Analysis of euploidy rates between D5 and D6 embryos by morphology grades and time-lapse algorithms in PGT-A treatments

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Abstract

In conventional in vitro fertilization (IVF) treatments, the euploidy of the embryos is unpredictable before transfer. Previous studies have suggested that parameters including female age, morphological grade, development speed of the embryos and KIDScore™ in time-lapse monitoring (TLM) system are correlated with euploidy of embryos, but their predictive ability remains controversial. In this study, pre-implantation genetic testing for aneuploidy (PGT-A) results were used to detect the euploidy of the embryos, and it was found that the female age, morphological grade, KIDScore™ and development speed of the embryos were correlated with the euploidy of embryos. These results can provide a basis for the selection of euploid embryos in conventional IVF treatments.

Introduction

The appearance of two pronucleus in 16 to 18 hours after fertilization is called normal fertilization. The genetic state of embryos is unpredictable before transfer on the in vitro fertilization or intra-cytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) treatments. Normally fertilized and well-developed embryos are usually selected for transfer in the first place [1]. However, normally fertilized embryos can also have chromosomal abnormalities, which present an increase or decrease in the number of whole chromosomes or fragments, resulting in infertility, recurrent pregnancy loss (RPL) or birth defects after embryo transfer [2]. Pre-implantation genetic testing (PGT) technology based on high-throughput sequencing or microarray is able to detect the genetic status of embryos before transfer, and select the euploid embryos for transfer to reduce the risk of pregnancy [3–5]. In previous studies, it was reported that the euploidy rate of embryos was related to morphology and development speed. These studies showed that the euploidy rate of embryos can predict the clinical pregnancy rate, and the morphological grades of embryos were positively related to live birth rate [6–12].

However, there was a study suggested that the euploidy rate of embryos was related to morphological grades, but not to development speed [13]. In addition, female age is also an important factor in assessing embryo development speed, euploidy and live birth rate [9, 14]. It is still controversial whether embryo development speed and morphological grades can predict euploidy and live birth rate.

Conventional morphological grading method of embryos is limited to observation and evaluation at a certain point in embryo development, and the operation requires the embryo to be exposed to changing environments. Time-lapse monitoring (TLM) system enables continuous evaluation during embryo development, avoids defining the embryo development which is a dynamic process by static observations, and its developmental dynamic parameters provide a basis for predicting the euploidy of the embryos. Meantime, TLM is capable of reducing its exposure time to the changing environments [15, 16]. The model based on the dynamic parameters of embryo development can predict the euploidy of embryos, which can help select embryos with developmental potentiality for transfer in conventional IVF/ICSI treatments [17–19]. In this study, we explored the prediction results of euploidy rate to development speed of embryos, morphological grades, KIDScore™ by TLM, female age in pre-implantation genetic testing for aneuploidy (PGT-A) treatments, so as to provide a basis for embryo selection in conventional IVF/ICSI treatments.

Results

303 PGT-A treatments were performed in 288 patients. 840 blastocysts were successfully tested, including 456 D5 blastocysts, with euploidy rate of 58.33% (266/456) and aneuploidy rate of 29.39% (134/456), and the mosaic rate was 12.28% (56/456). There were 384 D6 blastocysts with euploidy rate of 46.09% (177/384), aneuploidy rate of 41.67% (160/384) and mosaic rate of 12.24% (47/384) (Table 1). The results showed that the euploidy rate of D5 blastocysts was significantly higher than that of D6, and the aneuploidy rate of D5 blastocysts was significantly lower than that of D6 blastocysts (p<0.01) (Figure 1).

