo development potential but not to pregnancy outcomes [10]; clinical pregnancy and implantation rates are associated with high-quality embryos, but are not associated with early cleavage [2, 11]; early cleavage as a reference standard of day three ET is not necessary [12]. In this study, sample size was large, only day three high-quality embryos were used for ET, and all patients were aged less than 40 years. The present results indicated that in either IVF or ICSI, early-cleavage embryos significantly improved clinical pregnancy and implanta-

tion rates, which is similar to the results reported by Jing *et al.* [13]. The authors conclude that there are significant differences in pregnancy and implantation rates between high-quality embryos with early cleavage and high-quality embryos without early-cleavage; compared with late-cleavage high-quality embryos, early-cleavage high-quality embryos can significantly improve pregnancy outcomes. The present results demonstrate that early cleavage is not only related to embryo development potential but is also strongly-associated with pregnancy outcomes. The authors also can see from their results that the effects of early cleavage on ICSI pregnancy outcomes are marked, both partial early-cleavage ET and all early-cleavage ET can significantly increase pregnancy and implantation rates, namely that as long as there is early-cleavage ET, pregnancy outcomes will be improved. High-order birth rate was increased in early-cleavage group, but there was no significant difference compared with late-cleavage group. Based on this, the authors infer that early cleavage may not affect high-order birth rate, which remains to be demonstrated by large-sample statistical analysis. Different from ICSI, partial early-cleavage ET could increase clinical pregnancy and implantation rates in IVF, but there was no statistical significance compared with late-cleavage ET. This may be related to the differences in infertile factors between ICSI and IVF; because infertile factors are mainly from the male in ICSI, so improvement in embryo quality readily produces good effects on pregnancy outcomes, while in IVF, infertile factors are complex, so mixed ET can increase embryo quality to some extent, but does not necessarily improve pregnancy outcomes.

In summary, early cleavage has an important significance. Observation of early cleavage is a good evaluation method for embryos because it is simple, objective, rapid, and the time of embryo exposure to external environment is brief. Early cleavage may be served as an effective indicator of embryo development potential. In day three ET, the preferred choice of early-cleavage ET in ICSI and only early-cleavage ET in IVF, can effectively improve pregnancy outcomes.

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