

# Comparison of clinical outcome of frozen embryo transfer after embryo selection based on morphokinetic versus morphologic criteria for freezing

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## ABSTRACT

**Background:** Results from the latest meta-analysis, in fresh cycles, showed that the application of time-lapse monitoring (TLM) together with an embryo-evaluating algorithm was associated with a significantly higher rate of ongoing pregnancy and a lower rate of early pregnancy loss. The aim of this study was to compare the clinical outcomes of frozen embryos classified according to morphokinetic versus morphologic criteria. **Methods:** This was a retrospective cohort study, conducted at IVFAS, An Sinh Hospital, Vietnam, from July 2014 to July 2017. Patients undergoing *in vitro* fertilization (IVF) treatment with antagonist protocol and having freeze-only on day 5 were included. Exclusion criteria were patients (i) treated with in-vitro maturation, (ii) having obstructive azoospermia, or (iii) having uterine abnormalities. Embryos were cultured up to day 5 in TLM system (Primo Vision, Vitrolife, Sweden) or in benchtop (G185, K System, Denmark). The quality of frozen embryos was evaluated based on morphokinetic or morphologic criteria. In the subsequent cycle, endometrial preparation was done by using exogenous estradiol and progesterone. Embryos were thawed and up to 2 embryos were transferred to the uterus. The primary outcome was ongoing pregnancy. The rate of post-thaw survival, post-thawed good/moderate embryo, clinical pregnancy, implantation, miscarriage and ectopic pregnancy were used as secondary endpoints. **Results:** A total of 276 patients were recruited, with 138 patients in the morphokinetic group and 138 patients in the morphologic group. Baseline characteristics were comparable between the two groups. There was no significant difference in ongoing pregnancy rate in morphokinetic versus morphologic group (57.2% vs. 60.1%,  $p=0.71$ ). All secondary outcomes were comparable between the two groups. **Conclusion:** In frozen day-5 embryo transfer, the clinical outcomes were similar when embryos were classified according to morphokinetic versus morphologic criteria for freezing. Using morphokinetic criteria to select embryos for freezing did not improve the ongoing pregnancy rate, as compared to morphologic criteria.

