Cleavage pattern predicts developmental potential of day 3 human embryos produced by IVF

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Abstract  Time-lapse technique provides opportunities to observe the dynamic process of human early development. Previous studies have suggested several abnormal division patterns were associated with decreased developmental potential, but no systematic results are currently available. In this study, seven abnormal division patterns were observed during early cleavage, and these had different effects on the further development potential of daughter blastomeres. According to the severity and occurrence of abnormal division patterns during the initial three cleavages, an embryo hierarchical classification model was developed and day 3 embryos were classified into six grades (from A to F). The good-quality blastocyst formation rate for these grades decreased from 70.8–3.8% (P < 0.001). In a prospective observational study, 139 IVF cycles were recruited to assess the efficiency of this classification model. In the embryos that had confirmed implantation results, the implantation rate decreased from 67.0% (Grade A) to 0% (Grade D; P < 0.001). These results indicated that cleavage patterns can predict the developmental potential of day 3 human embryos.

KEYWORDS: blastocyst, cleavage, embryo, time-lapse

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**Introduction**

IVF and embryo transfer have been widely used for infertile couples to achieve successful pregnancy. Usually, more than one embryo is transferred during most IVF and embryo transfer cycles to guarantee a higher possibility of pregnancy; however, this also leads to a higher chance of multiple pregnancy (30–40%), increased risk of preterm birth, pre-eclampsia and low birth weight (Miyake et al., 2010; Sunderam et al., 2013; Wang et al., 2009). Multiple pregnancies are recognized as the primary factor that negatively affects the health of offspring produced by IVF and embryo transfer (Atanasova et al., 2013a, 2013b; Olivennes et al., 2002).

Single embryo transfer has been suggested to prevent multiple pregnancies, thereby reducing the occurrence of iatrogenic complications, compared with multiple embryo transfer (Grady et al., 2012; Rodriguez et al., 2012; Stillman et al., 2009; Sullivan et al., 2012; Tiitinen, 2012). A feasible strategy for single embryo transfer is blastocyst culture and single blastocyst transfer, which results in an improved clinical pregnancy rate of 50–60% (Gardner et al., 2004; Sadasivam and Sadasivam, 2008). Embryos, however, may not reach the blastocyst stage (French et al., 2010; Gardner et al., 2000), and prolonged embryo culture may increase the risk of epigenetic disorders, monozygotic twinning and preterm delivery (Horsthemke and Ludwig, 2005; Kallen et al., 2010; Kalra et al., 2012; Manipalviratn et al., 2009; Niemitz and Feinberg, 2004). Transferring one cleavage stage embryo is an alternative way to prevent multiple pregnancies, but previous studies have shown that single embryo transfer at the cleavage stage significantly reduce clinical pregnancy rate compared with single blastocyst transfer (Papanikolaou et al., 2006a, 2006b). It is thought that this reduction is partially due to methodological limitations in embryo selection, which is primarily based on morphological criteria and is unable to reliably identify the most competent embryos (Scott, 2003; Van Montfoort et al., 2004). Improved methods for selecting cleavage-stage embryos are therefore important to enhance the practice of single embryo transfer.

**Materials and methods**

**Embryo source and ethical approval**

For the experimental part of the study, immature oocytes (metaphase I, n = 967) from patients receiving intracytoplasmic sperm injection (ICSI) were collected with the informed consent from couples that agreed to donate oocytes for research use. All the metaphase I oocytes were collected from 12 noon to 5 pm during ICSI procedure and were further cultured in G1 media (Vitrolife, Goteborg, Sweden). The metaphase I oocytes were rechecked at 8 pm and only those oocytes that matured at this time point were further fertilized by ICSI and were used for time-lapse observation. This project was approved by the Institutional Review Board of the Reproductive and Genetic Hospital of CITIC Xiangya on 28 February 2012 (reference LL-SC-SG-2012-004).