Relationship between female age and euploidy of blastocysts

We concluded the female younger than 38 years old into the younger group, and the female equal or greater than 38 years old into the advanced group. The younger group had 184 PGT-A treatments. 585 blastocysts were biopsied, including 332 D5 blastocysts and 253 D6 blastocysts. The euploidy rate of D5 blastocysts was 69.28% (230/332), the aneuploidy rate was 19.28% (64/332), and the mosaic rate was 11.44% (38/332). The euploidy rate of D6 blastocysts was 56.13% (142/253), aneuploidy rate was 31.23% (79/253), and the mosaic rate was 12.28% (32/253). The advanced group had 119 PGT-A treatments. 255 blastocysts were biopsied, including 124 D5 blastocysts and 131 D6 blastocysts. The euploidy rate of D5 blastocysts was 75.00% (95/124), the aneuploidy rate was 26.72% (35/131), the aneuploidy rate was 56.45% (142/253), aneuploidy rate was 41.67% (160/384) and mosaic rate of 12.24% (47/384) (Table 1). The results showed that the euploidy rate of D5 blastocysts was significantly higher than that of D6, and the aneuploidy rate of D5 blastocysts was significantly lower than that of D6 blastocysts (p<0.01) (Figure 1).

Relationship between Gardner grades and euploidy of blastocysts

We divided the morphological grades of blastocysts into four groups according to Gardner blastocyst grading standard: 4AA, 4AB or 4BA, 4BB, 4BC or 4CB. The results showed that in younger group, the euploidy rate of D5 blastocysts graded 4AA was 98.04% (50/51), graded 4AB or 4BA was 68.00% (85/125), graded 4BB was 61.11% (77/126), graded 4BC or 4CB was 60.00% (18/30). The euploidy rate of D6 blastocysts graded 4AA was 92.31% (12/13), graded 4AB or 4BA was 58.82% (40/68), graded 4BB was 48.72% (57/117), graded 4BC or 4CB was 60.00% (33/55). In advanced group, the euploidy rate of D5 blastocysts graded 4AA was 52.17% (12/23), graded 4AB or 4BA was 21.21% (7/33), graded 4BB was 23.21% (13/56), graded 4BC or 4CB was 33.33% (4/12). The euploidy rate of D6 blastocysts graded 4AA was 75.00% (3/4), graded 4AB or 4BA was 26.47% (9/34), graded 4BB was 23.21% (13/56), graded 4BC or 4CB was 27.03% (10/37) (Table 3). The results suggested that the euploidy rate of D5 and D6 blastocysts graded 4AA was the highest in different age groups.

Relationship between KIDScore™ and euploidy of blastocysts

KIDScore™ was obtained for all 840 blastocysts (Table 3), and KIDScore™ of D5 blastocysts was significantly higher than those of D6 (Table 4). In younger group, euploidy of D5 blastocysts was significantly associated with KIDScore™ (r=0.32, p<0.01), and euploidy of D6 blastocysts was significantly associated
with KIDScore™ (r=0.23, p<0.01). In advanced group, euploidy of D5 blastocysts was significantly associated with KIDScore™ (r=0.31, p<0.01) and euploidy of D6 blastocysts was significantly associated with KIDScore™ (r=0.22, p<0.01) (Table 4). The results indicated that euploidy of blastocysts was significantly associated with KIDScore™.

### Prediction of KIDScore™ to euploidy of blastocysts

**Receiver operator characteristic (ROC) curve** was used to assess the predictive values of KIDScore™ and Gardner grades to the euploidy of blastocysts. The area under the curve (AUC) showed the predictive effects of ROC curve. In younger group, KIDScore™ and Gardner grades predicted euploidy of D5 blastocysts with AUC=0.698 and AUC=0.634, respectively, both of which were statistically significant (p<0.01) (Figure 3). KIDScore™ was significantly predictive to euploidy of D6 blastocysts (AUC=0.634, p<0.01), but Gardner grades was not significantly predictive to euploidy of D6 blastocysts (AUC=0.539, p=0.29). In advanced group, KIDScore™ was significantly predictive to euploidy of D5 blastocysts (AUC=0.697, p<0.01), but Gardner grades was not (AUC=0.580, p=0.16) (Figure 4). KIDScore™ was significantly predictive to euploidy of D6 blastocysts (AUC=0.644, p<0.01), but Gardner grade was still not (AUC=0.534, p=0.55).