**Key words:** Embryo evaluation, Embryo selection, Morphologic, Morphokinetic, Time-lapse

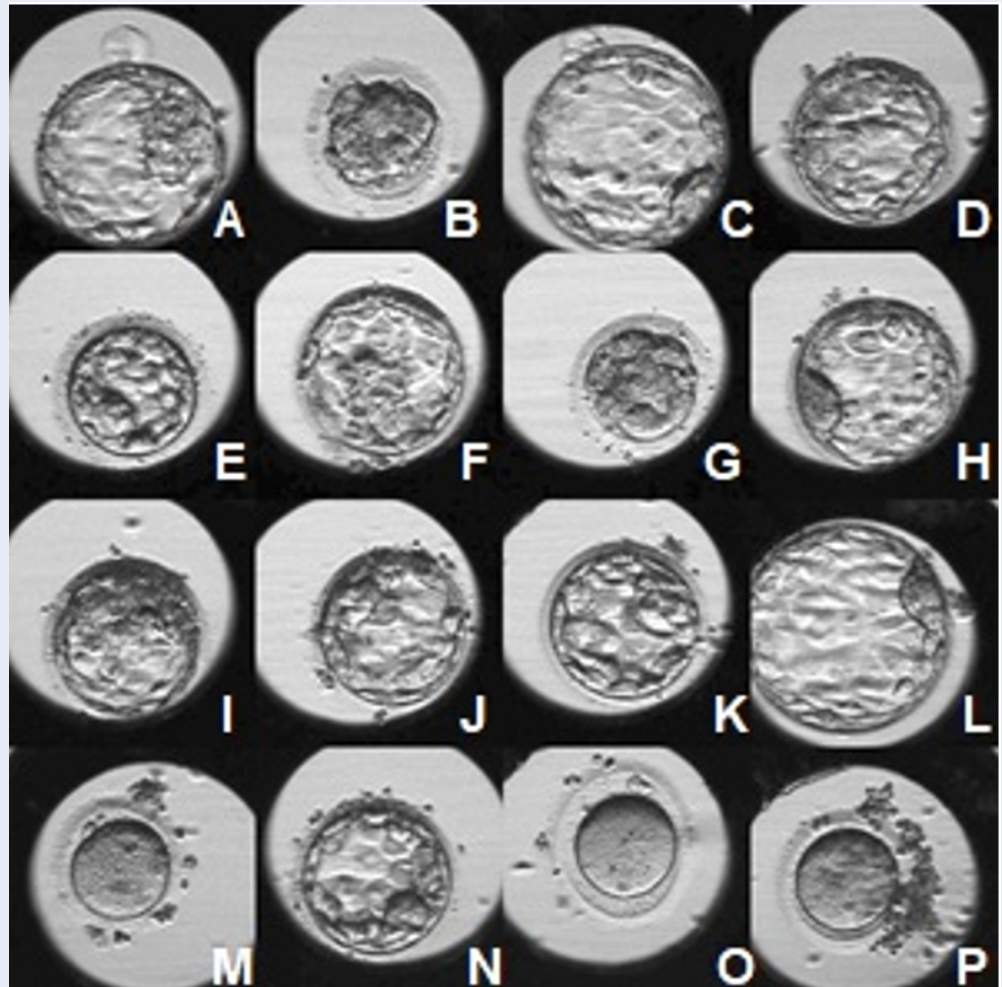
## INTRODUCTION

Embryo culture, assessment and selection are important steps in improvement of the safety and efficacy in assisted reproductive technology. Traditionally, an embryo must be removed from an incubator for assessing under a light microscope; its development may be affected from exposure to ambient temperature and pH conditions<sup>1</sup>. Embryos were evaluated and classified at the time of embryo assessment, based on morphologic criteria including cell numbers, fragmentation rate, nucleation status, cytoplasmic anomalies, spatial distribution of cells, compaction status in cleavage embryo and degree of expansion, inner cell mass morphology, and trophectoderm morphology in blastocysts. In recent years, embryo culture combined with time-lapse monitor-

ing has become more popular worldwide and has created a new trend of *in vitro* fertilization<sup>2-4</sup>. It can maintain an optimal culture environment, and allow for the best possible culture conditions<sup>5,6</sup>. Time-lapse system can take digital images of embryos at frequent time intervals throughout the culture period so that the embryos can be quality-assessed without being removed from the incubator **Figure 1**.

Time-lapse systems also record the data of embryo development (morphokinetics), including the precise determination of the onset, duration, and interval between cell divisions. In addition, dynamic morphologic characteristics of a developing human embryo, such as pronuclei (PN) and nucleus formation and disappearance, fragmentation, and size distribution of blastomeres may be quantified with time-lapse monitoring<sup>7,8</sup>. Based on the recorded data, potential

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**Figure 1:** Embryos were monitored by Primo Vision time-lapse system (IVFAS). Figure 1A, 1C, 1D, 1E, 1F, 1H, 1I, 1J, 1K, 1L, 1N: embryos reached the blastocyst stage. Figure 1B: embryo was blocked at morula stage. Figure 1M, 1O, 1P: oocytes were not fertilized.

embryos are used to classify and select with the support of time-lapse software.

These recent findings suggested that morphokinetic parameters may supplement current embryo selection methods to possibly increase clinical pregnancy rates for *in vitro* fertilization (IVF) treatments<sup>7,9-11</sup>. Meanwhile, other studies reported no significant differences in embryo incubation and selection between the time-lapse incubator and conventional incubator<sup>12,13</sup>. Pribenszky *et al.* recently updated their meta-analysis review and found that the quality of the evidence was moderate to low and that there were inconsistencies across the studies. Selective application and variability were also limitations. Although time-lapse is shown to significantly improve overall clinical outcome, further high-quality evidence are needed

before conclusions can be drawn<sup>14</sup>.

Recently, there has been greater evidence supporting elective frozen embryo transfer and its improved clinical outcomes, which not only achieved higher pregnancy rates but also lowered maternal and infant morbidity and mortality<sup>15</sup>. Frozen embryo transfer has become increasingly popular worldwide. Nevertheless, most of the published studies have mainly analyzed morphokinetic data in embryo selection in fresh embryo transfer cycles<sup>13,16-18</sup>. Therefore, it was necessary to verify the effectiveness of the morphokinetic criteria in embryo selection for freezing at our center. Accordingly, the aim of our study was to compare the clinical outcomes of frozen embryos classified according to morphokinetic versus morphologic criteria.

## METHODS

### Patient population and study design

This is a retrospective cohort study which was conducted at IVFAS (An Sinh Hospital, Vietnam) from July 2014 to July 2017. The identifying information was removed from patient data. As the data cannot be linked back to an individual, it was not necessary to obtain patient consent. The use of patient data was approved by An Sinh Hospital. All patients undergoing IVF treatment with antagonist protocol and having freeze-only embryo on day 5 were included in our study. Patients undergoing *in vitro* maturation (IVM) cycles, having obstructive azoospermia or having any uterine abnormalities were excluded. In this study, 256 patients undergoing assisted reproduction technology were recruited. The patients were grouped into morphokinetic group (138 patients) and morphologic group (138 patients).