**Embryo culture and time-lapse recording**

For the experimental part of the study, all pronuclear-stage embryos were placed individually into the microwells of a specially designed well-of-the-well culture dish (nine microwells in a 3 × 3 matrix, or 16 microwells in a 4 × 4 matrix) (Vitrolife; Budapest, Hungary) and were cultured in sequential media (G1 and G2) (Vitrolife; Goteborg, Sweden). All embryos were cultured to the blastocyst stage (about 5 days) in a 37°C incubator with a 6% CO₂, 5% O₂, and 89% N₂ atmosphere. Blastocyst morphology was evaluated at day 5 or day 6 after insemination according to Gardner’s scoring system (Gardner and Schoolcraft, 1999). Blastocysts that were scored as 3BB or higher quality were regarded as good-quality blastocysts. Negative effects on inner cell mass and trophectoderm were regarded as scoring less than grade ‘B’ and negative effects on expansion status were regarded as scoring less than grade ‘3’.

Time-lapse images were acquired using a Primo Vision system (Vitrolife), which was placed inside the incubator. The culture medium was changed on the afternoon of day 3 no matter whether the assessed parameters completed. When the dish was removed from the incubator, all embryos were transferred to the same position of another well-of-the-well dish containing a 50-μl microdroplet of G2.5 medium covered with oil (Ovoil, Vitrolife). The dish was then returned to the incubator, placed in the sample holder of the
digital microscope and the time-lapse monitoring was continued. Images of each embryo were recorded every 5 min. Abnormal division behaviours in the initial three cleavages and the percentage of blastomeres affected were annotated and analysed. Three morphokinetic parameters were also evaluated in this study: $t_5$ (time from insemination to completion of division to 5 cells), $cc_2$ (time of second cell cycle, from 2 to 3 cells), $s_2$ (time of synchrony of the second cell cycle, from 2 to 4 cells).

**Prospective observational study**

A prospective observational study was conducted in unselected patients undergoing IVF at the Reproductive and Genetic Hospital of CITIC Xiangya to validate the effectiveness of the time-lapse criteria established from the immature oocytes. The International Review Board of the hospital approved this study on 16 May 2013 (reference LL-SC-SG-2013-007), and patients signed an informed consent form before their participation. The patients were randomly selected from those with more than four retrieved oocytes between June 2013 and June 2014. Randomization was according to a list generated on www.random.org. The embryo culture and time-lapse imaging procedures were as described above. Embryo transfers were carried out on day 3. Embryos were selected and transferred using a Cook catheter. The luteal phase support included vaginal micronized progesterone (600 mg daily) (Utrogestan; Laboratories Besins International, Paris, France), starting from the day of oocytes retrieval. The primary endpoint was the implantation rate. The implantation of transferred embryos was confirmed at an ultrasound that scanned gestational sacs with a fetal heartbeat at 7 weeks of pregnancy. Only the known implantation data (144 embryos, 70 cycles) were included in the further analysis. The transfer cycles from which embryos were fully implanted (85 embryos, 42 cycles) or that had no implantation (59 embryos, 28 cycles) were included to calculate the implantation rate of embryos from each classification category.

**Statistical analysis**

The Statistical Package for Social Sciences (SPSS) for Windows, version 18.0 (Chicago, IL, USA) was used for data analysis. The rates of good quality blastocyst formation were analysed by partitions of the chi-squared method and linear-by-linear association. Categorical variables were analysed by a chi-squared test, and continuous variables were analysed by t-test if they followed normal distribution. Data that were not normally distributed were tested with a Mann–Whitney–Wilcoxon test. $P < 0.05$ was considered statistically significant.

**Results**

**Abnormal division behaviours in early embryo development**

For the experimental part of the study, a total of 967 immature oocytes from 445 retrieval cycles were collected. The characteristics of the experimental oocytes are presented in Table 1. Of the 345 fertilized dipronuclear (2PN) embryos, the initial three cleavages were focused on and eight major cleavage events defined (Figure 1): (i) normal cleavage (cleavage from one cell to two even blastomeres with produced fragments less than 10%) (Supplementary Video S1); (ii) direct cleavage (direct cleavage from one cell to three or more blastomeres) (Supplementary Video S2); (iii) uneven blastomeres (the average diameter of the large blastomere being over 20% larger than the average diameter of the small blastomere) (Supplementary Video S3); (iv) fragmentation (a daughter blastomere turning into several fragments after division or two daughter blastomeres plus over than 50% scattered fragments) (Supplementary Video S4); (v) big fragment (two daughter blastomeres plus a big fragment or 10–50% scattered fragments after division) (Supplementary Video S5); (vi) distorted cytoplasmic movement (a series of distorted cytoplasm movements during or after cell division and before the embryo enters the quiescent state) (Supplementary Video S6); (vii) developmental arrest (the blastomere does not enter the next cell cycle for division) (Supplementary Video S7); (viii) disordered division (the division of one blastomere in the previous cleavage was delayed after the other blastomere had undergone the next) (Supplementary Video S8). Cleavage patterns ii–viii were seen as abnormal behaviours.

