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Original Article

Effect of morphokinetics and morphological dynamics of cleavage stage on embryo developmental potential: A time-lapse study

Shiao-Hsuan Yang [a], Cheng-Hsuan Wu [a], Yu-Ching Chen [a], Chueh-Ko Yang [b], Tsui-Huang Wu [c], Pei-Chi Chen [c], Horng-Der Tsai [a, *]

[a] Reproductive Medicine Center, Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan, R.O.C
[b] Reproductive Medicine Laboratory, Department of Medical Research, Changhua Christian Hospital, Changhua, Taiwan, R.O.C
[c] In Vitro Fertilization Laboratory, Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan, R.O.C

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ABSTRACT

Objective: Using a non-invasive method to select the most competent embryo is essential in in vitro fertilization (IVF). Since the beginning of clinical application of time-lapse technology, several studies have proposed models using the time-lapse imaging system for predicting the IVF outcome. This study used both morphokinetic and morphological dynamic parameters to select embryos with the highest developmental potential.

Materials and Methods: A total of 23 intracytoplasmic sperm injection treatment cycles with 138 fertilized oocytes were included in this study. All embryos were cultured to the blastocyst stage, and embryo development was recorded every 10 min by using a time-lapse imaging system. Morphokinetic parameters and eight major abnormal division behaviors were studied to determine their effects on blastocyst formation. The most influential variables were used in hierarchical classification for blastocyst formation prediction.

Results: Several parameters were significantly related to the developmental potential. Embryos with the timing of pronuclear fading (tPNF) of >26.4 h post insemination (hpi), the timing of division to two cells (t2) of >29.1 hpi, and the timing of division to four cells (t4) of >41.3 hpi showed the lowest blastocyst formation rate. The abnormal division behaviors of fragmentation >50%, direct cleavage, reverse cleavage, and delayed division or developmental arrest were found to be detrimental to blastocyst formation. On the basis of these results, we propose a hierarchical model classification, in which embryos are classified into groups A-D according to their developmental potential. The blastocyst formation rates of groups A, B, C, and D were 80.0%, 77.8%, 53.7%, and 22.2% ($p < 0.001$). The good blastocyst rates of groups A, B, C, and D were 60.0%, 44.4%, 14.6%, and 11.1% ($p = 0.007$).

Conclusion: We propose a hierarchical classification system for blastocyst formation prediction, which provides information for embryo selection by using a time-lapse imaging system.

Introduction

Selecting the most competent embryo to achieve a singleton pregnancy is the ultimate goal of in vitro fertilization (IVF). Extended embryo culture with blastocyst transfer is considered a useful method in IVF for selecting embryos with a high implantation potential [1] and for reducing multiple pregnancies [2,3]. However, prolonged in vitro culture has been reported to potentially increase the risks of epigenetic disorders and preterm deliveries [4–6]. In patients with insufficient embryos, prolonged culture may be associated with the risk of no embryos remaining for transfer. A solution for the aforementioned conflicts is the identification and transfer of embryos with a high developmental potential in the cleavage stage.

Over the past three decades, embryologists have evaluated embryos' quality through conventional morphology assessment [7–10] at distinct time points. Since the first time-lapse microscopy system was approved for clinical use in June, 2009, more details of the cytokinetic process of embryo development have been revealed. Using the time-lapse microscopy system, morphokinetic and morphological dynamic parameters have been applied

* Corresponding author. No.135, Nanxiao Street, Changhua 500, Taiwan, R.O.C. Fax: +886 4 7228289.
E-mail address: 127119@cch.org.tw (H.-D. Tsai).

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predicting blastocyst formation [11,12], implantation potential [13–15], and aneuploidy [16,17].

Compared with embryos that cleave late, those that cleave early are more developmentally competent [18,19]. Wong et al. (2010) first reported the feasibility of recording the cleavage timing by using a time-lapse imaging system for predicting the embryo developmental potential [11]. Thereafter, numerous studies have used morphokinetic parameters to predict the IVF outcome [13,16,17]. However, these results have several limitations and should be evaluated with caution. First, the morphokinetic parameters were reported to be affected by several factors, such as stimulation protocols [20,21], patient population [22], culture condition [23,24], and sperm DNA fragmentation [25]. Second, some time-lapse equipment, such as the Embryoscope (Vitrolife), can only set the same starting time point for all cultured embryos, which made the sperm entry time in each individual embryo imprecise. Because of the aforementioned limitations, the results from each laboratory varied. Third, the clinical effectiveness and cost effectiveness of improving overall outcomes by using morphokinetic parameters for predicting IVF outcome remain controversial [26–28].

To reduce the variation and improve the transferability of the time-lapse algorithm, some researchers have used division patterns, rather than division timings, as the time-lapse parameters [12]. Yang et al. (2015) demonstrated that some specific cleavage patterns adversely affect the embryo developmental potential [12]. Moreover, some studies have found that direct cleavage (DC) and reverse cleavage (RC) exert deleterious effects on the implantation potential [15,29]. Recently, instead of the insemination timing, the timing of pronuclear fading (tPNF) was proposed as a reference starting time point to eliminate the error associated with variation in sperm entry time and settings of different time-lapse equipment [30].

