The influence of early cleavage on embryo developmental potential and IVF/ICSI outcome

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Abstract

Purpose To observe whether early cleavage can be a predictor of embryo developmental potential, pregnancy and implantation rates.

Methods A total of 9,544 embryos in 1,095 in vitro fertilization or intracytoplasmic sperm injection cycles were observed with regard to the appearance of early cleavage at 25–29 h post-insemination.

Results A significantly higher proportion of excellent quality embryos were observed in the early cleavage group compared to the late cleavage group (52.5 versus 28.9%, $P < 0.01$). In the early cleavage group there was also a higher rate of pregnancy per transfer compared with the late cleavage group (38.7 versus 26.3%, $P < 0.01$). In addition, we found that transfer of only one early cleavage embryo resulted in a high pregnancy rate (38.5%) and a low multiple pregnancy rate (18.0%).

Conclusion Early cleavage is a strong indicator of embryo quality, and may be used as an additional criterion in the selection of embryos for transfer to increase pregnancy rate and reduce multiple pregnancy rate.

Keywords Early cleavage · Embryo quality · Pregnancy rate · Implantation

Capsule Analysis of a large sample cohort reinforces the value of early cleavage behavior as a useful predictor of developmental potential and IVF/ICSI outcome although the mechanisms underlying cell cycle acceleration remain unknown.

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Introduction

One of the greatest problems in assisted reproduction technology (ART) today is how to select the optimal embryos for transfer to achieve high pregnancy rates without increasing multiple pregnancy rates. However, how to define the “optimal embryos” and how to select embryos which have the best developmental potential remains an open question. Therefore, it is important to increase our knowledge on embryo selection. The current practice in most in vitro fertilization (IVF) laboratories is to score cleavage-stage embryos evaluating the number of blastomeres, grade of fragmentation and cytoplasmic appearance [1]. In addition the presence of multinucleated blastomeres, the location of fragments and size of blastomeres in relation to each other may be analyzed. Embryo quality has also been reported to correlate with oocyte [2] and zygote morphology [3], for example, the appearance of the cytoplasm, pronuclei and polar bodies.

More recently, assessment of the time of pronuclear breakdown and early cleavage has been suggested to be a reliable parameter for the selection of embryos with the highest capability of implantation and successful pregnancy after transfer [4, 5].

The aim of the present study was to further exam whether early cleavage influences embryo quality, pregnancy and implantation rates.

Materials and methods

Ovarian stimulation, oocyte retrieval, oocyte insemination and embryo culture

This study was performed at the International Peace Maternal Child Hospital IVF centre, Shanghai, China,

between January 2006 and December 2008. Patients were not over 40 years of age, in their first in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles, with a basal FSH of less than 10 IU/L. In all, 1095 cycles in which patients underwent IVF or ICSI treatment with embryo transfer were analyzed.

Pituitary desensitization with a GnRH analogue and ovarian stimulation with gonadotropins were carried out as described previously [6]. A long protocol regime were used in all cycles. Oocyte retrieval was scheduled 36 h after hCG injection. Oocytes were recovered transvaginally under ultrasound guidance. Conventional IVF or ICSI was performed 3–5 h after oocyte aspiration. No more than 3 embryos were transferred transcervically 2 days after oocyte retrieval. Luteal supplementation with progesterone intra muscular injection (80 mg/day, Tongyong Pharmaceutical Co., China) was performed from the day of ovum pick-up to 14 days after ET.

Definition of early cleavage embryos

Fertilization was determined by confirmation of two pronuclei (2PN) 17–19 h after insemination/microinjection. The zygotes were checked again in the afternoon of day 1 (25–27 h after insemination/microinjection), and embryos displaying two cells at inspection were considered as early cleaved, and the remaining as late cleaved.

Assignment of embryonic quality

The embryo quality was evaluated 44–46 h after insemination or ICSI (day 2) according to their morphological grade, considering the number of blastomeres, the degree of

fragmentation, the uniformity of blastomeres and the presence of multinucleated blastomeres. If the number of the blastomeres were 4 cells, the fragmentation percent <10%, and the blastomeres were symmetrical, the embryo was classified as grade I–II and considered to be of excellent quality. The embryo scoring system was modified from Veeck, namely: score 4: preembryo with blastomeres of equal size and no fragmentation; score 3: preembryo with blastomeres of equal or unequal size and fragmentation ≤10% of the preembryo surface; score 2: preembryo with blastomeres of equal or unequal size and 11%–49% overall fragmentation; score 1: preembryo with blastomeres of equal or unequal size and fragmentation ≥50% of the preembryo surface [7]. A cumulative embryo score was obtained by adding the individual embryo scores of all transferred embryos. Embryos with the best morphological quality on day 2 were transferred. These embryos were further divided into those with early cleavage and those with late cleavage. If both were available the early cleavage ones were chosen for transfer. Pregnancy was determined by a urine and blood hCG test 14 days post transfer. An ongoing pregnancy was defined as the presence of at least one intrauterine gestational sac with fetal cardiac activity at ultrasound examination 5–6 weeks after oocyte retrieval. Implantation rate was defined as the number of fetal sacs with viable cardiac activity at ultrasound examination in gestational week 7, per number of transferred embryos.

