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Article

Clinical value of early cleavage embryo

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Abstract

Objective: This study was undertaken to see if embryo transfer containing early cleavage embryos resulted in better clinical pregnancy rate. *Method:* The treatment outcomes of IVF-ET were retrospectively reviewed. Out of 258 transfer cycles, 160 cycles contained no early cleavage embryos (Group I) and 98 cycles contained at least one early cleavage embryo (Group II). The definition of early cleavage embryo is the presence of two blastomeres 24–26 h after insemination. The implantation rate, clinical pregnancy rate were compared between two groups. Student's *t*-test and the Mann–Whitney *U*-test were used for continuous variables, and the Chi-squared (χ^2) test was used for binary variables. Differences were considered statistically significant at $P < 0.05$. *Result:* The implantation rate and clinical pregnancy rate were 11.6% and 25.6% in Group I, 18.6% and 38.8% in Group II ($P < 0.05$). *Conclusion:* Early cleavage embryos possess greater implantation potential. Embryo transfer containing early cleavage embryos had a better clinical pregnancy rate.

Keywords: In vitro fertilization; Cleavage speed; High order multiple pregnancy

1. Introduction

Compared to the natural history of multiple pregnancy, the frequency of twins in IVF is 20-fold higher and that of triplets is 400-fold higher [1]. Multiple pregnancies not only bring medical complications but also induce many social and financial problems [2,3]. Children from multiple births have a greater risk of death, low birth weight, physical and mental disabilities [4]. Women who carry multiple pregnancies also have a higher chance of pregnancy-induced complications such as hypertension, toxemia and diabetes [5]. In order to prevent these untoward results brought about by artificial reproductive technology (ART),

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it is necessary to restrict the number of embryos for transfer.

However, concerns have been raised that restricting the transfer number of embryos might jeopardize the successful rate of IVF treatment. Therefore, in order to maximize the pregnancy rate without increasing the multiple conceptions rate, it becomes very important to develop a method of choosing the embryos that possess the greatest potential to implant. Many different methods have been proposed to be effective in selecting good embryos for transfer, none of which has a universal conclusion, however. In this study, we retrospectively compared our IVF patients over the past 2 years to test if embryo cleavage speed can be used as a reference to choose the right embryos for transfer.

2. Materials and methods

The data of patients who received IVF-ET treatment in our hospital from January 1999 to December 2000 were retrospectively analyzed. For patients who received intracytoplasm sperm injection (ICSI) or patients who received no embryo transfer after oocyte retrieval were excluded from this study. Totally, 258 cycles of IVF-ET in 211 patients were included in this study. Of the 258 cycles, 160 cycles in 127 patients contained no early cleavage embryos in their transfer, 98 cycles in 84 patients contained at least one early cleavage embryo in their transfer. The definition of early cleavage embryo is presence of two blastomeres 24–26 h after insemination.

Ovulation induction was performed with a standard long protocol to anyone who went into the cycle. Briefly speaking, buserelin (Suprecur, Hoechst, Frankfurt Am Main, Germany), 0.9 mg, was applied from Day 21 of the previous cycle until the next menstruation began. Then the dosage was decreased to 0.45 mg per day. Complete down-regulation of the pituitary gland was defined as an estradiol level < 75 pg/ml and no follicle > 1 cm was measured when menstruation began. After complete down-regulation, ovulation stimulation was initiated with follicular stimulating hormone (Metrodin, Serono, Geneva, Switzerland) and/or human menopausal gonadotropin (Humegon, Organon, Oss, Holland) on cycle Day 3. Human chorionic gonadotropin (Pregnyl, Organon, Oss, Holland), 10 000 IU was injected intramuscularly when at least two leading follicles ≥ 16 mm were measured. Transvaginal oocyte retrieval was performed 34–36 h after the injection of HCG. Insemination was routinely performed 6 h after oocyte retrieval.

The following morning, 16–18 h after insemination, the oocytes were checked for the presence of two pronuclei. On the same day, approximately 24–26 h after insemination, the embryos were further examined to see if cleavage to the two-cell stage had occurred. Embryos that had cleaved to two-cell stage or above were defined as early cleavage embryos. Embryos that had not yet cleaved to the two-cell stage were defined as non-early cleavage embryos. The embryos then were cultured for another day and transferred on Day 3 after oocyte retrieval by the same person (Tsai).

