Morphokinetic parameters from a time-lapse monitoring system cannot accurately predict the ploidy of embryos

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Received: 7 December 2016 / Accepted: 25 May 2017 / Published online: 4 July 2017
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Abstract

Purpose This study aimed to test whether there is an association between embryo morphokinetic parameters and ploidy status.

Methods Patients with high risk of aneuploidy were analyzed by time-lapse microscopy combined with preimplantation genetic screening (PGS). Accordingly, 256 blastocysts from 75 patients were subjected to trophectoderm biopsy and microarray comparative genomic hybridization (array-CGH). Blastocyst development process was analyzed using time-lapse images.

Results Morphokinetic parameters: tPNf, t2, t3, t4, t5, t8, t9, tcom, tM, tSB, tB, tEB, CC1, CC2, CC3, S2, S3, t5-t2, and tB-tSB showed no significant difference in euploid embryos compared to aneuploid counterparts. In addition, two risk models based on previously published morphokinetic parameters failed to segregate euploid from aneuploid embryos.

Conclusions Morphokinetic parameters subjected to investigation in the present study failed to improve the chance of selecting euploid embryos.

Keywords Time-lapse monitoring system · PGS · Morphokinetic parameters · Ploidy

Introduction

The failure of implantation and recurrent abortion has conferred serious obstacles in assisted reproduction technologies (ART) in humans. There is growing evidence that the embryonic aneuploidy may be responsible for such problems [1–3]. It has been revealed that aneuploidy in younger women (27 to 35 years of age) going through an in vitro fertilization-embryo transfer (IVF-ET) was considerably high; such aneuploidy incidence showed was even higher in women >35 years old [4].

Widely published research has shown that preimplantation genetic screening (PGS) is effective in determination of aneuploid embryos. In a randomized-controlled trial, blastocyst biopsy by PGS resulted in improved IVF outcomes [5]. In another study, while PGS failed to improve the clinical outcome in women younger than 35 years of age, it was associated with a decreased odd of miscarriage in women >35 years and an increased odd of live birth in women >37 years [6]. Today, there are different PGS approaches, in which various technologies show great clinical success such as (1) microarray comparative genomic hybridization (array-CGH) [7, 8], (2) single nucleotide polymorphism microarrays (SNP arrays) [9], (3) quantitative polymerase chain reaction (qPCR) [10], and (4) next generation sequencing (NGS) [11]. Nevertheless, PGS applications have some limitations. For example, genetic composition of the inner cell mass (ICM) and trophectoderm (TE) can differ, and mosaicism could occur in the trophectoderm [12, 13]. In addition, PGS requires careful conduct. The invasive and complicated nature of PGS operation may lead to false results if not properly executed [3]. Thus, a reliable and noninvasive approach is required to predict the embryo ploidy.

Over the past decade, morphokinetic parameters have been increasingly used to predict the developmental potential and ploidy of embryos [14–25]. This has been associated with the

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feasibility of time-lapse embryo monitoring applications; however, there are some controversial conclusions. Several studies from 2013 to the present reported possible links between embryo morphokinetic parameters and the ploidy status, although such links were often different in these studies [17–19, 24, 25]. On the other hand, it was also shown that morphokinetic parameters could fail to predict the embryo ploidy [21, 22]. Taken together, it remains largely unclear whether there is a direct relationship between morphokinetic parameters and the embryonic ploidy, and the aim of this study was to determine such relationship, as well as to show if the time-lapse monitoring system could replace PGS to select euploid embryos.

Materials and methods

Patients

In this retrospective study, 75 patients were included undergoing an assisted reproduction treatment at The Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University from October 2014 to September 2015. Subjects were female, and their age ranged between 23 to 44 years inclusive (average 33.6 ± 4.2 years). Only patients with a history of recurrent miscarriage or repeated implantation failure were selected. An advanced maternal age was also a parameter in patient selection. Isolated embryos were cultured in a time-lapse incubator (EmbryoScope, Unisense, Denmark). Only the blastocysts that were assessed over 4 BC grade according to Garden and Lane criteria (1997) were analyzed for aneuploidy [26]. Two hundred fifty-six blastocysts from 75 patients with definite ploidy status and full video were included.