### Transfer outcomes of blastocysts

We had frozen embryo transfer (FET) in 157 PGT-A treatments (15 for advanced age, 26 for RIF, 78 for RPL, 38 for severe teratospermia). In younger group, the intrauterine pregnancy rate of D5 blastocysts transfer was 80.46% (70/87) and 70.59% of D6 blastocysts (24/34). In advanced group, the intrauterine pregnancy rate of D5 and D6 blastocysts transfer was both 72.22% (13/18) (Table 5). The results indicated that in younger group, KIDScore™ of D5 was significantly higher than that of D6 blastocysts in PGT-A treatments of younger group (p<0.01), and D5 blastocysts resulted in higher intrauterine pregnancy rate than implantation of D6 blastocysts. In advanced group, KIDScore™ of D5 transfer blastocysts was significantly higher than that of D6 blastocysts (p<0.01), but there was no significant difference in intrauterine pregnancy rate between D5 and D6 blastocysts after transfer.

### Discussion

Euploidy of embryos is one of the important factors affecting embryo implantation and development. Embryo chromosome aneuploidy can affect embryo implantation rate, increase abortion rate and the risk of birth defects. In IVF/ICSI treatments, the aneuploidy rate of normal fertilized embryos is 25%-40%, and the rate of chromosomal aneuploidy embryos increases with the female age [20–23]. Therefore, how to select blastocysts as much as possible is very important for conventional IVF treatments.

Euploidy of blastocysts during PGT-A treatments was found to be correlated with female age, development speed, morphological assessment (Gardner grade), and KIDScore™. When the female was younger than 38 years old, the euploidy rate of D5 was significantly higher than that of D6, which may be because the blastocyst development rate of D5 was slower than that of D6, the in vitro culture time was longer, and the possibility of genetic damage increased, leading to the decline of the euploidy rate of D6 [24, 25]. We also found that the euploidy of D5 and D6 in female age equal or greater than 38 years old was significantly lower in that younger than 38 years old. This may be due to the poor egg quality of elderly women, whose euploid rate decreases with the increase of age [9]. In addition, there was no difference in the euploidy rate between D5 and D6 blastocysts in elderly women, suggesting that the embryo development of older females is slower than that of younger females, and some blastocysts with a slower development rate than conventional blastocysts may also have great developmental potential [26]. The previous study had proved that D5 blastocysts in FET may be more tolerant to freezing damage and had a higher developmental potential during vitrification freezing and resuscitation [25]. In this study, the clinical pregnancy rate of euploidy D5 blastocysts was significantly higher than that of D6 in younger group, while there was no significant difference in the advanced group. Therefore, in the conventional IVF treatments, when high-quality blastocysts of both D5 and D6 are available, D5 blastocysts can be preferred for transfer in the first place.

In the conventional IVF treatments, according to the morphological and developmental status of blastocysts, embryologists with rich experience can screen out embryos with poor morphological development that can be seen macroscopically under the microscope at the early stage of embryo development, assisting to improve the euploidy rate and implantation rate. In all PGT-A treatments in this study, the euploidy rate of blastocysts graded as 4AA was the highest, significantly higher than that of blastocysts graded as 4AB or 4BA, 4BB, 4BC or 4CB, which was consistent with previous research results, indicating that the euploidy rate of blastocysts was related to morphology [27]. In addition, because of the subjective nature of Gardner assessment, different embryologists may have different evaluation results. However, we found that in clinical work, graded as 4AA blastocysts were well developed, with large number of full cells, clear boundaries and fully expanded blastocyst cavity, which could be clearly distinguished from other grade blastocysts, the grading error of blastocysts graded as 4AA by different staff was very small. Therefore, blastocysts graded as 4AA have the characteristics of easy grading and high euploidy rate, and can be preferred in conventional IVF-ET treatments in priority.

