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Original Article

Detection of early cleavage embryos improves pregnancy and delivery rates of Day 3 embryo transfer during *in vitro* fertilization

Chun-I Lee [a, b], Tsung-Hsien Lee [a, b, c], Chun-Chia Huang [d, e], Hsiu-Hui Chen [d, f], Chung-Hsien Liu [a], Maw-Sheng Lee [a, c, d, *]

[a] *Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan*
[b] *Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan*
[c] *Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[d] *Division of Infertility Clinic, Lee Women's Hospital, Taichung, Taiwan*
[e] *Department of Biotechnology, Chungtai Medicine University, Taichung, Taiwan*
[f] *Department of Life Sciences, College of Life Sciences, National Chung Hsing University, Taichung, Taiwan*

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ABSTRACT

Objective: This study established a simple criterion for improving the pregnancy and delivery rates of Day 3 embryo transfer for *in vitro* fertilization (IVF) by assessing the early cleavage of two-cell stage embryos.

Materials and Methods: In total, 258 cycle patients undergoing an IVF and Day 3 embryo transfer program were recruited. All cycles were divided into four groups containing viable Day 3 embryos and those (A) with distinct early cleavage (equal-sized blastomeres and ≤10% fragmentation: ECA grade); (B) with indistinct early cleavage (equal sized blastomeres, >2 blastomeres, or >10% fragmentation: ECB grade); (C) without early cleavage [no early cleavage (NEC grade)]; or (D) without early cleavage being assessed (control) at 25–27 after insemination.

Results: The percentage of viable Day 3 embryos from ECA grade (75.1%, 507/675) was significantly higher than that from ECB grade (19.2%, 151/403) or NEC grade (27.1%, 127/469) embryos ($p < 0.01$). The pregnancy and delivery rates in the ECA group [65.7% (65/990) and 48.5% (48/990), respectively] were significantly higher than those in the ECB group [30.8% (4/13) and 7.7% (1/13), respectively] or NEC group [36.8% (14/38) and 23.7% (9/38), respectively; all $p < 0.01$]. The implantation rate in the ECA group (32.3%, 129/400) was higher than those in the ECB (6.8%, 4/59) and NEC (13.0%, 18/136) groups ($p < 0.01$).

Conclusion: Simple selection using the early cleavage morphology may improve the pregnancy and delivery rates of Day 3 embryo transfer programs.

Introduction

A simple and efficient method for increasing pregnancy and implantation rates to a high level by using advance predictors of the transferred embryo quality is crucial for those involved in assisted reproductive technology [1–3]. A novel indicator, early cleavage at the two-cell stage, has been suggested for assessing human pre-implantation embryonic quality during *in vitro* fertilization (IVF) [4,5]. The earliest zygotic division occurs 20–27 hours after insemination or intracytoplasmic sperm injection (ICSI) [5–7]; hence, the recommended time for observing early cleavage is 25–27 hours [4,8]. At 24 and 27 hours after insemination, 5% and 38% of fertilized zygotes demonstrate early cleavage, respectively [6,9]. Early cleavage embryos have a higher blastocyst formation rate, superior morphology, and higher implantation rate compared with embryos without early cleavage [4,10,11]. However, the relationship between the morphological characteristics of early cleavage at the two-cell stage and embryo quality prior to the transfer warrants further discussion. Although the assessment of the degree of fragmentation and number and size of blastomeres is useful in determining the embryo quality prior to the transfer on Day 3, the usefulness of such assessment for determining the outcomes of

* Corresponding author. Lee Women's Hospital, 263 Pei-Tun Road, Taichung 400, Taiwan.
E-mail address: msleephd@gmail.com (M.-S. Lee).

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viable Day 3 early cleavage embryos with different morphologies remain unknown. The assessment of the Day 3 embryo quality and early cleavage is a useful criterion for evaluating Day 3 embryo transfers. For selecting transferrable embryos, the morphological characteristics of early cleavage embryos may be as crucial as those of Day 2, 3, 4, or 5 embryos. For example, the degree of fragmentation is a major indicator of embryo quality on Days 2–5 prior to the transfer. Furthermore, certain fragmentation patterns occur during the one- or two-cell stage, resulting in a loss of certain standard proteins from the blastomeres [12], which are associated with apoptosis [13]. Thus, the assessment of the morphological characteristics of early cleavage at the two-cell stage should be a criterion for embryo quality prediction.

A single evaluation of cell number and morphology on Day 3 of culture is not correlated with pregnancy rate or blastocyst formation [7,8]. The assessments of early cleavage appearance, Day 2 and 3 embryo morphology, or irregular development are primary indicators for selecting embryos for transfer on Day 3; however, the selection protocol should be efficiently shortened and simplified to reduce the range of efficient viable Day 3 embryos selected for transfer. In this study, two factors—morphology of early cleavage and quality of Day 3 embryos—were assessed prior to the transfer. This study established a simple and efficient selection criterion for Day 3 embryo transfer and predicted the optimal embryo quality and outcomes after the transfer.

