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Is day 5 blastocyst better than day 6 blastocyst? Evidence from NGS-based PGT-A results

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Research article

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Abstract

Background

Aneuploidy is the principal genetic factor leading to the failure of embryo implantation. For most patients who accept the non-preimplantation genetic testing (PGT) cycle, non-invasive methods to select euploid embryos with the best pregnancy potential are desirable

Methods

This retrospective study recruited women undergoing PGT for aneuploidy (PGT-A) with trophectoderm biopsy from January 2019 to December 2020. The ploidy status of embryos was determined by next generation sequencing (NGS).

Results

Altogether 2531 blastocysts from 839 PGT-A cycles were evaluated. The euploid rate of day 5 blastocysts seemed to be significantly higher than that of day 6 blastocysts, either from the same ovarian stimulation (OS) cycles (49.9% vs 35.7%, $P < 0.001$) or from different OS cycles (48.2% vs 27.8%, $P < 0.001$). Both the younger maternal age (adjusted OR = 0.917, 95% CI: 0.892–0.944, P < 0.001) and day 5 stage (adjusted OR = 1.735, 95% CI: 1.415–2.127, P < 0.001) were independently associated with the greater euploid rate of blastocysts. However, after single euploid embryo transfer, the clinical outcomes of day 5 blastocysts were comparable to those of day 6 blastocysts, no matter whether they were from the same OS cycles or not.

Conclusions

Our results revealed that day 5 blastocysts possess a higher euploid rate than day 6 blastocysts independent of the OS cycles. Giving priority to a day 5 blastocyst over day 6 blastocyst will increase the likelihood to select single euploid embryo for transfer in non-PGT cycles.

Introduction

At 5 or 6 days after fertilization, human embryos reach the blastocyst stage of preimplantation development, and will typically arrive at the uterus to initiate communication with the maternal endometrium [1]. Embryos reaching and surviving from the blastocyst stage are considered to achieve developmental milestones and have an excellent implantation potential. Nowadays, the culture of embryos to the blastocyst stage has become a routine practice during *in vitro* fertilization (IVF). But for patients who embark on a blastocyst transfer cycle, success is still by no means assured.

Aneuploidy has been identified as the principal genetic factor leading to the failure of embryo implantation. Over the past decades, preimplantation genetic testing for aneuploidy (PGT-A) has been frequently carried out for IVF patients, so as to increase the pregnancy rates per embryo transfer and decrease the miscarriage rates by genome-wide technologies. More recently, the increased elective single embryo transfer and reduced time to pregnancy have been added to the PGT-A outcome measures. Currently, the cited indications for PGT-A include advanced maternal age (AMA), recurrent implantation failure (RIF), severe male factor (SMF) and couples with normal karyotypes who have experienced recurrent miscarriage (RM) [2].

However, for most patients who accept the non-PGT IVF cycle, non-invasive methods to select euploid embryos with the best pregnancy potential are desirable. Most centers have evaluated embryos based on morphologic criteria, since the capacity of blastocyst culture cannot eliminate aneuploid embryos. Several studies have shown that day 5 blastocysts transfer achieves higher pregnancy rates than day 6 blastocysts transfer, suggesting the improved viability for faster-developing embryos [3–5]. In this study, the next generation sequencing (NGS)-based PGT-A results were used to compare the ploidy status and clinical outcomes between day 5 blastocysts and day 6 blastocysts. Findings in this study may offer a strategy to rank-order and select embryos that are more likely to result in a vital pregnancy when PGT-A is not used.

Materials And Methods

Study population

This retrospective study was approved by the Shanghai Jiaotong University School of Medicine, Renji Hospital Ethics Committee and performed at the University Center for Reproductive Medicine between January 2019 and December 2020. A total of 2531 blastocysts from 839 PGT-A cycles were identified by NGS. Only cycles that obtained blastocysts for biopsy were included in this analysis. According to the blastocyst stage and ovarian stimulation (OS) cycle, the blastocysts were classified as both day 5 and day 6 blastocysts, only day 5 blastocysts and only day 6 blastocysts groups (Figure 1).