Statistical analysis

Distribution of the variables is given as means and standard deviations (SD). The differences of means

Table 1 Demographic characteristics of the embryo transfer cycles with pregnancy or non-pregnancy

	Pregnancy	No pregnancy	P
No. of cycles	407	688	—
Mean age (years)	30.8±3.4	30.6±3.6	> 0.05
Sencondary infertility proportion (%)	53.1 (216/407)	49.6 (341/688)	> 0.05
Mean duration of infertility in years	4.2±2.5	4.3±2.7	> 0.05
Basal FSH (IU/L)	8.3±3.4	7.5±1.3	> 0.05
Mean Gn dose (IU)	2405±736.4	2580±808.6	> 0.05
Mean E2 level on the day of HCG admistration (pmol/l)	9982±764.6	9274±445.7	> 0.05
Mean endometrial thickness (mm)	9.9±1.9	9.4±1.9	> 0.05
No. of oocyte collected (mean ± SD)	12.3±5.1	11.3±6.4	> 0.05
ICSI proportion (%)	31.7 (129/407)	31.0 (213/688)	> 0.05
Fertilization rate (%)	76.4 (3851/5038)	71.9 (6034/8388)	< 0.05
Cleavage rate (%)	97.1 (3740/3851)	96.2 (5804/6034)	< 0.05
Early cleavage rate (%)	47.6 (1779/3740)	38.5 (2236/5804)	< 0.05
No. of embryos transferred (mean ± SD)	2.2±0.5	2.1±0.4	> 0.05
Accumulative score of transferred embryos mean ± SD	8.1±1.7	7.4±2.0	< 0.05

Table 2 Demographic characteristics of the embryo transfer cycles with at least one early cleavage embryo or without early embryo cleavage

	Early cleavage ET	No early cleavage	P
No. of cycles	962	133	—
age (years) (mean $\pm$ SD)	30.7 $\pm$ 3.5	31.1 $\pm$ 3.5	> 0.05
Sencondary infertility proportion (%)	51.5 (495/962)	50.4 (67/133)	> 0.05
Duration of infertility years (mean $\pm$ SD)	4.2 $\pm$ 2.6	5.0 $\pm$ 2.9	> 0.05
Basal FSH (IU/L)	7.5 $\pm$ 1.4	7.5 $\pm$ 1.2	> 0.05
Mean Gn dose (IU)	2325 $\pm$ 825.0	2600 $\pm$ 816.6	> 0.05
Mean E2 level on the day of HCG administration (pmol/l)	9926 $\pm$ 864.6	9440 $\pm$ 673.3	> 0.05
Endometrial thickness (mm) (mean $\pm$ SD)	10.0 $\pm$ 2.0	9.9 $\pm$ 2.1	> 0.05
No. of oocyte collected (mean $\pm$ SD)	12.6 $\pm$ 6.6	11.2 $\pm$ 5.3	> 0.05
ICSI proportion (%)	31.2 (300/962)	31.6 (42/133)	> 0.05
Fertilization rate (%)	74.9 (9142/12198)	60.5 (743/1228)	< 0.01
Cleavage rate (%)	96.9 (8863/9142)	91.7 (681/743)	< 0.01
Proportion of excellent quality embryos (%)	52.5 (4655/8863)	28.9 (197/681)	< 0.01
No. of embryos transferred (mean $\pm$ SD)	2.0 $\pm$ 0.9	1.9 $\pm$ 0.6	> 0.05
the accumulative score of embryo transferred (mean $\pm$ SD)	7.1 $\pm$ 2.1	5.5 $\pm$ 2.4	< 0.01
Pregnancy rate (%)	38.7 (372/962)	26.3 (35/133)	< 0.01
Ongoing pregnancy rate (%)	33.1 (318/962)	24.1 (32/133)	< 0.01
Implantation rate (%)	24.4 (485/1984)	17.0 (40/235)	< 0.01

between two variables were calculated using the Mann-Whitney U test. The differences in pregnancy rates, implantation rates, and early-cleavage rates were calculated using the chi-square test. A *P* value <0.05 was considered statistically significant.

Results

During the study, a total of 13426 oocytes were observed. Among them, 9885 oocytes were fertilized, 9544 embryos were obtained, and 4015 embryos were recorded with an early cleavage behavior. IVF/ICSI was performed in 1095 cycles. These cycles were divided into two groups (Table 1). No differences were found between the groups with regard to maternal age, the duration of infertility, and the proportion of secondary infertility. However, the cumulative morphological score of embryos transferred and the early cleavage rate were significantly higher than that of the non pregnant group (Table 1).

