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Embryo Morphokinetic Parameters Evaluated Using a Time-Lapse Embryo Monitoring System Can Predict the Embryo Developmental Potential in ICSI Cycles

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Abstract

At present, embryo morphology assessment based on the observation of the embryonic morphological characteristics at several specific time points has been mainly used for selecting the high-quality embryo. However, we all know that embryo development is a dynamic process. Many research results on the correlation between the embryo morphokinetic parameters and embryo quality and development potential were inconsistent. With the help of time-lapse imaging, the development processes and outcomes of a total of 365 embryos were cultured and analyzed in this study. The results showed that the mean tPNf and t2 of the high-quality embryo were significantly shorter than the low-quality embryo; the mean t2PBe and tPNa of the high-quality embryo from the implantation group were significantly shorter than those from the non-implantation group. In addition, based on the quartile grouping of each morphokinetic parameter, the embryos that had 21.15≤tPNf≤25.30 value were significantly higher in embryo quality when compared with the embryos that had the tPNf values outside the range on Days 3. Similarly, the embryos that had values of t2≤25.60 were significantly higher in embryo quality than those with outside the range values on Days 3. Thus, we demonstrated that the morphokinetic parameter evaluated using a time-lapse embryo monitoring system can predict the embryo quality, and be benefit for the selection of the high-quality embryos and improvement for the implantation success of the patient in assisted reproductive technologies.

Introduction

Assisted reproductive technology (ART) has been advanced rapidly in recent decades, and tens of thousands of infertile couples have obtained offspring with the aid of ART. Selecting a single best-quality embryo for transfer, improving the implantation rate, and reducing the multiple pregnancy outcomes have been the intense focus of research in the field of ART. At present, standard embryo morphology assessment has been used for selecting the high-quality embryo for transfer, and this conventional method is mainly based on the observation of the fertilized embryo and its cleavage at several specific time points during the development, and then the embryos are scored using these morphological characteristics[1]. However, such evaluations have limitations [2], because scoring may be affected by the subjective effects of the embryologists, and embryos need to be temporarily removed from the incubator during the assessment, which may cause stress to the embryo and negatively affect the embryo development. In addition, embryo development is a dynamic process, and embryo morphology at some time points does not fully reflect the entire process of embryo development.

In recent years, time-lapse monitoring system (TLM) was widely used in the embryology laboratory. The TLM is a technology, and it can continuously monitor the morphokinetic parameters of the early embryo during its development. Further. the embryo assessment can be performed without removing the embryos from the incubators using the TLM system. Previous time-lapse studies indicated that the embryo morphokinetic parameters obtained using TLM have certain predictive significance on the potential of blastocyst formation, implantation after embryo transfer, and live birth rate[3–6]. Aguilar et al. (2014) showed that the time of second polar body (2PB) extrusion and the time of pronuclear fading have a certain value in predicting embryo implantation rate [7]. However, some studies believed that embryo morphokinetic parameters were not related to the embryo developmental potential [8, 9], and Barberet et al. (2019) believed that the time of second polar body extrusion and pronuclear fading were not related to the early embryo development [10]. The variations of these results may have been caused by the differences in the sample size, fertilization methods used, embryo culture conditions adopted, and characteristics of the research participants.

Therefore, the TLM was used to observe and record the embryos' development process from the intracytoplasmic sperm injection (ICSI) cycles in this study. We retrospectively analyzed the relationship between the identified embryo morphokinetic parameters such as the time of second polar body extrusion (t2PBe), the time of pronuclear appearance (tPNa), the time of pronuclear fading (tPNf), and the time of cleavage to 2-cells (t2), and the embryo quality, embryo developmental potential, and pregnancy outcome. The aims of this study were to explore whether the embryo morphokinetic parameters could predict the embryo development potential in the ICSI cycle and determine whether these morphokinetics variables can serve as effective evaluation indicators in embryo selection for improving embryo implantation rate and pregnancy rate.