By using the standard G-banding procedures, all participants were identified to have a normal karyotype. For females, the baseline (on days 2-4 of a previous menstrual cycle) serum contents of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and anti-Mullerian hormone (AMH) were measured. For males, the semen samples were evaluated according to the WHO criteria for ejaculate volume, sperm concentration and progressive motility.

OS protocols

OS was performed according to the standard gonadotropin-releasing hormone (GnRH) agonist protocols, GnRH antagonist protocols or mild stimulation protocols, with recombinant follicle stimulating hormone (rFSH) or highly purified human menopausal gonadotropin (HP-HMG) as the stimulation gonadotropin

(Gn). The OS regimens and Gn dosage were selected based on the ovarian reserve parameters and the previous ovarian stimulation, if available.

Follicle development was monitored by ultrasound scans at intervals of 1-3 days till the stimulation day. Simultaneously, the serum contents of E2 and LH were detected. Trigger for final oocyte maturation was achieved by administering GnRH agonist and/or human chorionic gonadotropin (hCG) when the leading follicles reached the mean diameter of 18-20 mm, with the serum E2 level of 200-300 pg/mL per mature follicle (greater than 14 mm). At 33-36 h later, ultrasound-guided oocyte collection was performed.

Ploidy status of blastocysts detected by NGS

Intracytoplasmic sperm injection (ICSI) was applied in all oocytes in the metaphase-II (MII) stage. At 17-20 h later, fertilization was confirmed by the presence of two pronuclei. The embryo cleavage was also evaluated at 41-44 h (day 2) and 65-68 h (day 3) after ICSI, respectively. All embryos were cultured to the blastocyst stage by assisted hatching and scored according to the Gardner grading system [6], from the aspects of degree of expansion, as well as quality of the inner cell mass (ICM) and trophectoderm (TE). TE biopsy was performed on day 5 or day 6 blastocysts with the grade better than 3BB.

Afterwards, the whole-genome amplification (WGA) procedure was performed on all the individual samples. Then, NGS was conducted to directly read the sequenced DNA fragments and their quantification based on the sequence read numbers, in accordance with the Illumina NGS platform protocol. Notably, the ploidy status was reported as “euploid” with the aneuploid percentage under 20%, or “aneuploid” with the aneuploid percentage over 80%, or “mosaicism” with the aneuploid percentage between 20% and 80%.

Clinical outcomes

Single euploid embryo was transferred in each hormone replacement cycle. Luteal support was applied after embryo transfer till 12 weeks of gestation for pregnant women. At 14 days after embryo transfer, pregnancy was checked by the serum b-hCG assay. A biochemical pregnancy was identified when the b-hCG concentration was > 5 mIU/mL. At 4 weeks after embryo transfer, a clinical pregnancy was confirmed by the presence of a gestational sac and heartbeat detected by ultrasonography. Ongoing pregnancy referred to a pregnancy lasting for over 12 weeks of gestation. Miscarriage was defined as fetal loss before 20 weeks of gestation. A live birth was defined as a live infant delivered after 20 weeks of gestation.

Statistical analysis

Quantitative variables among the three study groups were compared by one-way analysis of variance (ANOVA), while those between two groups were compared by post hoc Bonferroni correction. Categorical data were compared by Pearson’s chi-square test or Fisher’s exact test. Multivariable logistic regression analysis was performed to detect the predicting factors for blastocyst formation by day 5 and euploid

blastocyst. Meanwhile, odds ratios (OR) with 95% confidence intervals (CI) were determined and adjusted for female age, AMH, male age and the stage of blastocysts. All statistical analyses were performed by SPSS26.0 (IBM Corporation, USA) and a probability value (*P*-value) < 0.05 indicated statistical significance.

Results

A total of 2531 blastocysts from 839 PGT-A cycles were obtained in the present study. There were 269 PGT-A cycles with both day 5 and day 6 blastocysts, 422 cycles with day 5 blastocysts alone, and 148 cycles with day 6 blastocysts only. The euploid rate of day 5 blastocysts seemed to be significantly higher than that of day 6 blastocysts, either from the same OS cycles (49.9% vs 35.7%, $P < 0.001$) or from different cycles (48.2% vs 27.8%, $P < 0.001$). However, after single euploid embryo transfer, the clinical outcomes, including clinical pregnancy rate, miscarriage rate, ongoing pregnancy rate and live birth rate, were comparable between day 5 and day 6 blastocysts, no matter whether they were from the same OS cycles or not.