Table 2 showed the results, including clinical pregnancy and implantation rates in patients who had at least one early cleavage embryo transferred compared with those who had none. In contrast to the fertilization and cleavage rates, the proportion of excellent quality embryos and the pregnancy and implantation rates were significantly higher in the group who had at least one early cleavage embryo transferred compared to the group which had no early cleavage embryos to transfer.

Table 3 showed the number of early cleavage embryos included among the transferred embryos per patient and the corresponding pregnancy rates. There was no difference among these three groups in pregnancy rate (*p*>0.05). However, the multiple pregnancy rate differed significantly (*p*<0.01) between those with only one early cleavage embryo and those with three early cleavage embryos transferred.. Therefore, increasing the number of early cleavage embryos among the transferred embryos did not increase pregnancy rates, but resulted in an increased multiple pregnancy rate.

Table 3 The numbers of the early cleavage embryos transferred per patient and the corresponding pregnancy rates

The number of the early cleavage embryos transferred	Transfer cycles	Pregnancy rate	Multiple pregnancy rate
1	130	38.5 (50/130) ^a	18.0 (9/50) ^a
2	668	38.2 (255/668) ^a	28.2 (72/255)
3	164	40.9 (67/164) ^a	31.3 (21/67) ^b

Significant differences are indicated (a,b) (*P*<0.01)

Discussion

Although many factors influence the result of an IVF cycle (e.g. stimulation response, endometrial receptivity, oocyte maturity, culture conditions), embryo quality is regarded as one of the most important factors. Earlier, increasing the number of transferred embryos has been the approach to increase pregnancy rates. However, this also increases the multiple pregnancy rates with associated increased medical risks, and cost to the patient and society. Much effort has been made in trying to find a strategy to select the most optimal embryos to increase the pregnancy rate per transferred embryo without increasing the multiple pregnancy rate. A number of reports have been published concerning the importance of different factors for embryo development, implantation and pregnancy [8]. Subsequent reports confirmed that selection of embryos with the highest implantation rates can be made as early as the oocyte [9] and pronuclear stages [3]. Early cleavage is one of the most promising new selection parameters, which is also the finding of our own study.

In previous studies it was found that embryos with an early cleavage had a higher blastocyst development rate [10] and transfer of embryos with an early cleavage led to significantly higher pregnancy rates [4, 5, 8, 11, 12] as compared to late cleavage embryos. This is in agreement with the results obtained in the present study (38.7 versus 26.3% pregnancy rate, $P<0.01$, and 24.4 versus 17.0% implantation rate, $P<0.01$).

Why would the early cleavage embryos have a higher implantation potential? There is a close link between the 25 h embryo development and the 48 h embryo morphology. Some studies have shown that the embryo quality at 48 h correlates to the state of the zygote at 25 h after insemination or microinjection. The percentage of the embryos with at least 4 blastomeres was significantly higher in early cleavage embryos. However, the embryo status at 25 h did not predict affect the percentage of the fragmentation observed later [13]. Although many researchers have considered the connection between early cleavage and embryo morphology [5, 12, 14–17], the early cleavage mechanism is still unknown. The first cell cycle seems to take place when the zygote prepares for the initial cleavage. Therefore, it may be effective to reveal the early cleavage mechanism to study the cell cycle progression. The molecular and genetic condition of gametes and consequently the zygote is expected to affect the duration of the initial cell cycle. Maternal genes and proteins inherited from the oocyte regulate the first and subsequent cleavages. The state of the ooplasmic organelles such as mitochondria efficiency and adenosine triphosphate concentrations may affect the time of the initial cleavage [18]. Besides, the condition of the zygote may affect the duration of the first

cell cycle, which is important in the process of maternal to zygotic transition and zygote genome activation [19]. Overall, these findings may indicate that early cleavage is a biologic indicator of fertilization with fitting gametes, which requires a shorter time for the cell cycle. Therefore, high embryo quality and high pregnancy and implantation rates are observed after transfer of these embryos. Our results showed that early cleavage embryos had significantly higher fertilization rates, cleavage rates and proportion of excellent quality embryos. Thus early cleavage seems to reflect a good oocyte, and as a result the oocyte can undergo early mitosis.

In the current study, the effect of the early cleavage on the viability of transferred embryos was evaluated in a selected group of good prognosis patients undergoing elective single embryo transfer (eSET) aiming to prevent multiple pregnancies [5]. Our results show that increasing the number of the early cleavage embryos transferred did not increase the pregnancy rate, but increased the multiple pregnancy rates. In other words, choosing only one early cleavage embryo for transfer could reduce the multiple pregnancy rate without reducing the pregnancy rate. Early cleavage stage analysis provides a means of improving the embryo selection process that might lead to a transfer policy with a higher proportion of eSET. In turn, this will lead to a reduction of the twin pregnancy rate, which is one of the most serious adverse outcomes of an IVF treatment for both mother and child.

In conclusion, the present study provides evidence that early cleavage is a strong indicator of both embryo developmental potential and implantation rate. Therefore, we suggest that early cleavage could be an additional factor for selecting embryos with a higher potential of implantation and successful pregnancy while avoiding multiple pregnancies.

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