Materials And Methods

Study design

This study included 365 embryos involving 55 women (one cycle/person) who underwent ICSI treatment at the Center for Reproductive Medicine, the First Affiliated Hospital of Zhengzhou University. The inclusion criteria for this study were the female patients who underwent the first ICSI cycle, ≤ 35 years of age, and only male infertility factors including oligozoospermia, asthenozoospermia, and obstructive azoospermia. Women with the abnormal uterine condition (hysteromyoma, endometriosis, etc.) and recurrent abortion were excluded. GERI time-lapse system (Genea Biomedx, Sydney, NSW, Australia)was used to culture the zygotes which were fertilized using the ICSI procedure. Furthermore, the embryo morphokinetic parameters were recorded and analyzed, including the times taken for second polar body extrusion (t2PBe), pronuclear appearance (tPNa), and pronuclear fading (tPNf), and cleavage to 2-cells (t2).

ICSI and embryo culture

Transvaginal ultrasound-guided follicles' aspiration was performed 36 h after recombinant human chorionic gonadotropin (rhCG) administration. Oocytes retrieved were cultured for 1–2 hours before denudation in G-IVF plus medium (Vitrolife Sweden AB, Goteborg, Sweden). Then, the oocyte denudation was carried out using hyaluronidase (Vitrolife Sweden AB) andere cultured continuously in the G-IVF plus medium for 1–2 hour until the intracytoplasmic sperm injection. Ejaculated sperm and epididymal sperm were washed with SpermGrad gradient solution (Vitrolife) to obtain progressive motile sperm, whereas testicular sperm were obtained shredding the testicular tissue in the G-IVF plus medium using a 1 mL syringe needle and then washed directly with the G-IVF plus medium. Under an inverted microscope (TE2000-U, Nikon, Chiyoda, Japan), mature oocytes were fertilized using the ICSI procedure and transferred into the time-lapse culture dishes containing G-1 plus medium (Vitrolife) after the ICSI. All zygotes were incubated at 37°C and under the microenvironment

containing 5.0% O2, 6.0% CO2, and 89.0% N2 controlled by the GERI time-lapse incubator. The images were automatically acquired at intervals of 10 min through the time-lapse monitoring system. From the fertilization until Day 3, embryos were selected to transfer or freeze or continuously be cultured until the blastocyst stage. For the blastocyst culture, the Day 3 embryos were transferred to the G-2 plus medium (Vitrolife) and cultured at 37℃ and under 5.0% O2, 6.0% CO2, and 89.0% N2.

Embryo scoring and embryo transfer

Embryo morphology was only evaluated on Day 3 based on Peter's embryo scoring criteria (the number of blastomeres, percentage of fragmentation, and presence of multi-nucleation)[11], and grades Ⅰ and Ⅱ were defined as the high-quality embryo. Blastocysts (on Day 5 or Day 6) were graded according to Gardner's criteria [12] and the high-quality blastocysts were accordingly grouped (stage 3–6, AA, AB, BA, or BB). At the blastocyst stage, a single high-quality embryo on Day 5 was selected for embryo transfer. Embryo morphology scoring was performed by two trained embryologists. A total of 54 embryos were transferred to 46 patients. The number of embryos transferred was normally two, but in some cases, one embryo was transferred because of embryo quality.

The serum β-hCG value was determined 14 days after the embryo transfer. The β-hCG of ≥ 50mIU/mL was defined as a biochemical marker for a positive pregnancy. The pregnancy was also confirmed by ultrasound examination when a gestational sac with a fetal positive heartbeat was visible after 35 days of the embryo transfer.

Statistical analysis

All statistical analyses were performed using the SPSS v.20.0 (IBM SPSS software, Chicago, IL, USA). The Student t-test was used to analyze the data. The continuous variables were presented as mean ± standard deviation (SD), and the categorical variables were presented as percentages (%). Furthermore, timing variables were converted from continuous variables into categorical variables. They were divided into groups based on their quartiles. An optimal range was defined as the combined range spanned over the two quartiles with the highest implantation rate. The categorical variables were analyzed using Chi-square tests. The P-value < 0.05 was considered to be significant.