Of the 345 embryos studied, 337 abnormal division behaviours were captured, and, among these behaviours, direct cleavage, fragmentation, big fragment and distorted cytoplasmic movement showed the highest occurrence (18.7%, 17.2%, 19.6% and 27.0% respectively) (Figure 2A). Additionally, these abnormal behaviours primarily occurred during the first and second cleavage (52.5% and 32.6%, respectively) (Figure 2B). In summary, abnormal division behaviours occurred in 72.2% (249/345) of embryos. Only 27.8% (96/345) of the embryos had normal cleavage in all of the initial three cleavages, and 50.4% of the embryos showed one abnormal behaviour in one of the initial three cleavages. The remaining embryos showed one or more abnormal behaviours in one or two cleavages.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of oocyte retrieval cycles</th>
<th>Number of immature oocytes collected</th>
<th>Number of oocytes injected</th>
<th>Fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>445</td>
<td>967</td>
<td>564 (58.3)</td>
<td></td>
</tr>
<tr>
<td>Number of two pronuclei</td>
<td>345 (61.2)</td>
<td>One pronucleus</td>
<td>12 (2.1)</td>
<td>Three pronuclei</td>
</tr>
<tr>
<td>Number of blastocysts</td>
<td>235 (68.1)</td>
<td>Number of good quality blastocysts</td>
<td>134 (38.8)</td>
<td></td>
</tr>
</tbody>
</table>

Variables are presented as number or number and percentage.
Figure 1 Abnormal cleavage during early embryonic development as assessed by time-lapse imaging. Classification of division behaviours occurring in early embryonic development is presented (see definitions in text): (1) normal cleavage; (2) direct cleavage (3); uneven blastomeres (4) fragmentation (5) big fragment (6) distorted cytoplasm movement during cleavage (7) development arrest and (8) disordered division.

Figure 2 The proportion and distribution of abnormal divisions. (A) The relative proportion of the seven abnormal division behaviours; and (B) the distribution of abnormal divisions that occurred during the first, second or third cleavage are presented. BF, big fragment; DA, development arrest; DC, direct cleavage; DD, disordered division; DCM, distorted cytoplasm movement during cleavage; FR, fragmentation; UB, uneven blastomeres.
Effect of division patterns on blastocyst formation

As abnormal division behaviours occurred at such a high frequency in the population of preimplantation embryos, their influence upon embryo developmental potential was investigated by following blastomeres with unusual cleavage behaviour using time-lapse monitoring. For this purpose, embryos that had single abnormal divisions were selected and the resultant daughter blastomeres were followed to identify whether these blastomeres would participate in blastocyst formation (Figure 3A). In cases in which the abnormal division occurred in the first cleavage, then all blastomeres were subsequently followed. In cases where the abnormal division occurred in the second or third cleavages, the daughter blastomeres did not integrate into the late blastocyst formation and the abnormal divisions were considered to have a detrimental effect on blastocyst formation.

Developmental arrest, direct cleavage, disordered division and fragmentation events significantly \( P < 0.001 \) influenced the blastocyst formation rate of daughter cells, such that 100%, 71.1%, 100% and 93.2% of the daughter cells did not form blastocysts. Distorted cytoplasmic movement, uneven blastomeres and big fragment had less effect \( P < 0.001 \) on blastocyst formation, with 3.4%, 18.8% and 20.0% of the daughter cells not forming blastocysts, respectively (Figure 3B). On the basis of this observation, the listed division behaviours were assigned to one of two categories according to their influence on blastocyst formation: category 1 included distorted cytoplasmic movement, uneven blastomeres and big fragment events, and represented the divisions with a low impact on blastocyst formation; and category 2 included developmental arrest, direct cleavage, disordered division and fragmentation events, which had a significant influence on blastocyst formation.