The implantation rate, which was defined as the total number of gestation sacs over the total number of transfer embryos, and pregnancy rate were compared between the two groups. Only clinical pregnancy, which was defined as a gestation sac with fetal heartbeat visualized on ultrasound, was calculated. Results are reported as mean $\pm$ S.D. for normally distributed data and median (range) for non-normally distributed data. The data analyses were performed using the SPSS software program for windows (SPSS Inc., Chicago, IL, USA). To compare the differences between the groups, Student's t-test and the Mann–Whitney U-test were used for continuous variables, and the Chi-squared (χ^2) test was used for binary variables. Differences were considered statistically significant at $P < 0.05$. Multiple logistic regression was performed to compute the adjusted odds ratio.

3. Results

Two hundred and fifty-eight IVF-ET cycles in 211 patients were recruited in this study. There was no difference in age distribution and the

Table 1
Comparisons of characteristics between non-pregnant group and pregnant group

	Non-pregnant	Pregnant	*P* value
No. of cycles	179	79	
Age (y/o)	32.4 ± 3.6	32.3 ± 3.4	NS
No. of oocytes collected	6(1–21)	8(2–22)	NS
No. of 2PN	4(1–18)	6(1–18)	0.014
No. of embryos transferred	3(1–6)	4(1–6)	0.025

Values are mean ± S.D. and median (range).

number of retrieved oocyte between pregnant and non-pregnant groups in our study. The number of fertilized oocytes was statistically significant more in the pregnant group than the non-pregnant group ($P < 0.05$). Besides, the number of embryos being transferred was also greater in the pregnant group than the non-pregnant group ($P < 0.05$) (Table 1).

In order to evaluate the influence of early cleavage embryos on treatment outcome, the implantation rate, clinical pregnancy rate, ongoing pregnancy rate, multiple pregnancy rate and high order multiple pregnancy rate were calculated (Table 2). Statistically significant higher implantation rates (18.6%) and clinical pregnancy rates (38.8%) were observed in embryo transfers with at least one early cleavage embryo compared with transfers without early cleavage embryos (11.6% and 25.6%, respectively). The ongoing pregnancy rate, multiple pregnancy rate and high order multiple pregnancy rate revealed no difference between the two groups. To further explore the relationship between clinical pregnancy and transfer of early cleavage embryo, a multiple logistic regression was performed adjusting for number of 2PN embryos and number of embryo being transferred (adjusted OR = 1.34, 95% CI: 1.01–1.76, *P* value = 0.043).

4. Discussion

One of the major complications in IVF-ET treatment is high order multiple pregnancy. Multiple pregnancies bring not only medical, but also social and financial problems. Babies from multiple pregnancy are often born prematurely, which has significant impacts on the newborn's morbidity and mortality [3,4]. It also results in a prolonged hospital stay of the newborn that may affect the economy of the family [1]. Women who carry high order pregnancies have a higher chance of pregnancy-induced complications, such as hypertension, pre-eclampsia, diabetes and antepartum hemorrhage [5]. Although fetal reduction can

Table 2
The comparisons of treatment outcomes between non-early cleavage and early cleavage groups

	Transfer without early cleavage embryo	Transfer with early cleavage embryo	*P* value
No. of cycles	160	98	
Implantation rate (%)	11.6(62/534)	18.6(62/333)	0.004
Clinical pregnancy rate (%)	25.6(41/160)	38.8(38/98)	0.026
Ongoing pregnancy rate (%)	90.2(37/41)	84.2(32/38)	NS
Multiple pregnancy rate (%)	43.9(18/41)	47.4(18/38)	NS
High order multiple pregnancy rate (%)	7.3(3/41)	13.2(5/38)	NS

Multiple logistic regression was performed to further explore the relationship between clinical pregnancy and early cleavage, adjusting for No. of 2PNs and no. of transferred embryo (Adjusted OR = 1.34, 95% CI: 1.01–1.7, *P* value = 0.043).

be an option, it brings religious objections and might leave a psychological consequence on the parents [6]. Besides, fetal reduction also introduces the risk of endangering the entire pregnancy [7].