Results

Characteristics of the enrolled patients

In total, this retrospective analysis has utilized clinical data from 55 ICSI treatment cycles. The embryonic development data of 365 fertilized two pronuclear (2PN) zygotes from the time-lapse system recordings were used to analyze. In this current study, the mean age was 29.4 ± 4.3 years, the mean body mass index (BMI) was 22.0 ± 2.9 kg/m2, the mean number of oocytes retrieved was 12.4 ± 3.4, the mean value for anti-Müllerian hormone (AMH) was 3.47 ± 2.3 ng/mL, the mean value for estradiol(E2)was 6.86 ± 2.0 mIU/mL, and the mean value for luteinizing hormone (LH) was 5.32 ± 2.73 mIU/mL.

The morphokinetics of the embryo during the early development monitored using a time-lapse monitoring system (TLM) in the ICSI cycle

The time of monitoring the embryonic developmental events such as the second polar body extrusion, pronuclear appearance, pronuclear fading, and the cleavage of fertilized oocytes to 2-cells after ICSI using the TLM system, is shown in Fig. 1. For all evaluated embryos in the present study, the time interval from the ICSI to the second polar body extrusion(t2PBe)was 2.85 ± 0.9 h, the time interval from the ICSI to pronuclear appearance (tPNa) was 6.43 ± 1.41 h, the time interval from the ICSI to pronuclear fading (tPNf) was 23.36 ± 3.08 h, and the time interval from the ICSI to the cleavage of the fertilized oocyte to 2-cells embryo (t2) was 26.45 ± 4.11 h.

Comparison of embryo morphokinetic parameters between high-quality and low-quality embryos

According to Peter's embryo assessment system on cleavage stage, all Day 3 embryos from the 2PN zygotes were divided into the high-quality group and low-quality group. The embryo morphokinetic parameters were compared between the two groups and were shown in Table 1. In the high-quality embryo group, the tPNf (22.92 ± 2.68 vs. 24.33 ± 3.69, P < 0.05) and the t2 (25.58 ± 2.74 vs. 28.41 ± 5.76, P < 0.05) were found to be significantly shorter compared with the low-quality group. No significant differences for the variables t2PBe and tPNa were found between the groups (P□0.05).

Table 1
Comparison of embryo morphokinetic parameters between the high-quality and low-quality embryos

Group	N	t2PBe(h)	tPNa(h)	tPNf(h)	t2(h)
High-quality	238	2.80 ± 0.79	6.39 ± 1.26	22.92 ± 2.68	25.58 ± 2.74
Low-quality	109	2.95 ± 1.1	6.56 ± 1.75	24.33 ± 3.69	28.41 ± 5.76
T		-1.379	-1.048	-3.976	-6.151
P		0.169	0.295	□0.05	□0.05
* The P□0.05 was considered statistically significant.					

Relationship between embryo morphokinetic parameters and high-quality embryo ratio, and the blastocyst formation rate

To explore the relationship between embryo morphokinetic parameters during early development and the high-quality embryos ratio and blastocyst formation rate, the Day 3 embryos were divided into groups based on the quartiles of each morphokinetic parameter, and the high-quality embryos ratio were compared among the groups (Table 2). For each timing variable, an optimal range for the high-quality embryo distribution was defined as the two consecutive quartiles with the highest high-quality embryo ratio (numbers in bold and underlined in the table). The results showed that the high-quality embryo rate was statistically different when compared with each tPNf quartile group, and when compared with each t2 quartile group ($P < 0.05$). Among them, the high-quality embryo rate was highest (73.4%) when the tPNf was 22.83h-25.3h, and when the t2 < 24.0 h and no significant differences for high-quality embryo rate were found for the other parameter quartile groups (P□0.05).