Materials and methods

Patients and oocyte retrieval

We analyzed a database containing the clinical and laboratory information of all IVF treatment cycles conducted at the Lee Women's Hospital between September and December 2009. The study protocol was approved by the Institutional Review Board of Chung Shan Medical University Hospital (CS10084). The ovarian stimulation and embryo culture procedures used in our IVF program have been published elsewhere [14]. The participating women were administered the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron, Takeda Chemical Industries, Ltd., Osaka, Japan) from the midluteal phase for pituitary downregulation. A serum estradiol (E_2) level of <50 pg/mL on cycle Day 2 confirmed pituitary suppression, and recombinant follicle-stimulating hormone (Gonal-F, Serono) treatment was initiated. The participants' ovarian responses were monitored through serial serum E_2 levels and ultrasound examinations. When the leading follicles reached approximately 18 mm in diameter with an appropriate serum E_2 level, 10,000 IU of human chorionic gonadotrophin (Profasi, Serono) was administered. Transvaginal oocyte retrieval was performed 34–36 hours later.

Embryo fertilization and culture

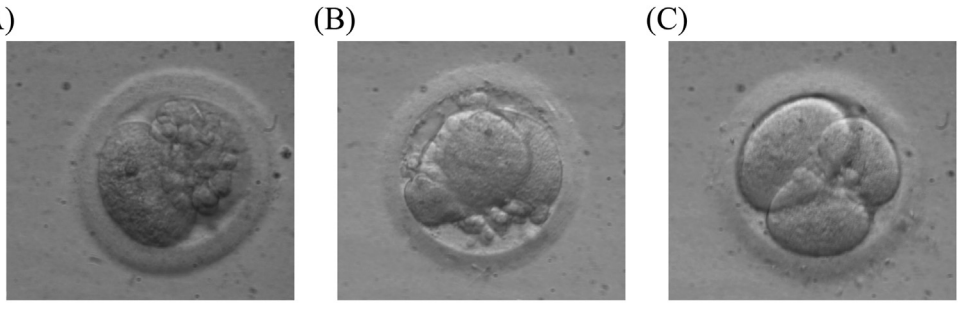
All mature oocytes were used for artificial insemination or ICSI. All inseminations and IVF were performed using microdrops of human tubal fluid medium (mHTF; Irvine Scientific, Santa Ana, CA, USA) containing a 5% (v/v) serum substitute supplement (Irvine Scientific). Immediately prior to ICSI, cumulus cells were removed by pipetting the oocytes in mHTF containing 80 IU/mL hyaluronidase (Type 8, H-3757; Sigma Chemical, St. Louis, MO, USA). Following artificial insemination or ICSI, all embryos were further cultured in microdrops of Quinns Advantage Cleavage (SAGE) medium.

Criteria for early cleavage and embryonic development

After insemination, embryonic development—including the embryonic pronuclei appearance (18–20 hours), two-cell stage or early cleavage (26–27 hours), four-cell stage (45–46 hours), and eight-cell stage (69–70 hours)—was observed. On the basis of the early cleavage morphology, two-cell stage embryos were classified as follows: (1) ECA grade, an embryo with two equal-sized blastomeres and ≤10% fragmentation; (2) ECB grade, an embryo with two unequal-sized blastomeres, more than two blastomeres, or >10% fragmentation; and (3) no early cleavage (NEC) grade; embryos without division at the time for assessment were defined as NEC. In the ECB grade, all embryos were further divided into ECP (>10% fragmentation), EC > 2 (>2 blastomeres), and ECC (unequal sized blastomeres) grades according to their two-cell stage morphology (Figure 1). Day 3 embryos with ≥8 equal-sized blastomeres and ≤20% fragmentation were considered advanced or viable embryos. By contrast, the embryos with <8 equal- or unequal-sized blastomeres or >20% fragmentation were considered poor embryos.

Assessment of the relationship between Day 3 embryo quality and early cleavage morphology at the two-cell stage

On the basis of their embryo development protocols, the 258 IVF cycle patients were classified into two groups: (1) the control



	ECP	ECC	EC > 2
Day 3 group			
Total embryos	150	165	88
No. of good embryos on Day 3 [%]	15 [10.0]	83 [50.3[a]]	53 [60.2[a]]

Figure 1. The two-cell stage morphology and distribution of early cleavage embryos with >10% fragmentation (A), unequal-sized blastomeres (B), and >2 blastomeres (C). All of them were included in the ECB group. ECB = early cleavage grade B (unequal sized blastomeres, >2 blastomeres, or >10% fragmentation); ECP = early cleavage with poor quality (>10% fragmentation).