In a study with an average female age younger than 35 years old, the KIDScore™ of blastocysts between 6.0 and 9.9 had higher euploidy rates than those below 6.0. Several previous studies have recognized the predictive potential of KIDScore™ for euploidy and supported the effectiveness of KIDScore™ for euploidy of blastocysts [17, 18, 28]. Compared with previous studies, the correlation coefficients between KIDScore™ and euploidy of blastocysts in all age groups in this study were smaller (r < 0.4, p < 0.05), and the AUC of KIDScore™ to the euploidy of blastocysts suggested that the predictive effects were moderate in all groups (AUC < 0.7, p < 0.05). This may be due to the limitations of this study and the small sample size included (288 patients, 303 PGT-A treatments, 840 blastocysts). However, we still believe that KIDScore™ is perfectable. It is inadequate for predicting euploidy of blastocysts, requiring further assessing and refinement, and should be insufficient as a decision tool in clinical. In our study, although the KIDScore™ of euploidy blastocysts was significantly higher than that of mosaic and aneuploidy blastocysts (p < 0.01), it could be seen that KIDScore™ of D5 euploid blastocysts was significantly higher than that of D6 euploidy blastocysts (Table 4), and some euploidy and aneuploidy blastocysts had similar KIDScore™. It is less likely to identify a specific KIDScore™ value to effectively predict euploidy of blastocysts based on the available research data (Fig. 5).
The clinical experience from this study is important and suggest that we should pay attention to whether the predictive models are applicable to all patients at different ages and development speed of blastocysts. We think KIDScore™, as one of the reference index for embryo transfer, has predictive potential and can be an assessing model to help doctors design the best embryo transfer strategy in conventional IVF-ET treatments in the future, but large sample size multicenter studies are absolutely needed. It may be an effective method to add in more developmental dynamic parameters and further improve the evaluation system.

Methods

Patients

288 patients with repeated implantation failure (RIF), RPL, severe teratospermia, and advanced age (age is equal or greater than 38 years old and require assisted reproductive technology, (ART)) who received PGT-A treatments in Reproductive Medicine Center of Xuzhou Maternal and Child Health Care Hospital from January 2019 to December 2021 were brought into this study. The diagnostic criterias of PGT-A indications are followed the Expert Consensus on Preimplantation Genetic Diagnosis/Screening in China. All patients had no chromosomal karyotype abnormalities according to International System for Human Cytogenomic Nomenclature (ISCN) 2016. Patients in this study had received detailed genetic counseling and signed relevant informed consents, and this study was approved by the Ethics Committee of Reproductive Medicine of Xuzhou Maternal and Child Health Care Hospital.

Procedure

All procedures are followed Measures for The Administration of Assisted Reproductive Technology and the Expert Consensus on Preimplantation Genetic Diagnosis/Screening in China. Controlled ovarian hyperstimulation (COH) was carried out in patients by long acting or antagonist protocols according to Expert Consensus on Assisted Reproductive Ovulation Drug Therapy in China. Human chorionic gonadotropin (HCG) was injected when the largest diameter size of follicles reached to 18mm or two follicles diameter reached to 16mm. The transvaginal ovum pick-up (OPU) was performed guided by b-ultrasound. ICSI was carried out in all PGT-A treatments. Embryo culture and morphological observation were performed using G-TL culture system (Vitrolife, Sweden) and TLM system (Vitrolife, Sweden). After 5 to 6 days of embryo culture after fertilization, morphological grading of blastocysts was performed (referring to Gardner blastocyst grading standard). Biopsy of blastocysts graded above 4CB or 4BC was performed. PGT test was used on the genetic testing of biopsy products. Euploidy blastocysts according to PGT results were prepared for transfer. Clinical pregnancy was defined as blood HCG>20mIU/ mL 14 days after transfer. Pregnancy was defined when (gestational sac, GS) was able to be detected by b-ultrasound 28 days after blastocyst transfer. At 16-20 weeks of gestation, amniocentesis was performed to analyze fetal chromosome euploidy and karyotype.

Statistics and analysis

SPSS 21.0 software was used for data statistics. T test was used to analyze the significance of the difference among groups. Spearman correlation analysis was used for correlation test. p<0.05 indicated that the difference was statistically significant, while ns indicated that the difference was not significant. GraphPad Prism 8 software was used to plot figures.

Declarations

Data availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

Acknowledgements

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Author contributions statement

X. J. H. and M. Y. conceived the experiments and COH project. Z. Y. Y., X. M. S., and W. Q. Y. conducted OPU, ICSI and embryo culture. M. Y. conducted biopsy of blastocysts. Z. Y. Y. conducted PGT of blastocysts. X. J. H. and W. Q. Y. conducted blastocyst transfer. Z. Y. Y. analyzed the results. All authors reviewed the manuscript.

Competing interests

The authors declared no competing interests.