Table 2
The relationship between embryo morphokinetic parameters and high-quality embryo rate

Morphokinetic parameters	1		2		3		4		χ^2	P
	Time interval/h	Rate of High-quality embryo(%)	Time interval/h	Rate of High-quality embryo(%)	Time interval/h	Rate of High-quality embryo(%)	Time interval/h	Rate of High-quality embryo(%)		
t2PBe	□2.25	65.5	2.25–2.70	67.8	2.70–3.30	69.0	□3.30	65.8	0.522	0.914
tPNa	□5.60	67.0	5.60–6.25	60.6	6.25-7.0	68.1	□7.0	53.2	3.789	0.285
tPNf	□21.15	67.0	21.15–22.83	66.0	22.83–25.30	73.4	□25.30	42.6	22.465	□0.001
t2	□24.0	73.4	24.0-25.60	67.0	25.60–28.20	67.0	□28.20	40.4	27.091	□0.001

* The embryos were divided into four groups (1–4) based on the quartiles of the morphokinetic parameters. The percentage of the high-quality embryos in each quartile is shown. Numbers in bold and underline indicate the two quartiles with the highest high-quality embryo rate. The P□0.05 was considered statistically significant.

To further explore the relationship between the embryo morphokinetic parameters and the blastocyst formation rate, the blastocyst formation rate was compared among the morphokinetic parameters quartile groups and was shown in Table 3. The results revealed that the blastocyst formation rate was not statistically different ($P > 0.05$) among the morphokinetic parameters quartile groups.

Table 3
The relationship between the embryo morphokinetic parameters and the blastocyst formation rate

Morphokinetic parameters	1		2		3		4			
	Time interval/h	Rate of Blastocyst Formation(%)	Time interval/h	Rate of Blastocyst Formation(%)	Time interval/h	Rate of Blastocyst Formation(%)	Time interval/h	Rate of Blastocyst Formation(%)	χ^2	P
t2PBe	□2.25	66.7	2.25–2.70	82.1	2.70–3.30	81.8	□3.30	72.7	3.173	0.366
tPNa	□5.60	67.6	5.60–6.25	86.7	6.25-7.0	78.9	□7.0	70.3	4.054	0.256
tPNf	□21.15	87.1	1.15–22.83	75.9	22.83–25.30	70.3	□25.30	71.1	3.257	0.354
t2	□24.0	90.6	24.0-25.60	71.4	25.60–28.20	70.3	□28.20	71.1	5.201	0.158

* The P□0.05 was considered statistically significant

The influence of the patient's BMI status on embryos' morphokinetics during early development

Based on the Chinese body mass index (BMI) criteria formulated by the National Health and Family Planning Commission of the People's Republic of China, there are group A (BMI < 18.5 kg/m2), group B (18.5 kg/m2 ≤ BMI < 24kg/m2) and group C (24 kg/m2 ≤ BMI ≤ 32 kg/m2). We found that the high-quality embryos ratio, blastocyst formation, and high-quality blastocysts rate were different in participants with different BMI (Table 4). For the Day 3 embryos, the ratio of high-quality embryos in group A is higher than that in the other groups, but no statistical differences were found. Besides, no differences for the blastocyst formation rate and the high-quality blastocyst rate were also found among the groups ($P > 0.05$).

Table 4
The influence of the BMI status on early embryonic development

BMI	N	Rate of High-quality Embryo(%)	Rate of Blastocyst Formation(%)	Rate of High-quality Blastocyst(%)
A (≤18.5)	4	85.2	55.6	0
B(18.5–23.9)	37	69	51.5	23.1
C(24.0–32.0)	14	60.9	55.6	20.0
χ^2		5.618	0.207	2.723
P		0.06	0.902	0.605
* The P≤0.05 was considered statistically significant				

We further analyzed and compared the embryo morphokinetic parameters with the BMI groups (Table 5). The results showed that the tPNa was statistically significant among all groups ($P < 0.05$) with a positive correlation. It is interesting to note that no statistical differences for the other embryo morphokinetic parameters were found among ($P > 0.05$), but the t2PBe, tPNf, and t2 increased gradually with the BMI increase.