An abnormal event that occurred during the first cleavage affected all blastomeres in an embryo and influenced the development of the whole embryo. Abnormal events occurring during the second cleavage, one (partial) or two (all)

![Figure 3](image.png)
blastomere(s) might be involved. Accordingly, these two situations differed in their power to influence embryo potential. If only one abnormal blastomere was involved, the embryo had another normal cell with some compensatory ability. If both blastomeres were identified as abnormal, the development of the whole embryo was influenced. If an abnormal event occurred during the third cleavage, it generally affected one to two blastomeres (partial), rather than all four blastomeres (all). In this scenario, the remaining cells in the embryo still had compensatory ability to continue further development.

**Hierarchical classification model**

On the basis of observations made about immature oocytes, a hierarchical classification model for D3 embryos was proposed by combining the two division categories and applying them to the initial three cleavages (Figure 4). This model contained the following six categories, denoted A–F: (A) embryos that had normal cleavage in the initial three cleavages; (B) embryos that had category 1 behaviours in all three cleavages; (C) embryos undergoing category 1 behaviours in the initial two cleavages and category 2 behaviours in the third cleavage; (D) embryos that showed category 1 behaviours in the first cleavage and one of the two blastomeres (partial) showed category 2 behaviours in the second cleavage; (E) embryos that showed category 1 behaviours in the first cleavage and two blastomeres (all) showed category 2 behaviours in the second cleavage; and (F) embryos that showed category 2 behaviours in the first cleavage. On the basis of this model, it was observed that from grade A to grade F, the blastocyst formation rate decreased from 94.8% to 21.2% \( (P < 0.001) \) (Figure 4). Similarly, the good-quality blastocyst formation rate decreased from 70.8% to 3.8% across the grades \( (P < 0.001) \) (Figure 4). This indicated that the embryos that did not show any abnormal behaviours in their early development had the highest development potential, and grade F embryos showed the lowest development potential.

As the percentage of good-quality blastocysts decreased from Grade A to F, it was investigated whether the trophectoderm or inner cell mass was preferentially affected by different categories of abnormal behaviour (Figure 5). A total of 52.2% of the poor-quality blastocysts from grade A had a C score for trophoderm cells, which meant that for blastocysts with normal behaviour, trophoderm cells were negatively affected through an unknown mechanism. In embryos with a more severe degree of abnormal cleavage behaviour (including grade B to F), the inner cell mass and the whole blastocyst (inner cell mass plus trophoderm cells) were preferentially affected.

**Division patterns and morphokinetic parameters**

To further understand the influence of abnormal division behaviour upon morphokinetic parameters, three parameters were selected \( (t_5, \text{time from insemination to completion of division to five cells}; \text{cc}_2, \text{time of second cell cycle}; \text{s}_2, \text{time of synchrony of the second cleavage}) \).
Figure 5 The relationship between poor-quality blastocysts between groups showing the factors that affected blastocyst development. Proportion of poor-quality blastocysts showing negative effects in the inner cell mass, trophectoderm, both inner cell mass and trophectoderm or expansion status, as defined in the Materials and Methods, according to division categories A to F. Group A: normal cleavage in all of the initial three cleavages; group B: category 1 behaviours in all of the initial three cleavages except for group A embryos; group C: category 1 behaviours in the initial two cleavages and category 2 behaviours in third cleavage; group D: category 1 behaviours in the first cleavage and one of the two blastomeres (partial) showing category 2 behaviours in the second cleavage; group E: category 1 behaviours in the first cleavage and two blastomeres (all) showing category 2 behaviours in the second cleavage; and group F: category 2 behaviours in the first cleavage.

Embryo hierarchical classification model for D3 embryo selection: a prospective observational study

To assess whether the hierarchical classification model established from the immature oocytes could apply to normal IVF–ICSI embryos and its clinical effectiveness, 1465 ICSI–IVF D3 embryos from 138 patients were analysed. Baseline data for the prospective observational study are listed in Table 2. The mean number of oocytes retrieved per cycle was 16 (range four to 31). Eight cleavage events observed during the development of embryos obtained from matured metaphase I oocytes could also be observed in the development of normal IVF–ICSI embryos. The incidence of A, B, C, D, E and F grades in the total embryo cohort were 29.3%, 25.0%, 12.0%, 14.2%, 4.6% and 14.9%, respectively, which were comparable rates to the incidence of each sub-grade among the embryos derived from mature metaphase I oocytes (27.8%, 29.6%, 10.7%, 11.3%, 5.5%, 15.1%, respectively). The number of cycles with fresh D3 embryo transfer was 121, and the mean number of transferred embryos per cycle was 2 (range 2 to 3). The number of canceled cycles was 18, of which six with no viable embryo for transfer, five with high risk of ovarian hyperstimulation syndrome and seven with thinner endometrium. The total number of transferred embryos was 255, of which 144 either failed to implant (n = 59, from 28 cycles) or were fully implanted (n = 85, from 42 cycles). Only the known implantation data (n = 144, from 70 cycles) was included in the further analysis. The implantation rate of each sub-grade is shown in Table 2. The implantation rate for these grades decreased from 67.0% (Grade A) to 0.0% (Grade D) (P < 0.001).