References


### Tables

**Table 1.** Clinical parameters in this study.
### Table 2. Clinical parameters in different female age groups.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Female age</th>
<th>Male age</th>
<th>Number of patients/PGT treatments</th>
<th>Blastocysts in PGT</th>
<th>D5 blastocysts</th>
<th>D5 euploidy blastocysts</th>
<th>D5 aneuploidy blastocysts</th>
<th>D6 blastocysts</th>
<th>D6 blastocysts in total</th>
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</thead>
<tbody>
<tr>
<td>Younger group</td>
<td>31.96±3.04</td>
<td>33.58±5.28</td>
<td>177/184</td>
<td>585(3.18±2.22)</td>
<td>332(1.80±1.87)</td>
<td>230(1.25±1.40)</td>
<td>64(0.35±0.61)</td>
<td>253(1.38±1.41)</td>
<td>142</td>
</tr>
<tr>
<td>Advanced group</td>
<td>40.61±2.44</td>
<td>42.76±4.41</td>
<td>111/119</td>
<td>255(2.14±1.38)</td>
<td>124(1.04±1.30)</td>
<td>36(0.30±0.63)</td>
<td>70(0.59±0.90)</td>
<td>131(1.10±1.06)</td>
<td>35</td>
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</tbody>
</table>

### Table 3. Gardner grades in different groups.

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<tr>
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<th>4AA grades</th>
<th>4AB or 4BA grades</th>
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<th>4BC or 4CB grades</th>
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<td></td>
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<td>euploidy</td>
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<td>85/1.35±0.68</td>
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<td>19/1.00±0.00</td>
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<td></td>
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<tr>
<td>euploidy</td>
<td>12/1.33±0.71</td>
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<td>40/1.08±0.28</td>
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<td>12/1.00±0.00</td>
<td>7.16±0.61</td>
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<td><strong>Advanced groups</strong></td>
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<tr>
<td>euploidy</td>
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<td>7.59±0.50</td>
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Table 4. Correlations between euploidy and KIDScore™ in different groups.

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<th>p value</th>
<th>correlation of euploidy</th>
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Table 5. Transfer outcomes of blastocysts in this study.

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<th>Analysis of blastocysts transfer outcomes</th>
<th>Female age (mean±SD)</th>
<th>Endometrium thickness</th>
<th>KIDScore™ (mean±SD)</th>
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<th>Intrauterine pregnancy</th>
<th>Live birth</th>
<th>Still in pregnancy</th>
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<td>D5 blastocysts FET</td>
<td>31.45±2.90</td>
<td>9.17±1.06</td>
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<td>87</td>
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Figures

**Figure 1**
Comparison of euploid and aneuploid rates of D5 and D6 blastocysts. X-axis represents euploidy and aneuploidy of D5 and D6 blastocysts. Y-axis represents the median rates of euploidy and aneuploidy.

**Figure 2**
Comparison of euploidy rates between D5 and D6 blastocysts at different age groups. X-axis represents D5 and D6 blastocysts in younger and advanced groups. Y-axis represents median euploidy rates in groups.

**Figure 3**
Predictive effects of Gardner grade and KIDScore™ on euploidy of blastocysts in younger group. The green and red curves represent ROC of KIDScore™ of D5 and D6 blastocysts, while the blue and orange curves represent ROC of Gardner grade of D5 and D6 blastocysts, respectively. The black imaginary line represents standard line. X-axis represents 1-specificity (%), Y-axis represents sensitivity (%).
Figure 4

Predictive effects of Gardner grade and KIDScore™ on euploidy of blastocysts in advanced group. The green and red curves represent ROC of KIDScore™ of D5 and D6 blastocysts, while the blue and orange curves represent ROC of Gardner grade of D5 and D6 blastocysts, respectively. The black imaginary line represents standard line. X-axis represents 1-specificity (%), Y-axis represents sensitivity (%).

Figure 5

Comparison of KIDScore™ of D5 and D6 blastocysts. 5a and 5b represent the comparison in younger group and advanced group, respectively. Others represent the mosaic and aneuploid. X-axis represents euploid and others of D5 and D6 blastocysts. Y-axis represents KIDScore™ of blastocysts in different age groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RawData.xlsx