Oocyte retrieval, embryo culture, embryo biopsy, and microarray comparative genomic hybridization

Oocyte retrieval was performed by transvaginal ultrasound-guided follicular aspiration 36 h after hCG administration. Intracytoplasmic sperm injection (ICSI) was performed on metaphase II oocytes approximately 4 h after the oocyte retrieval. Following the insemination, embryos were placed in the time-lapse incubator and cultured in 6% carbon dioxide and 5% oxygen concentrations until day 5 (D5) or 6 (D6). Sequential culture media (G1 and G2) from Vitrolife (Scandinavian IVF Science, Goteborg, Sweden) was used for embryo culture. The EmbryoScope was programmed to acquire images of each embryo every 15 min through seven different focal planes. Trophectoderm biopsy and vitrification were performed on blastocysts with high morphological grades [26]. Trophectoderm biopsies were analyzed by the array-CGH technique for aneuploidy.

Evaluation of time-lapse imaging and morphokinetic parameters

An expert embryologist viewed time-lapse videos by EmbryoViewer (Unisense, Denmark), and annotated developmental events and cleavage anomalies. The conduct of ICSI was designated as “time zero” (t0), and a computer software was used to calculate the timing of following events:

- From insemination to the pronuclear fading (tPNf)
- 2, 3, 4, 5, 8, 9 cells (t2, t3, t4, t5, t8, t9)
- First fusion of two cell boundaries (tcom)
- When the embryo formed into a morula and all cell boundaries are unobvious (tM)
- The start of a cavity forming (tSB)
- When the blastocoel cavity is filled up and the embryo starts to expand (tB)
- When the blastocyst is expanded and the zona pellucida is thin (tEB)
- The duration of the first (CC1, t2–tPNf), second (CC2, t3–t2), and the third cell cycle (CC3, t5–t3)
- Time of the synchrony of the second (S2, t4–t3) and third cell cycle (S3, t8–t5)
- The interval between 2 and 5 cells (t5–t2)
- Multinucleation at 2-cell stage (MN)
- Reverse cleavage (RC), a blastomere was reabsorbed after cleavage
- Direct cleavage (DC), a single blastomere divided directly from 1 to 3 cells in less than 5 h.

Previously established risk models claiming embryonic ploidy prediction

Two different models were used to predict ploidy status. First model was established by Basile et al. (2014) [18]. In this model, embryos within the optimal ranges proposed for t5–t2 (>20.5 h), and CC3 (11.7–18.2 h) are more likely to be euploid, as shown in Table 1.

Second model was developed by Campbell et al. (2013) [19]. In this model, embryos are classified into three types: low risk of being aneuploid, tB < 122.9 h and tSB < 96.2 h; medium risk, tB < 122.9 h and tSB ≥ 96.2 h; and high risk, tB ≥ 122.9 h, as shown in Table 2.

Table 1 Risk model of Basile et al. (2014) [18]

Risk class	Definition
Low risk of being aneuploid	t5–t2 (>20.5 h) and CC3 (11.7–18.2 h)
High risk of being aneuploid	Outside the range

Table 2 Risk model of Campbell et al. (2013) [19]

Risk class	Definition
Low risk of being aneuploid	tB < 122.9 h and tSB < 96.2 h
Medium risk of being aneuploid	tB < 122.9 h and tSB ≥ 96.2 h
High risk of being aneuploid	tB ≥ 122.9 h

Statistical analysis

Significance of data was assessed by SPSS Statistics 17 software (IBM Inc., New York, NY) using the Student's *t* test and the chi-square test. Differences were considered significant when *P* values < 0.05.

Results

One hundred six out of 256 embryos (41.4%) were found to be aneuploid. This proportion increased with the age of females, as demonstrated in Table 3.

There was no significant difference between euploid and aneuploid embryos according to the early morphokinetic parameters, such as timings tPNf, t2, t3, t4, t5, t8, t9 or timings CC1, CC2, CC3, S2, S3, t5-t2 (Table 4).

No significant differences were observed between euploid and aneuploid embryos in the initiation (tcom) and completion (tM) of compaction, the initiation (tSB) and completion (tB) of blastulation, as observed in Table 5. However, the aneuploid embryos had a longer interval between tB and tEB, with a significant difference, *P* < 0.05.

When the early timings were analyzed using the risk model proposed by Basile et al. (2014), the proportion of normal embryos did not increase in the optimal range proposed for t5-t2 (>20.5 h) and CC3 (11.7–18.2 h) (Fig. 1). When the other model proposed by Campbell et al. (2013) was used, euploid and aneuploid embryos were distributed evenly. tSB followed by tB cannot help predict the risk class of aneuploidy (Fig. 2).