Table 5
Comparison of embryo morphokinetic parameters with the different BMI groups

Group	N	t2PBe(h)	tPNa(h)	tPNf(h)	t2(h)
A (≤18.5)	4	2.67 ± 0.60	5.28 ± 0.96	22.12 ± 2.30	24.77 ± 2.24
B(18.5–23.9)	37	2.82 ± 0.93	6.52 ± 1.38	23.44 ± 3.09	26.58 ± 4.39
C(24.0–32.0)	14	2.97 ± 0.89	6.54 ± 1.47	23.53 ± 3.16	26.63 ± 3.67
F		1.280	10.048	2.423	2.466
P		0.279	≤0.001	0.090	0.086
* The P≤0.05 was considered statistically significant					

The effect of embryo morphokinetic parameters during early development on the clinical outcome after embryo transfer

In this study, a total 46 fresh cycles of embryo transfer were carried out. Upon analysis of the pregnancy outcomes after embryo transfer, there were 19 patients from the ICSI cycles that were completely implanted (the number of gestational sacs matched with the number of transferred embryos) and 13 patients from the ICSI cycles that were not implanted (the biochemical marker for pregnancy was negative) and included in the analysis. Subsequently, all high-quality embryos from those patients were divided into the implantation group and non-implantation group. The average value of t2PBe, tPNa, tPNf, and t2 for the high-quality embryos from two groups, is presented in Table 6. The t2PBe (2.68 ± 0.82 vs. 3.16 ± 0.9, $P < 0.05$) and the tPNa (6.31 ± 1.11 vs. 7.06 ± 1.32, P≤0.05) of the high-quality embryos were significantly shorter in the implantation group compared with those in the non-implantation group. Although no significant difference was found for the variables, tPNf and t2 between the implantation group and non-implantation group (P≤0.05), the tPNf and t2 were shorter in the implantation group.

Table 6
The effect of embryo morphokinetic parameters on the high-quality embryos from implantation or non-implantation patients after transfer

Group	N	t2PBe(h)	tPNa(h)	tPNf(h)	t2(h)
Implantation	115	2.68 ± 0.82	6.31 ± 1.11	23.32 ± 2.61	25.92 ± 2.64
Non-implantation	49	3.16 ± 0.9	7.06 ± 1.32	23.83 ± 2.55	26.55 ± 2.57
T		-3.217	-3.767	-1.144	-1.406
P		0.002	0.000	0.254	0.162
* The P≤0.05 was considered statistically significant					

Discussion

Selection of embryos with the high developmental potential for embryo transfer to increase the implantation rate and thus the pregnancy rate is crucial in the ART program. The introduction of the TLM system successfully allowed the uninterrupted observation of the process of embryonic development and performed the dynamic assessment of early embryos. With the aid of the TLM system, the morphokinetic parameters of a single embryo can be effectively monitored. The morphokinetic parameters of the embryo during early development include the time taken for the second polar body extrusion (t2PBe), the time taken for the pronuclear appearance (tPNa), the time taken for the pronuclear fading (tPNf), and the time taken for the cleavage to 2-cells (t2), 3-cells (t3), 4-cells (t4) and 5-cells (t5) stages [9]. These embryonic developmental parameters can offer embryologists and clinicians the ability to effectively predict the potential of embryonic development and successful implantation.

As early as 1997, Payne et al. (1997) used the time-lapse technology for the first time to monitor embryonic development including the time of polar body appearance, pronuclear appearance (tPNa), and pronuclear fading (tPNf). These parameters were found to be significantly different among different zygotes and also differed significantly between the high-quality embryos and low-quality embryos [13]. Subsequently, many studies recognized that the morphokinetic parameters of the embryo during early development could be used as key markers to predict the embryonic developmental potential.