### Embryo culture

After retrieval, oocytes were rinsed and cultured in G-IVF Plus (Vitrolife, Sweden) at 37°C with 6% CO<sub>2</sub>, 5% O<sub>2</sub> in incubator (Galaxy 170R, New Brunswick)<sup>19</sup>. After 2 hours, denudation of cumulus cells surrounding the oocytes was performed by Pasteur pipette and Hyase (Vitrolife, Sweden)<sup>19</sup>. Subsequently, intracytoplasmic sperm injection (ICSI) was performed with all mature oocytes. After ICSI, oocytes were cultured overnight in G1 – Plus media (Vitrolife, Sweden) at 37°C with 6% CO<sub>2</sub>, 5% O<sub>2</sub> in benchtop (G185, K System, Denmark)<sup>20</sup>. Fertilization was checked approximately 16 to 18 hours after ICSI<sup>21</sup>.

### Conventional embryo culture

Three days after ICSI, all embryos were transferred to G2 – Plus media (Vitrolife, Sweden) for culturing at 37°C with 6% CO<sub>2</sub>, 5% O<sub>2</sub> in benchtop (G185, K System, Denmark) to blastocysts (20).

### Time-lapse embryo culture

After evaluation of the fertilization, embryos were transferred to WOW dish with G-TL media (Vitrolife, Sweden) and placed in Primo Vision time-lapse system (Vitrolife, Sweden) which was placed in an incubator at 37°C with 6% CO<sub>2</sub>, 5% O<sub>2</sub> (Galaxy 170R, New Brunswick).

### Embryo evaluation

#### Conventional embryo culture

On day 5 after oocyte retrieval, embryos were removed from the incubator for quality assessment, under a light microscope, based on three main features:

the degree of expansion, inner cell mass (ICM) morphology and trophoctoderm (TE) morphology<sup>22</sup>. The timing of embryo assessment on day 5 was 116 ± 2 hours after ICSI.

#### Time-lapse embryo culture

Every embryo was assessed based on morphologic criteria and morphokinetic parameters. The whole development process of embryos was observed via the monitor of Primo Vision time-lapse system. The timing of embryo cleavage and appearance of abnormal characteristics were marked. Specific kinetic markers were evaluated: time to pronuclear (2PN) fading (t1) and cleavage to 2 cells, 3 cells, 4 cells, 5 cells, 8 cells, morula (t2, t3, t4, t5, t8, tM), time of embryo to start of blastulation, expanded blastocyst, and time of hatching (tEB, tB, tH.Blast). The abnormalities in the cleavage stage embryo were also marked at the time of occurrence and included: reverse cleavage (RCLV — decrease of the number of embryos during division), direct cleavage (DC — a single blastomere directly cleaved into three or more blastomeres), and MNB (multinucleated blastomeres)<sup>23</sup>.

Moreover, kinetic parameters were calculated based on the time of the listed events, including: duration of the second cycle (cc2; t3 — t2), third cell cycle (cc3; t5 - t3), time of synchronous divisions s2 (t4 — t3), and s3 (t8 — t5)<sup>13,24–26</sup>.

### Embryo selection

#### Morphologic group

Embryo selection for freezing was based on embryo morphologic criteria (Istanbul consensus workshop on embryo assessment in 2011)<sup>22</sup>. Selected embryos for freezing had expansion grade from 2 to 5, ICM category 1 or 2, TE category 1 or 2<sup>22</sup>.

#### Morphokinetic group

Embryos were selected for freezing based on morphologic criteria, timing of kinetic events, and presence of cleavage anomalies<sup>26</sup>. Morphokinetic parameters were referenced from the study of Meseguer *et al.* (2011) and installed in Primo Vision software<sup>26</sup>. When selecting embryos, embryos were sorted in order by the software based on the parameters that had been installed.

### Embryo freezing

In two group, embryos had been frozen using the Cryotec vitrification method, with a maximum of two embryos per cryotec.

### Vitrification protocol

#### Preparation

Preparation of the solution in vitriplate included: 300  $\mu\text{L}$  equilibration solution (ES), 600  $\mu\text{L}$  vitrification solution (VS) and then divide VS to the next two wells, VS1 (300  $\mu\text{L}$ ) and VS2 (300  $\mu\text{L}$ ). Then, they were placed at room temperature (25°C - 27°C) for at least 1 hour before use.