Irregular division patterns (DC and RC) exhibited no significant differences between aneuploid and euploid embryos; however, the incidence of multinucleation in aneuploid embryos was higher than in euploid embryos, *P* < 0.05, as shown in Table 6.

Table 3 The patient age (year) with the ploidy of embryos

	Euploid	Aneuploid
All embryos	150/256 (58.6%)	106/256 (41.4%)
Embryos from patients ≤35	120/189 (63.5%)	69/189 (36.5%)
Embryos from patients >35	30/67 (44.8%)	37/67 (55.2%)

Table 4 Early morphokinetic parameters of euploid and aneuploid embryos

	Euploid	Aneuploid	<i>P</i> value
tPNf	22.0 ± 2.4	22.5 ± 3.0	0.12
t2	24.5 ± 2.5	25.0 ± 3.0	0.15
t3	35.2 ± 3.3	36.0 ± 3.6	0.06
t4	36.3 ± 3.1	37.1 ± 3.9	0.08
t5	48.3 ± 5.5	49.2 ± 5.3	0.16
t8	54.9 ± 7.8	55.7 ± 7.7	0.44
t9	69.0 ± 8.5	70.4 ± 7.6	0.17
CC1 (t2-tPNf)	2.5 ± 0.4	2.5 ± 0.4	0.60
CC2 (t3-t2)	10.7 ± 1.7	11.0 ± 1.0	0.10
CC3 (t5-t3)	13.1 ± 3.1	13.2 ± 3.4	0.72
S2 (t4-t3)	1.1 ± 1.5	1.1 ± 1.2	0.88
S3 (t8-t5)	6.7 ± 6.6	6.5 ± 5.9	0.82
t5-t2	23.8 ± 4.1	24.2 ± 3.8	0.37

Discussion

Widely published researches have explored the relationship between embryo morphokinetic parameters and ploidy, particularly based on widely used time-lapse embryo monitoring. This led to a number of different conclusions. Chawla et al. (2015) showed that mean time durations for tPNf, t2, t5, CC2, CC3, and t5-t2 differed significantly between normal and abnormal embryos [17]. One year before that, Basile et al. (2014) reported that two early morphokinetic parameters were related to the chromosomal content. Accordingly, embryos within optimal ranges proposed for t5-t2 (>20.5 h) and CC3 (11.7–18.2 h) were more likely to be euploid [18]. Del Carmen Nogales et al. (2017) recently showed that the variable t3 and t5-t2 were the best timings to predict whether an embryo was normal or abnormal [24]. Campbell et al. (2013) pointed that the ploidy of embryo has no relationship with timings of early cleavage; however, an association between

Table 5 Morphokinetic parameters of euploid and aneuploid embryos after compaction

	Euploid	Aneuploid	<i>P</i> value
tcom	83.1 ± 7.3	81.6 ± 6.7	0.11
tM	88.6 ± 8.3	86.8 ± 7.6	0.09
tSB	97.8 ± 8.6	97.7 ± 8.4	0.92
tB	107.5 ± 9.9	106.5 ± 9.7	0.42
tEB	120.5 ± 9.6	121.5 ± 9.6	0.41
tB-tSB	9.6 ± 4.2	8.8 ± 4.4	0.10
tEB-tB	13.1 ± 4.3	15.1 ± 4.6	0.001 ^a

^a Differences were considered significant when *P* values < 0.05

Fig. 1 Distribution of euploid and aneuploid embryos in the model of Basile et al. (2014) [18]

human embryo ploidy and morphokinetic parameters were demonstrated during the blastula stage. The initiation time of compaction (tSC), the initiation time of blastulation (tSB), and the time of full blastulation (tB) were delayed in aneuploid embryos [19, 20]. Moreover, Minasi et al. (2016) showed observations of a large number of morphokinetic parameters, in which two early parameters t4-t3 and t4 as well as the timing of (a) starting blastulation, (b) reaching full blastocyst stage, (c) blastocyst expansion, and (d) hatching were all delayed in aneuploid embryos [25].