Baseline characteristics

The baseline characteristics of PGT-A cycles are shown in Table 1. The ovarian reserve varied significantly among the three groups. Among them, female patients in cycles with day 6 blastocysts only seemed to have the poorest ovarian reserve, with the average age of 36.63 ± 4.81 years and the AMH concentration of 2.33 ± 1.64 ng/mL ($P < 0.001$). Accordingly, the percentage of AMA as the PGT-A indication (35.8%) was the greatest in those cycles, compared with those in the other two groups (26.4% for both day 5 and day 6 blastocysts group, and 26.3% for day 5 blastocysts alone group, $P = 0.02$). Besides, in cycles with only day 6 blastocysts, the average male age was the oldest (38.54 ± 6.30 years), compared with that of 35.11 ± 5.67 years in cycles with both day 5 and day 6 blastocysts, and 36.52 ± 6.15 years in cycles with only day 5 blastocysts ($P < 0.001$). The other basic characteristics regarding female body mass index (BMI), basal FSH, LH, E2 concentration, semen volume, sperm count, total motility, and percentages of progressive and slow progressive spermatozoa were comparable among the three study groups.

As confirmed by the multivariate logistic regression model, women with a higher AMH concentration inclined to have blastocyst formation by day 5 (adjusted OR = 1.130, 95% CI: 1.080-1.182, P<0.001), after adjusting for female age and male age (Table 4).

OS characteristics

As for OS cycles, those with day 6 blastocysts alone mostly received the mild stimulation protocol (43.2%), compared with the other two groups (20.8% for both day 5 and day 6 blastocysts group, 37.9% for only day 5 blastocysts group, $P < 0.001$). Meanwhile, the average stimulation duration was the shortest in those cycles (7.86 ± 1.92 days) relative to the other two groups (8.31 ± 1.13 days for both day 5 and day 6 blastocysts group, 7.97 ± 1.67 days for only day 5 blastocysts group, $P < 0.01$). The initial Gn

dose and total Gn dose were comparable among the three groups. On the stimulation day, cycles with only day 6 blastocysts had the lowest E2 concentration (1647.97 ± 1165.22 pg/mL) compared with the other two groups (2737.98 ± 1709.63 pg/mL for both day 5 and day 6 blastocysts group, 2062.09 ± 1444.06 pg/mL for only day 5 blastocysts group, $P < 0.001$). But the LH concentration (5.23 ± 4.22 IU/L) was the highest in cycles with only day 6 blastocysts, compared with the other two groups (3.87 ± 3.17 IU/L for both day 5 and day 6 blastocysts group, 4.75 ± 3.74 IU/L for only day 5 blastocysts group, $P < 0.001$). Finally, in cycles with both day 5 and day 6 blastocysts, the average numbers of oocytes retrieved, MII oocytes, oocytes fertilized (2PN), fertilized oocytes that cleaved, and blastocysts for biopsy were 12.20 ± 6.69, 10.57 ± 5.98, 8.29 ± 4.79, 8.07 ± 4.64 and 4.31 ± 2.14, respectively; those in cycles with only day 5 blastocysts were 8.81 ± 6.61, 7.41 ± 5.79, 5.72 ± 4.69, 5.62 ± 4.59 and 2.77 ± 2.30, respectively; and those in cycles with only day 6 blastocysts were 6.95 ± 5.95, 5.66 ± 4.50, 4.34 ± 3.48, 4.23 ± 3.36 and 1.41 ± 0.61, respectively. Differences among the three groups were statistically significant ($P < 0.001$) (Table 2).