This study proposes a concise and feasible time-lapse model for predicting the embryo developmental potential by using both morphokinetic and morphological dynamic parameters of the early cleavage stage. Moreover, the relationship between each studied parameter and the developmental potential was analyzed.

Materials and methods

Patient selection and patient management

This retrospective cohort study was performed at Changhua Christian Hospital from September 2014 to April 2016. A total of 23 intracytoplasmic sperm injection (ICSI) treatment cycles with 138 fertilized oocytes were included in this study. The mean age (±SD) of women (including oocyte donors) in the treatment cycles was 34.2 ± 4.2 years. Embryos with cleavage-stage biopsy or assisted hatching conducted at the cleavage stage were excluded. This retrospective study was approved by the Institutional Review Board of Changhua Christian Hospital. All patients provided informed consent for data collection.

Ovarian stimulation was performed using a standardized gonadotropin-releasing hormone (GnRH) antagonist (0.25 mg of ganirelix, Merck Sharp and Dohme, or 0.5 mg of cetrorelix, Merck Serono) protocol or GnRH agonist (leuprorelin, Takeda) protocol. Different dosages of recombinant follicle-stimulating hormone (follitropin-α, Merck Serono, or follitropin-β, Merck Sharp and Dohme) and human menopausal gonadotropins (Ferring) were administered according to the patients' body weight, ovarian reserve, and previous ovarian response. Human chorionic gonadotropin at 5000 IU or 6500 IU (Merck Sharp and Dohme or Merck Serono) was administered when at least two leading follicles had reached a mean diameter of ≥18 mm. Transvaginal oocyte retrieval was scheduled 34–36 h after triggering of oocyte maturation.

Oocyte retrieval and ICSI

Oocyte cumulus complexes were washed and cultured in Quinn's Advantage Fertilization Medium (QAFM; SAGE, Trumbull, CT, USA) at 37 °C under 5.5% CO_2 and 5.0% O_2 before denudation. Oocyte denudation was performed at approximately 2 h after retrieval through pipetting in bicarbonate and N-hydroxyethylpiperazine-N-ethanesulfonate-buffered medium (ASP; Vitrolife, Vastra Frolunda, Sweden) with hyaluronidase solution (Sigma–Aldrich, St. Louis, MO, USA). The conventional ICSI procedure was performed in QAFM under a × 200 magnification microscope.

Embryo culture and time-lapse recording

Following ICSI, oocytes were individually placed into microwells (LifeGlobal, Guilford, CT, USA) and were cultured in Quinn's Advantage Sequential Medium (SAGE). All embryos were cultured to the blastocyst stage (Day 5 or 6 post insemination) in a time-lapse incubator (CCM-IVF; ASTEC, Fukuoka, Japan), with the culture conditions of 37 °C, 5.5% CO_2, 5.0% O_2, and balanced N_2. The culture medium was changed on Day 3 (at least 70 h post insemination [hpi]). Images of each embryo were obtained every 10 min. The precise timing of completing each ICSI procedure was recorded individually by a technician and was regarded as the starting time of each embryo set in the time-lapse system.

Regarding conventional morphology assessment, the eight-cell stage was verified during the period of 68 ± 1 hpi by using the Veeck grading system [8]. The blastocyst stage was verified during the period of 116 ± 2 hpi by using the Gardner grading system [9]. Good embryos were defined as grade I to II embryos with 6–10 blastomeres. Good blastocysts were defined those with trophectoderm and inner cell mass both rated higher than grade B.

Table 1
Definitions of variables used in analysis.

Variables	Definition
Morphokinetic parameters	
tPNF	The timing of both pronuclei had faded
t2	The timing of division to 2-cell stage (Complete the 1st cleavage)
t3	The timing of division to 3-cell stage
t4	The timing of division to 4-cell stage (Complete the 2nd cleavage)
T2_PNF (t2-tPNF)	Duration of the period as 1-cell
T3_PNF (t3-tPNF)	Duration of the period from pronuclear fading to 3-cell stage
T4_PNF (t4-tPNF)	Duration of the period from pronuclear fading to 4-cell stage
cc2 (t3-t2)	The time of second cell cycle (Duration of the period as 2-cell)
s2 (t4-t3)	The time of synchrony of second cell cycle (Duration of the period as 3-cell)
Morphology dynamic parameters	
Fragmentation > 50%	Over 50% scattered fragments after division
Fragmentation 10–50%	10–50% scattered fragments after division
Direct cleavage	Direct cleavage from one cell to three or more blastomeres
Reverse cleavage	Blastomeres fusion after division
Uneven blastomeres	The largests blastomere being over 20% larger than the smallest blastomere
Big fragmentation	Big fragment develops after division
Delayed division or developmental arrest	The blastomere with division delayed compare with others or did not enter next cell cycle while other blastomere kept going on
Distorted cytoplasm movement	A series of distorted cytoplasm movements during cell division