Table 2 Baseline and cycle characteristics for the prospective observation study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of patients</th>
<th>Maternal age (years)</th>
<th>Number of oocyte retrieval cycles</th>
<th>Number of observed two pronuclei embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Grade B embryos per cycle</td>
<td>2 (0;14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Grade C embryos per cycle</td>
<td>1 (0;6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cycles with fresh day 3 embryo transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Clinical pregnancy rate</td>
<td>76.0% (92/121)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>255</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implantation rate</td>
<td>54.9% (140/255)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cycles (embryos) with known implantation data</td>
<td>70 (144 embryos)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implantation rate of Grade A embryos</td>
<td>67.0% (69/103)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Implantation rate of Grade B embryos</td>
<td>50.0% (14/28)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Implantation rate of Grade C embryos</td>
<td>16.7% (2/12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implantation rate of Grade D embryos</td>
<td>0.0% (0/1)</td>
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</tbody>
</table>

Continuous data are presented as medians and range. Categorical variables are presented as percentage. Grade A: normal cleavage in all of the initial three cleavages; grade B: category 1 behaviours in all of the initial three cleavages except for group A embryos; grade C: category 1 behaviours in the initial two cleavages and category 2 behaviours in third cleavage.

Discussion

In this study, for the experimental part, 72.1% of embryos that had been generated from immature oocytes collected after ovarian hyperstimulation had abnormal division behaviours, and these behaviours primarily occurred during the first and second cleavage. On the basis of this relationship between the types and timing of these abnormal division behaviours during the initial three cleavages, and the blastocyst formation rate of the affected embryos, an embryo hierarchical
classification model was proposed for the improved selection of embryos for IVF and embryo transfer. This model divides normal fertilized embryos into six groups based on the monitoring of embryo cleavage by time-lapse microscopy. This model was then validated in a prospective observational study, which highlighted that it was effective to select D3 embryos with a high implantation potential.

Previous studies have shown that abnormal division behaviours (e.g. direct division from one to three or more cells) may have negative effects on embryonic developmental potential (Hlinka et al., 2012; Meseguer et al., 2011). Meseguer et al. (2011) observed that several abnormal division behaviours, including the direct cleavage from the zygote into a three-blastomere embryo (1C–3C), uneven blastomere size at the two-cell stage, and multinucleation at the four-cell stage, which were detrimental for the further development of cleavage embryos into the blastocyst stage. Similarly, it has been shown that, although some 2C–5C embryos yielded blastocysts, no 1C–3C embryos reached the blastocyst stage (Hlinka et al., 2012). Campbell et al. (2013b) reported no significant difference between aneuploid and euploid embryos in irregular division patterns (direct or rapid division defined as less than 5 h). Furthermore, in bovines, embryos that show direct division from one cell to three or four blastomeres have similar blastocyst formation abilities to those that show normal division, although they do have a higher frequency of chromosome abnormalities (Somfai et al., 2010). Not all types of abnormal division behaviour were systematically analysed, and the consequences of their occurrence during the initial three cleavages were not reported. Our study indicated that some division behaviours, such as developmental arrest, direct cleavage, disordered division and fragmentation, have a significant influence on blastocyst formation rate, consistent with previous research (Aliłak et al., 1999; Athayde et al., 2014; Rubio et al., 2012; Shen et al., 2005). Mechanistically, embryos with direct cleavage had a negative outcome compared with embryos with normal cleavage, possibly caused by the formation of tripolar spindles that may be associated with abnormal distribution of chromosomes to the blastomeres during cell division (Somfai et al., 2010). Additionally, developmental arrest and fragmentation have been correlated with higher reactive oxygen species levels in spent culture media (Lee et al., 2012), and increased risk of chromosomal abnormalities (Munne, 2006), which were negatively correlated with the implantation potential of embryos.