On the other hand, Rienzi et al. (2015) suggested that there was no statistical correlation between 16 commonly detected morphokinetic characteristics (Syngamy, t2, t3, t4, t5, t8, CC1, CC2, CC3, CC3/CC2, S2, S3, t5-t2, initiation of compaction, initiation of blastulation, and completion of blastulation) and ploidy in a longitudinal cohort of 455 blastocysts from 138 patients. Rienzi and colleagues also suggested that the model proposed by Campbell et al. (2013) and Basile et al. (2014) were not suitable for separating aneuploid embryos from euploid embryos [21]. In the present study, we reached similar conclusions with Rienzi's work. Likewise, Kramer et al. (2014) showed that the "Aneuploidy Risk Classification Models" proposed by Campbell et al. (2013) were not predictive of embryo ploidy among IVF facilities [22]. Swain (2013) performed a meta-analysis exploring the correlation between

Fig. 2 Distribution of euploid and aneuploid embryos in the model of Campbell et al. (2013) [19]

Table 6 Incidence of multinucleation and cleavage anomalies in euploid and aneuploid embryos

	Euploid	Aneuploid	<i>P</i> value
MN	14/150 (9.3%)	20/106 (18.9%)	0.03 ^a
DC	6/150 (4.0%)	6/106 (5.7%)	0.54
RC	10/150 (6.7%)	6/106 (5.7%)	0.74

^a Differences were considered significant when *P* values < 0.05

morphokinetic parameters and embryo chromosomal status, and concluded that the time-lapse embryo monitoring may help predict the ploidy of embryos. Nonetheless, PGS still remains as the most reliable method in such prediction [23].

In the present cohort, 106 of 256 embryos (41.4%) were aneuploid, showing lower ratio than several other studies [18, 21, 22]. We think the younger maternal age is responsible for such lower rate. The average patient age was relatively lower than other studies [18, 19, 21, 22, 24, 25] and was calculated 33.6 ± 4.2 . Today, it has been demonstrated that embryo aneuploidy increases with female aging [4].

A significant proportion of morphokinetic parameters failed to predict the embryo ploidy. Furthermore, two risk models from Campbell et al. (2013) [19] and Basile et al. (2014) [18] were used in the present study, and both approaches failed to segregate euploid embryos from aneuploid counterparts. Thus, we believe that the time-lapse monitoring system does not improve the chance of selecting euploid embryos. This conclusion is parallel with Rienzi's [21] and Kramer's [22] findings, although Minasi [25], Basile [18], Campbell [19], and many others claimed opposite. Many factors may be responsible for such differences between our findings and others. For instance, a different culture media [19, 21, 22] and different gas concentrations [18, 19] were used in the present research. It has been shown that conditions of incubation can impact morphokinetic parameters [27–29]. Another determinant of such different conclusions may be biopsy techniques and PGS approaches per study [18, 19, 24].

The following parameters were similar between euploid and aneuploid embryos:

- the time from insemination to every endpoint of the embryo development (tPNf, t2, t3, t4, t5, t8, t9, tcom, tM, tSB, tB, tEB)
- the length of the first, second and third cell cycles (CC1, CC2, CC3)
- the synchronization parameters (S2, S3)
- the duration of blastulation (tB-tSB)

An important finding was the longer duration of blastocyst expansion (tEB-tB) in aneuploid embryos compared to euploid embryos ($P < 0.05$), while the interval between tB and tEB may have been affected by several external factors. Thus,

future investigations are needed to detail this morphokinetic parameter.

Irregular division behaviors MN, DC, and RC have received great interest from geneticists and researchers of reproduction. The presence of DC and RC equally reduced developmental potential of embryos [30, 31]; however, we found that there were no significant differences between aneuploid and euploid embryos in DC and RC. Moreover, the presence of multinucleated blastomeres on the second day of the embryo development showed an increase of aneuploidy and poor pregnancy outcome in IVF-PGS cycles [32]. Embryos with multinucleated cells are more likely to be aneuploid (76.6%) than euploid (50.9%) [33]. In this study, the aneuploid embryos showed higher incidence of multinucleation than euploid embryos. It is unlikely that such single parameter can predict the ploidy of embryos; therefore, the association between MN and embryonic aneuploidy needs further investigation.

To conclude, none of the morphokinetic parameters could accurately predict the ploidy of embryos in the present study. The disadvantage of relatively small patient cohort was balanced with younger patient age and wider range of analyses. The potential of the present findings is considerable as we believe that these results are helpful for a better understanding of the association between embryo morphokinetic parameters and ploidy status.

Acknowledgement This research was funded by National Research and Development Plan (2016YFC1000600) and Major Program of National Natural Science Foundation of China (8149073).

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