In this retrospective study, we have found that the tPNf and t2 were significantly related to the embryo quality. Lemmen et al. (2008) found that the tPNa, tPNf, t2, t3, and t4 were related to the embryo quality and implantation potential, and the embryo development potential was greater when the t2 was shorter [14]. Barberet et al. (2019) concluded that the tPNf and t2 had significant correlations with the embryo quality, while the t2PBe and tPNa had not, and with relatively faster development, embryo quality was found to be high [10]. These conclusions are consistent with our results. Our results showed that the t2PBe and tPNa were not significantly different between the high quality and low-quality embryos, but the t2PBe and tPNa in the high-quality embryos are shorter than those in the low-quality embryos. Based on the analysis, the tPNf and t2 in the present study were shorter by 1 h than the morphokinetic parameters reported by Coticchio et al. (2018) (tPNf: 23.36 ± 3.08h vs 24.5 ± 4.7h, t2: 26.45 ± 4.11h vs 27.7 ± 5.0 h) [3], and this difference may be related to the characteristics of the patients enrolled. We have found that the t2PBe, tPNa were not significantly related to the Day 3 embryo quality and the developmental potential, but the t2PBe (2.68 ± 0.82 vs. 3.16 ± 0.9, P□0.05) and the tPNa (6.31 ± 1.11 vs. 7.06 ± 1.32, $P < 0.05$) of the high-quality embryos were significantly different between the implantation and non-implantation groups, indicating that the embryos with shorter t2PBe and tPNa have higher implantation potential. Meseguer et al. (2011) indicated that the t2, t3, t4, s2 (3-cells to 4-cells), and cc2 (2-cells to 3-cells) were significantly correlated with the embryo implantation potential, but their study did not evaluate the t2PBe, tPNa, and tPNf [15]. Carrasco et al. (2017) also showed that the morphokinetic parameters such as t2, t3, t4, and t8 could predict the embryo implantation ability, and the implantation ability was higher in the embryos with the faster cleavage rate, but the tPNa and tPNf were not significantly correlated with the embryo implantation [6]. Aguilar et al. (2014) revealed that the t2PBe and tPNf had a certain predictive value for embryo implantation [7]. Therefore, this current study analyzed the t2PBe, tPNa, tPNf, and t2, and found that the t2PBe, tPNa were significantly related to the implantation potential. Although the tPNf and t2 were not found to be significantly related to the implantation potential, median values of the tPNf and t2 in the high-quality embryos from the implantation group were shorter than those from the non-implantation group. These results were consistent with the outcomes of previous studies. The differences in conclusions may be due to the different embryo fertilization methods, culture environments, and population characteristics.

Some studies combined the duration of the cc2 and the duration of the 3-cells to 5-cells (cc3) in the Day 3 embryo morphology score to predict the blastocyst formation [16]. The results from Cruz et al. (2012) were consistent with this protocol, indicating that timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality [17]. Desai et al. (2014) proposed that the morphokinetic parameters such as tPNf, t2, t4, and t8 could be used as indicators to predict the formation of the blastocyst [18]. Nevertheless, some scholars believed that embryo morphokinetic parameters have no correlation with embryo implantation rate, clinical pregnancy rate and live birth rate [8, 19]. In our study, the morphokinetic parameters were not found to be significantly related to the blastocyst formation potential. It may be related to small sample size, different culture system and so on.

At present, the impact of BMI on embryo quality and the pregnancy outcome is controversial. Some scholars proposed that the blastocyst formation rate in the females with the normal BMI was higher than that in the obese women, and the BMI was significantly correlated with the embryo quality [20]. However, some scholars suggested that the BMI had no effect on the embryo morphokinetic parameters and embryonic development [21]. Bartolacci et al. elucidated the correlation between the BMI and the embryo morphokinetic parameters using the time-lapse monitoring system and found that the embryo development in overweight or obese females was slower (t5 and t8 were prolonged), and these morphokinetic parameters may be used to improve the selection of the high-quality embryos [22]. Therefore, the present study further analyzed the participants with different BMI and found that the Day 3 high-quality embryo ratio and the blastocyst formation rate were not statistically different between participants with different BMI ($P > 0.05$). The only statistical difference was found for the tPNa among different groups ($P < 0.05$). Perhaps, due to the small sample size in the present study, significant differences for the tPNf and t2 may not have been found between the groups, but it is interesting to note that both tPNf and t2 gradually increased with the increase of BMI. Similarly, no significant difference regarding the ratio of the high-quality embryo on the Day 3 was not found between patients with different BMI. But the ratio of the high-quality embryo on Day 3 gradually declined with the increase of BMI. This analysis also showed that the Day 3 high-quality embryo rate gradually declined when the tPNf and t2 were longer (Table 1). Therefore, we speculated that the BMI could impact the early embryo quality affecting embryo morphokinetic parameters, and the sample size needs to be increased to further verify this correlation.