Specific division behaviours occurring during the different cleavage stages had different influences, not only on blastocyst formation but also on blastocyst quality. First, the severe abnormalities occurring during the first and second cleavage, and conferred to all blastomeres in an embryo, had the greatest effect on embryonic developmental potential. Second, abnormalities that were conferred to partial blastomeres in an embryo during the second and third cleavage influenced only part of the embryo, and the rest of the embryo could play a compensatory role in embryonic development based on their pluripotency (Becker and Chung, 2006; Geens et al., 2009; Rossant, 1976). Because of such differences, the cleavage stage at which abnormal behaviour occurred, and the number of blastomeres affected, is an important consideration for the assessment of embryo potential. It is interesting to note that embryos with apparently normal cleavage behaviour also had a 29.2% possibility of not forming a blastocyst, or of forming a poor quality blastocyst. In these embryos, it was primarily the trophectoderm cells that were affected, although the reason for this phenomenon needs further investigation, perhaps aneuploidy played a part in it (Fragouli et al., 2014). Previous studies have shown a close relationship between morphokinetic parameters and blastocyst formation, blastocyst quality, embryo implantation and chromosome composition (Campbell et al., 2013b; Hashimoto et al., 2012; Meseguer et al., 2011; Wong et al., 2010). Embryos with in-range measurements for three morphokinetic parameters (t5, s2 and cc2) had the highest implantation rate whereas those with out-range measurements for three morphokinetic parameters had the lowest implantation rate (Meseguer et al., 2011). In general, in our classification model, embryos with lower degrees of abnormal patterns (Grade A, B and C) had a more concentrated range of morphokinetic parameters (t5, s2 and cc2) and higher developmental potential than embryos with grade D, E and F. These results were consistent with previously published findings (Meseguer et al., 2011), and indicated that a diverse range of morphokinetic parameters might result from abnormal division behaviours. Further investigation of division patterns could reflect the distribution of morphokinetic parameters. It should also be highlighted that embryos with abnormal division may not be detected by assessing the morphokinetic parameters alone. We observed that category 2 behaviours occurred in some embryos with a normal range of some morphokinetic parameters in the first or second cleavages, which significantly influenced the ability for late blastocyst formation. This indicated that the mere analysis of certain morphokinetic parameters may neglect these abnormal behaviours.

As described above, different grade embryos showed a different range of morphokinetic parameters, suggesting that abnormal morphokinetic parameters might be caused by abnormal division patterns, especially direct cleavage. For example, surplus blastomeres shortened the time from insemination to completion of division and disrupted cleavage synchrony and cell cycle. It should also be highlighted that embryos with abnormal division may not be detected by assessing morphokinetic parameters, as abnormal embryos with similar morphokinetic parameters as normally dividing embryos were observed. In general, embryos with high developmental potential had a more concentrated range of morphokinetic parameters than low potential ones, which means that studying division patterns can also reflect the information that studying morphokinetic parameters provides.

In this study, immature metaphase I oocytes were initially used to build the classification model. No agreement has been reached on the optimal incubation time for in-vitro matured metaphase I oocytes to be used. One study reported that, after an additional 6 h of incubation, 67% of metaphase I oocytes could reach the metaphase II stage and had equivalent fertilization and development potential (Strassburger et al., 2004) compared with in-vivo matured oocytes. Longer incubation periods to 24 and 36 h were reported to yield higher maturation rates to above 60% and 80%, respectively (Cekleniak et al., 2001; Chen et al., 2000). Embryos originating from these aged oocytes, however, had increased incidence of abnormal cytoskeletal organization and chromosomal imbalance (Plachot et al., 1988; Racowsky and Kaufman, 1992). Therefore, those oocytes matured within
8 h for study were selected to achieve higher maturation rates and avoid the disadvantages that aged oocytes may bring. To confirm whether the initial observations were representative in mature oocytes from normal IVF–ICSI cycles, a prospective observational study was conducted. No obvious difference was observed in the occurrence of abnormal division behaviours and implantation outcomes, which indicated that the classification model was effective for the selection of D3 embryos with a high implantation potential. A prospective randomized controlled trial will be needed to compare the effectiveness of the established classification model with routine morphological selection procedures.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rbmo.2015.02.008.

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Declaration: The authors report no financial or commercial conflicts of interest.