Outcome comparisons

The comparisons between day 5 and day 6 blastocysts regarding PGT-A outcomes and clinical outcomes are presented in Table 3. It was found that day 5 blastocysts had better euploid status than day 6 blastocysts, no matter whether they were from the same OS cycles or from different cycles ($P < 0.001$). Specifically, the euploid rate, aneuploid rate and mosaic rate of day 5 blastocysts were 49.9%, 31.1% and 19.0%, respectively; while those of day 6 blastocysts from the same OS cycles were 35.7%, 41.3% and 23.0%, respectively. By contrast, the euploid rate, aneuploid rate and mosaic rate of day 5 blastocysts were 48.2%, 37.6% and 14.2%, respectively; while those of day 6 blastocysts from different OS cycles were 27.8%, 51.2% and 21.0%, respectively (Figure 2A). The clinical outcomes were comparable between single day 5 blastocyst transfer and single day 6 blastocyst transfer, either from the same OS cycles or different OS cycles. For single day 5 blastocyst transfer, the clinical pregnancy rate, biochemical pregnancy rate, miscarriage rate, ongoing pregnancy rate and live birth rate were 59%,7.7%, 10.4%, 73.9% and 15.7%, respectively; while those for single day 6 blastocyst transfer from the same OS cycles were 44.2%, 7.0%, 21.0%, 63.2% and 15.8%, respectively. In comparison, the clinical pregnancy rate, biochemical pregnancy rate, miscarriage rate, ongoing pregnancy rate and live birth rate were 62%, 8.1%, 14.4%, 72.5% and 13.1%, respectively, for single day 5 blastocyst transfer; while those for single day 6 blastocyst transfer from different OS cycles were 43.9%, 7.3%, 11.1%, 66.7% and 22.2%, respectively (Figure 2B).

Further, multivariate logistic regression analysis confirmed the higher euploid rate for younger female age (adjusted OR = 0.917, 95% CI: 0.892-0.944, P < 0.001) and day 5 blastocysts (adjusted OR = 1.735, 95% CI: 1.415-2.127, P < 0.001), after adjusting for the significantly different basal characteristics (Table 4).

Discussion

To the best of our knowledge, this retrospective study is the first to compare the ploidy status and clinical outcomes of blastocysts from the perspectives of development stage and OS cycles produced by NGS-based PGT-A. According to our results, the stage of blastocyst was correlated with its ploidy status. The euploid rate of day 5 blastocysts was significantly higher than that of day 6 blastocysts, no matter whether they were from the same OS cycles or not. Giving priority to a day 5 blastocyst over day 6 blastocyst will increase the likelihood to select single euploid embryo for transfer in non-PGT cycles. Women with poor ovarian reserve incline to obtain only day 6 blastocysts, and PGT-A can help to increase the pregnancy rate per embryo transfer by identifying the ploidy status of embryos, especially for those with advanced age. However, the stage of blastocysts will not affect the clinical outcome after transfer when they are identified as euploidy.

Embryos in the blastocyst stage have succeeded in activating their genome and differentiating into two cell types, namely, TE and ICM. The average time to an expanded euploid blastocyst is 111–121 h after insemination [7]. Aneuploidy of post-zygotic origin primarily occurs during the cleavage divisions [8]. Slower blastocyst development may be caused by the delayed timing of the first cleavage division, subsequent cleavage divisions or abnormal early cleavage events. These events may affect the complex transition of embryonic genome activation, which then shifts gene transcription from the maternal to the embryonic genome, finally leading to differentiation of ICM and TE, as well as genetic mosaicism or aneuploidy [9–11]. In other words, aneuploid embryos may progress through blastocyst development at a slower rate. This study examined a large cohort of blastocysts and demonstrated that day 5 blastocysts were associated with greater euploidy. This significant relationship between the stage of blastocyst and euploid rate was still observed in cycles with both day 5 and day 6 blastocysts available for biopsy, demonstrating that this relationship was independent of the variability of OS cycles. Our results were consistent with other studies [12, 13] evaluating the blastocyst development stage and embryonic euploidy by using the high-density oligonucleotide microarray comparative genomic hybridization (aCGH) platform. These results are also encouraging for counseling patients undergoing non-PGT cycles. Compared with day 6 blastocysts, day 5 blastocysts possess a higher possibility to be euploidy and deserve to be transferred in the non-PGT cycles.