Conclusions

In summary, embryo morphokinetic parameters may be effectively used to predict the embryo quality to improve the selection of embryos in clinical practice. The embryos with a relatively faster development rate will have a greater high-quality embryo rate and implantation rate. This study included a relatively small sample and the morphokinetic parameters were affected by many factors. Therefore, more ICSI cycles need to be analyzed, and the strict inclusion criteria should be included to further explore the predictive value of the embryo morphokinetic parameters to determine the embryo quality and speculate the pregnancy outcome. It is expected that the embryo morphokinetic parameters in conjunction with the currently used conventional morphologic parameters could improve the selection of embryos and improve the outcome of the ICSI procedure.

Abbreviations

ART: assisted reproductive technology

TLM: time-lapse monitoring system

2PB: second polar body

ICSI: intracytoplasmic sperm injection

t2PBe: time of second polar body extrusion

tPNa: time of pronuclear appearance

tPNf: time of pronuclear fading

t2: time of cleavage to 2-cells

rhCG: recombinant human chorionic gonadotropin

BMI: body mass index

AMH: anti-Müllerian hormone

Declarations

Availability of data and materials

All data generated through this study are included in this article.

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Authors' contributions

QH and GDY design subject, analyze data and write manuscript. JH and GY analyze data and write manuscript. ZW, HF, YB, JZ, TZ, NS, HJ, WS and SS provide data and modify manuscript. GDY and YS supervised the subject. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

This study was approved by the Biomedical Ethics Committee of the First Affiliated Hospital of Zhengzhou University, and written informant consent was obtained from the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Figures

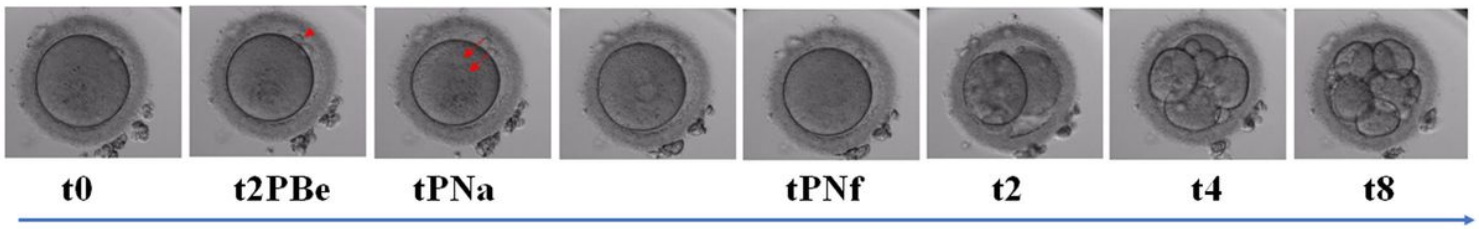

Figure 1

The morphokinetic parameters of the embryos during the early embryonic development monitored using a time-lapse monitoring system in the ICSI cycle. The t0 refers to the time of ICSI completed, t2PBe refers to the time interval from the ICSI to the second polar body extrusion, tPNa refers to the time interval from the ICSI to the pronuclear appearance, tPNf refers to the time interval from the ICSI to the pronuclear fading, and t2 refers to the time interval from the ICSI injection to the cleavage of the fertilized zygotes to 2-cell embryos. The t4 and t8 refer to the time interval from the ICSI to the cleavage of the fertilized zygotes to 4- and 8-cell embryos, respectively. The red arrowhead points to the second polar body, and the red arrow points to the pronucleus.