In accordance with previous reports, our study also demonstrated that the number of embryos being transferred plays an important role in determining the outcome of IVF-ET. The average number of embryos being transferred was four in the pregnant group and three in the non-pregnant group ($P < 0.05$). However, the transfer of only two or even one embryo has been proposed recently to prevent the multiple pregnancies brought about by assisted reproductive technology [8,9]. Recent studies have indicated that the transfer of only two embryos would not diminish the woman's chance of pregnancy, but reduce the chance of multiple births [10]. Hsu et al. also reported that elective transfers of two good quality embryos did not reduce the pregnancy rate [11]. The main problem is whether we have a correct way of choosing the right embryo for transfer.

The morphology of the embryo is one of the methods that have been proposed to choose a good embryo. Various scoring systems, which are based predominantly on the morphological appearance of embryos, have been proposed for the selection of embryos for transfer [12–14]. However, these scoring systems used dis-accord parameters, such as degree of blastomere uniformity, severity of blastomere fragmentation, and cytoplasmic appearance, which make the evaluation not only complicated but also subjective. Alternatively, blastomere biopsy is considered as a new method to predict the outcome of embryo development [15]. Nevertheless, this embryo manipulation method is so invasive that it should be limited to particular populations that are at risk of genetic disorders. An ideal method of embryo assessment should be simple, effective and non-invasive for selection and evaluation of embryos prior to transfer.

In our study, embryo transfer contained early cleavage embryos which resulted in a significantly higher implantation rate and clinical pregnancy rate. The total implantation rate was 11.6% in Group I compared to 18.6% in Group II ($P < 0.05$). The clinical pregnancy rate was 25.6% in Group I compared to 38.8% in group II, $P < 0.05$. After adjusting the number of transferred embryo numbers, we demonstrated that the transfer of early cleavage embryos can be a single parameter in influencing the clinical pregnancy rate. In this study, we purposely did not reduce the number of transferred embryos in the early cleavage group. Although statistically non-significant, the multiple pregnancy rate and high-order multiple pregnancy rates seemed to be higher in the transfer of early cleavage transfer group. If the sample size was large enough, we suppose the difference would be more obvious. According to the results of this study, we currently transfer only two embryos in the early cleavage group and the multiple pregnancy rate has reduced significantly.

Testart reported in 1986 that implantation failures occurred more frequently when embryos with less than four cells were transferred [16]. Lewin et al. later demonstrated that embryos with a slow cleavage rate in vitro are less likely to produce pregnancy following IVF-ET [17]. They thus concluded that the growth rate in vitro could be used as an indicator of embryo quality and the cleavage stage at transfer was a valuable reference in the selection of the best embryo. The same result was found in ICSI: Zhu et al. reported that following ICSI, the embryo developmental stage at transfer influences the outcome of treatment [18]. Recently, Shoukir et al. further indicated that early cleavage of embryos at the two-cell stage is associated with improved progression to blastocyst stage, implantation rate and pregnancy rate [19].

Although blastocyst transfer was suggested to enable physicians to select those embryos that would be more likely to implant, it still carried the risk of approximately 50% failure rate due to the suboptimal conditions in current culture medium [20]. Besides, prolong in-vitro culture of embryo was reported to increase the cleavage anomaly of embryos [21]. Since Day 3 and Day 5 transfers had similar pregnancy, implantation and twinning rates, the necessity of blastocyst culture needs to be reconsidered [22].

An ideal infertility therapy is to maximize the

pregnancy rate without high-order multiple conceptions. By identifying early cleavage embryos, one can choose the best embryo for transfer and reduce the risk of multiple births. Consequently, the quality and quantity of cryopreserved embryos can be increased and transferred at a next trial. Our results confirmed that these early cleavage embryos possessed greater implantation potential and resulted in better clinical pregnancy rate. Accordingly, we have suggested the use of the presence of early cleavage as a reference in determining which and how many embryos should be transferred.

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