Although the aim of this study was not to evaluate the predicting factors for blastocyst formation time, the supplementary logistic regression analysis was conducted to identify the association of baseline characteristics of couples with blastocyst stage timing. Our data indicated that the timing of blastocyst might occur later in patients with poor ovarian reserve, as AMH was identified as a predicting factor for blastocyst stage. Previous study [14] also reveals that the patient-related factors may affect blastocyst stage timing and individual embryo timing resulting from a combination of clustering effects of each patient. Different from many other studies [4, 5, 15, 16], this work did not reveal a better clinical outcome of single day 5 blastocyst transfer than single day 6 blastocyst transfer when they were both identified as euploidy. This discrepancy might be dedicated to the higher euploid rate of day 5 blastocysts and the research subjects of all non-PGT cycles in their studies.

Unlike other research, the combination of morphological grading with the stage of blastocysts was not taken to associate with their ploidy status. Firstly, only blastocysts eligible for freezing were biopsied according to our local standard procedure in a developing country. Secondly, it is widely acknowledged that the microscopic appearance of an embryo is only weakly correlated with its viability. Thirdly, a study enrolling a large cohort of 1213 embryos confirms that chromosomal abnormalities have little if any effect on morphological scores in the cleavage stage, and some forms of aneuploidy begin to affect microscopic appearance, but in most cases, the impact is subtle in the blastocyst stage [17]. Notably, our study supported a higher possibility of embryo euploidy with day 5 blastocysts, and the stage of blastocyst was by no means indicative of euploid status in the absence of PGT-A cycles. For women with poor ovarian reserve, especially those in advanced age who inclined to had day 6 blastocysts, PGT-A was a better choice for identifying euploid blastocyst for transfer.

In conclusion, the euploid rate of day 5 blastocysts is significantly higher than that of day 6 blastocysts, no matter whether they are from the same OS cycles or not. In non-PGT cycles, our data support the selection of day 5 blastocysts for transfer over day 6 blastocysts. However, the clinical outcomes are comparable between day 5 blastocysts and day 6 blastocysts after single euploid embryo transfer.

Declarations

Funding No funds, grants, or other support was received.

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval The present study was consistent with the Declaration of Helsinki and received approval from the Ethics Committee of Renji Hospital, Shanghai Jiaotong University School of Medicine. All patients provided written informed consent.

Author contribution statement

J T analyzed data and wrote the paper. Y N and A W collected the data. T Z conceived the study and revised the paper. All authors reviewed the results and approved the final version of the manuscript.

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Tables

Table 1 Comparison of basal characteristics in PGT-A cycles which had biopsy on both day 5 and day 6 blastocyst, only day 5 blastocyst and only day 6 blastocyst.

Parameters	Both day 5 and day 6 blastocyst	Only day 5 blastocyst	Only day 6 blastocyst	*P* value
No. of cycles	269	422	148	NA
Female Age, yr	33.67 ± 4.69	34.93 ± 4.89	36.63 ± 4.81	<0.001, 0.011[a], <0.001[b], 0.010[c]
Female BMI, kg/m^2	21.86 ± 2.86	22.21 ± 7.79	21.99 ± 2.76	NS
AMH concentration, ng/mL	3.56 ± 2.47	3.28 ± 2.43	2.33 ± 1.64	<0.001, <0.001[b], 0.005[c]
Basal FSH concentration, IU/L	6.54 ± 4.65	6.77 ± 2.84	7.39 ± 3.39	NS
Basal LH concentration, IU/L	4.82 ± 3.02	5.40 ± 4.29	4.44 ± 3.37	NS
Basal E_2 concentration, pg/mL	42.58 ± 28.01	44.15 ±35.86	50.20 ± 40.30	NS
Male age, yr	35.11 ± 5.67	36.52 ± 6.15	38.54 ± 6.30	<0.001, 0.029[a], <0.001[b], 0.016[c]
Semen volume, ml	2.65 ± 1.34	2.48 ± 1.13	2.71 ± 1.49	NS
Sperm count, M/mL	61.64 ± 39.48	55.65 ± 37.27	63.19 ± 40.60	NS
Total motility, %	40.72 ± 16.90	40.51 ± 18.00	39.13 ± 17.16	NS
Progressive spermatozoa, %	33.43 ± 15.67	32.91 ± 16.22	30.74 ± 14.69	NS
Slow progressive spermatozoa, %	7.94 ± 3.07	7.88 ± 3.62	8.17 ± 4.11	NS
Indication for PGT-A				0.02
RM, % (n)	49.4 (133/269)	41.2 (174/422)	35.8 (53/148)	
AMA, % (n)	26.4 (71/269)	26.3 (111/422)	35.8 (53/148)	
RIF, % (n)	16.8 (45/269)	25.9 (109/422)	20.9 (31/148)	
Severe teratozoospermia, % (n)	7.4 (20/269)	6.6 (28/422)	7.5 (11/148)	