#### Freezing

Freezing protocol included 3 steps: equilibration (maximum 15 minutes), vitrification 1 (30 – 40 seconds), vitrification 2 (10 – 20 seconds) (following the manufacturer's protocol). The details are as below:

Put embryo on the surface of ES in ES well for a maximum of 15 minutes. Then, transfer embryo to the half depth of the VS1 well for 30 – 40 seconds. Transfer embryo to the half depth of the VS2 well for 10 – 20 seconds. Finally, put it on the end of the Cryotec seat with a minimum volume of VS and immediately submerge the Cryotec into liquid nitrogen.

### Warming protocol

#### Preparation

The TS dish and the TS vial (with closed cap) were placed in the incubator at 37°C overnight before use. Preparation of the solution in vitriplate included: 300  $\mu\text{L}$  of diluent solution (DS) in the first well, 600  $\mu\text{L}$  washing solution (WS) and then divide WS to the next two wells, WS1 (300  $\mu\text{L}$ ) and WS2 (300  $\mu\text{L}$ ). They were placed at room temperature (25°C – 27°C) for at least 1 hour before use.

#### Warming

The warming protocol included 3 steps: warming (1 minute), dilution (3 minutes), washing 1 (5 minutes) and washing 2 (1 minute) (following the manufacturer's protocol). The details are as below:

Take the TS vial out of the incubator, and expel all of it to the TS dish. Quickly put the Cryotec from liquid nitrogen into the TS dish. Let the embryo release from the Cryotec and wait for 1 minute. Transfer the embryo to the bottom of the DS well and wait for 3 minutes. Transfer the embryo to the bottom of the WS1 and then wait for 5 minutes. Transfer the embryo on the surface of the WS2, and wait for 1 minute. Put the embryo into the droplet of culture media until the embryo transfer step.

### Embryo transfer and outcome measures

Endometrial preparation was done by using exogenous estradiol and progesterone until the endometrial

thickness reached 8 mm or more. After 5 days from the start of progesterone, embryos were thawed (up to 2 embryos). Two hours after thawing, the embryo was transferred to the uterus under ultrasonographic guidance.

The primary outcome was ongoing pregnancy after the first embryo transfer cycle. The ongoing pregnancy was defined as pregnancy with a detectable heart rate after 12 weeks of gestation<sup>27,28</sup>. The secondary outcomes were the percentage of survival, clinical pregnancy, implantation, miscarriage and ectopic pregnancy.

### Statistical analysis

The samples of the two groups were matched by MatchED Packages (R software). The results were compared depending on embryo evaluation criteria by using a Chi-squared test and significance level of  $p < 0.05$ . All the statistical analyses were performed using R software.

## RESULTS

All of the baseline characteristics of the patients were recorded including age, BMI, infertility duration, total FSH dose, and total days of stimulation. These characteristics were summarized in **Table 1**.

The morphokinetic group showed similar age ( $31.9 \pm 5.1$  years vs.  $32.3 \pm 5.9$  years,  $p = 0.65$ ), infertility duration ( $5.0 \pm 3.2$  years vs.  $5.3 \pm 4.1$  years,  $p = 0.66$ ) and total FSH dose ( $2206.8 \pm 1065.7$  IU vs.  $2341.5 \pm 971.2$  IU,  $p = 0.29$ ), as compared to the morphologic group. There were no significant differences between the two groups (**Table 1**). This was evident by the fact that the primary patient's characteristics were similar between the two groups.

From all of the patients, there were a total of 6,264 retrieved oocytes with 5,369 mature oocytes which were administered with intracytoplasmic sperm injection. The fertilization rate reached 84.4%. The morphokinetic group showed a similar average number of retrieved oocytes ( $22.9 \pm 9.8$  vs.  $22.5 \pm 9.4$  oocytes,  $p = 0.74$ ), ICSI oocytes ( $19.6 \pm 8.7$  vs.  $19.3 \pm 8.0$  oocytes,  $p = 0.77$ ), fertilized oocytes ( $14.4 \pm 7.0$  vs.  $14.7 \pm 6.8$  oocytes,  $p = 0.70$ ), day-5 embryos ( $13.7 \pm 6.8$  vs.  $13.0 \pm 6.6$  embryos,  $p = 0.40$ ), and frozen embryos ( $5.6 \pm 3.6$  vs.  $5.6 \pm 3.2$  embryos,  $p=0.99$ ), as compared to the morphologic group (**Table 2**).

Our primary outcome (ongoing pregnancy rate) was found in 57.2% in the morphokinetic group, compared to 60.1% in the morphologic group, and it was not significantly different between the two groups. The morphokinetic group showed similar number of embryos transferred ( $1.9 \pm 0.4$  vs.  $2.0 \pm 0.3$  embryos,

**Table 1: Patient characteristics**

Characteristic	Morphokinetic group n = 138	Morphologic group n = 138	p-value
Age (years)	31.9 ± 5.1	32.3 ± 5.9	0.65
BMI (kg/m <sup>2</sup> )	21.1 ± 2.2	21.1 ± 2.1	0.76
Infertility duration (years)	5.0 ± 3.2	5.3 ± 4.1	0.66
Total FSH dose (IU)	2206.8 ± 1065.7	2341.5 ± 971.2	0.29
Total days of stimulation (days)	9.0 ± 2.1	8.7 ± 1.5	0.20

**Table 2: Laboratory outcome data**

Parameters	Morphokinetic group n = 138	Morphologic group n = 138	p-value
Number of retrieved oocytes	22.9 ± 9.8	22.5 ± 9.4	0.74
Numbers of ICSI oocytes	19.6 ± 8.7	19.3 ± 8.0	0.77
Number of fertilized oocytes	14.4 ± 7.0	14.7 ± 6.8	0.70
Number of day-5 embryos	13.7 ± 6.8	13.0 ± 6.6	0.40
Number of frozen embryos	5.6 ± 3.6	5.6 ± 3.2	0.99

$p = 0.74$ ) and clinical pregnancy rate (64.5% vs. 65.9%,  $p = 0.90$ ), as compared to the morphologic group. There were no statistically significant differences in mean of endometrial thickness ( $11.0 \pm 1.2$  mm vs.  $10.8 \pm 1.3$  mm,  $p = 0.09$ ), implantation rate (51.2% vs. 49.3%,  $p = 0.73$ ), or ectopic pregnancy rate (2.9% vs. 0.7%,  $p = 0.37$ ) between the two groups. Besides, the two groups were equal in the post-thawing survival embryo rate (100%) and miscarriage rate (5.1%) (Table 3).

## DISCUSSION

Embryos were evaluated based on their morphologic characteristics<sup>22</sup>, a practiced routinely applied in many IVF centers worldwide. In this criteria, blastocyst transfer with fewer embryos can be performed with high implantation and clinical pregnancy rates<sup>29-31</sup>. Morphokinetic data, recorded through time-lapse technology, help make the embryologist's selection more precise and improve clinical outcomes<sup>32,33</sup>. Extended culture to blastocyst stage has been a new trend that has been applied in many IVF centers around the world, including Vietnam<sup>34,35</sup>. Vitrification technologies have been widely used in embryo freezing and the clinical outcomes have been very good<sup>36,37</sup>. Frozen embryo transfer has increased worldwide and predominated in all embryo transfer cycles<sup>15</sup>.

This was the first study to evaluate the effectiveness of morphokinetic criteria in day-5 embryo selection in

the first frozen embryo transfer cycle after freeze-all embryo in Vietnamese patients. There were no statistically significant differences in the mean of age, BMI, infertility duration, total FSH dose and total days of stimulation (Table 1). The number of retrieved oocytes, the numbers of mature oocytes using to performance of ICSI, the number of fertilized oocytes, the number of day-5 embryos and day-5 frozen embryos were recorded and analyzed. There were no statistically significant differences between the morphokinetic group versus morphologic group. The above analysis showed that there were similarities between the two groups in terms of patient characteristics and laboratory outcomes.

In this study, we froze all potential embryos conceived during the IVF cycle. By using Cryotec method (Reprolife, Japan), vitrification and thawing embryo protocol were applied correctly, so the post-thawing survival embryo rate was up to 100% in both groups. To ensure endometrial acceptability, endometrial thickness was assessed by the doctor before embryo transfer; this parameter was similar in the two groups.

The results of our study showed that there was no statistically significant difference between the morphokinetic group versus morphologic group. The ongoing pregnancy rate was relatively high (57.2% vs. 60.1%,  $p = 0.71$ ). Recently, the predictive value of morphokinetic parameters has been studied<sup>13,26,38-42</sup>. Our study was a retrospective cohort study which was

**Table 3: Clinical outcome data**

Parameter	Morphokinetic group n = 138	Morphologic group n = 138	p-value
Post-thawing survival rate (%)	100	100	1.00
Endometrial thickness on FET day (mm)	11.0 ± 1.2	10.8 ± 1.3	0.09
Number of embryos transferred	1.9 ± 0.4	2.0 ± 0.3	0.74
Ongoing pregnancy rate (%)	57.2	60.1	0.71
Clinical pregnancy rate (%)	64.5	65.9	0.90
Implantation rate (%)	51.2	49.3	0.73
Miscarriage rate (%)	5.1	5.1	1.00
Ectopic pregnancy rate (%)	2.9	0.7	0.37

compiled data from July 2014 to July 2017. All potential embryos were frozen and our clinical outcomes were analyzed based on the patient's first frozen embryo transfer cycle. Previous studies have been published; although their clinical outcomes were analyzed on the fresh embryo transfer cycle, they were similar to our study. In 2014, Rubio *et al.* used time-lapse system in embryo culture, selected embryos based on multivariable morphokinetic model, and reproductive outcomes were compared to embryo selection based on morphology. In this study, Rubio *et al.* conducted a prospective, randomized, double-blinded, controlled study which included eight hundred forty-three infertile couples undergoing ICSI. They concluded that reproductive outcomes were improved when using time-lapse system in culture and selection embryo based on embryo morphokinetic criteria. However, the ongoing pregnancy was not statistically significant different between the two groups (morphokinetic vs. morphologic criteria in embryo selection)<sup>10</sup>. Recently, in another prospective cohort study, there was a total of 235 patients who underwent fresh autologous IVF cycles. They were divided into 2 groups: time-lapse monitoring (119 patients) and conventional morphologic embryo screening (116 patients). There was no statistically significant difference between two groups in clinical pregnancy and implantation rates<sup>13</sup>.

Some studies only analyzed cleavage embryo selection in order to compare the effectiveness of morphokinetic versus morphologic criteria in embryo selection<sup>9,16,43</sup>. Wu *et al.* analyzed a total of 608 patients, which was divided into two equal groups (time-lapse and standard incubator culture system) with 304 patients in each group. They found that the ongoing pregnancy rate and the live birth rate in time-lapse

group were higher than that of the standard incubator culture system<sup>16</sup>. Siristatidis showed in a study population (of 239 women) that the women were classified into two groups: time-lapse and conventional monitoring. The clinical and ICSI cycle characteristics and reproductive outcomes were compared. The results showed better reproductive outcomes in the time-lapse monitoring group<sup>9</sup>.

From the results of the above studies, when embryo selection was done using the morphokinetic criteria, the clinical outcomes were not the same between the cleavage embryos and blastocysts. A possible cause is the chromosome self-normalization during the development from embryo to blastocyst stage<sup>44</sup>.

Recently, Chen *et al.* published a review of effectiveness of time-lapse imaging versus conventional methods in culture and embryo selection in clinical *in-vitro* fertilization<sup>45</sup>. Currently there is insufficient evidence to support that time-lapse is better than conventional methods. To evaluate the effectiveness of time-lapse imaging, more well-designed randomized clinical trials need to be done<sup>45</sup>.

The limitation of our study is that it was a retrospective cohort study, in which we did not actively select samples for the study. Instead, we collected data on treated outcomes of the patient.

## CONCLUSIONS

The clinical outcomes of frozen embryo transfer were similar when embryos were classified according to morphokinetic versus morphologic criteria for freezing. Using morphokinetic criteria to select day-5 embryo for freezing did not improve the ongoing pregnancy rate, as compared to morphologic criteria. Our study design was weak and sample size may not be large enough to make a statistically significant difference. A more well-designed study with a larger sam-

ple size needs to be considered and may provide impactful results.

## ABBREVIATIONS

**BMI:** Body Mass Index  
**FET:** Frozen embryo transfer  
**FSH:** Follicle stimulating hormone  
**ICSI:** Intracytoplasmic sperm injection  
**IVF:** *In-vitro* fertilization  
**IVM:** *In-vitro* maturation  
**RCT:** Randomized controlled trial  
**TLM:** Time-lapse monitoring

## COMPETING INTERESTS

The authors declare that they have no conflict of interests.

## AUTHORS' CONTRIBUTIONS

LNQ drafted the manuscript. LNQ, LTB and NHMT performed the IVF, embryo vitrification works. DQV planned and designed the experiments. PDT, LNQ collected and analysed the data. NTTH supervised the study and finalized the manuscript. All authors read and approved the final manuscript.

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