Values are presented as mean ± standard deviation (SD) or number (%).

NA: not applicable*; NS*: not statistically significantly different; *BMI*, body mass index; *AMH*, anti-Mullerian hormone; *FSH*, follicle stimulating hormone; *LH*, luteinizing hormone; E_2, estradiol; *RM*, recurrent miscarriage; *AMA*, advanced maternal age; *RIF*, recurrent implantation failure.

[a]: comparison between PGT-A cycles which had biopsy on both day 5 and day 6 blastocyst and which had only day 5 blastocyst; [b]: comparison between PGT-A cycles which had biopsy on both day 5 and day 6 blastocyst and which had only day 6 blastocyst; [c]: comparison between PGT-A cycles which had biopsy on only day 5 blastocyst and which had only day 6 blastocyst.

Table 2 Comparison of ovarian stimulation characteristics in PGT-A cycles which had biopsy on both day 5 and day 6 blastocysts, only day 5 blastocysts and only day 6 blastocysts.

Parameters	Both day 5 and day 6 blastocysts (n=269)	Only day 5 blastocysts (n=422)	Only day 6 blastocysts (n=148)	*P* value
Ovarian stimulation protocol				<0.001
Agonist protocol, % (n)	40.5 (109/269)	27.5 (116/422)	37.8 (56/148)	
Antagonist protocol, % (n)	38.7 (104/269)	34.6 (146/422)	18.9 (28/148)	
Mild stimulation, % (n)	20.8 (56/269)	37.9 (160/422)	43.2 (64/148)	
Initial Gn dose, IU	138.20 ± 80.99	146.61 ± 92.06	137.85 ± 77.25	NS
Total Gn dose, IU	1084.46 ± 731.84	1104.98 ± 703.19	1124.83 ± 766.37	NS
Duration of stimulation, day	8.31 ± 1.13	7.97 ± 1.67	7.86 ± 1.92	<0.01, 0.004[a], 0.028[b]
E_2 concentration on the stimulation day, pg/mL	2737.98 ± 1709.63	2062.09 ± 1444.06	1647.97 ± 1165.22	<0.001, <0.001[a], <0.001[b], 0.002[c]
LH concentration on the stimulation day, IU/L	3.87 ± 3.17	4.75 ± 3.74	5.23 ± 4.22	<0.001, 0.004[a], 0.003[b]
No. of oocytes retrieved	12.20 ± 6.69	8.81 ± 6.61	6.95 ± 5.95	<0.001, <0.001[a], <0.001[b], 0.005[c]
No. of MII oocytes	10.57 ± 5.98	7.41 ± 5.79	5.66 ± 4.50	<0.001, <0.001[a], <0.001[b], 0.004[c],
No. of oocytes fertilized (2PN)	8.29 ± 4.79	5.72 ± 4.69	4.34 ± 3.48	<0.001, <0.001[a], <0.001[b], 0.001[c]
No. of fertilized oocytes that cleaved	8.07 ± 4.64	5.62 ± 4.59	4.23 ± 3.36	<0.001, <0.001[a], <0.001[b], <0.001[c]
No. of blastocysts for biopsy	4.31 ± 2.14	2.77 ± 2.30	1.41 ± 0.61	<0.001, <0.001[a], <0.001[b], <0.001[c]

Values are presented as mean ± standard deviation (SD) or number (%).

NS: not statistically significantly different; Gn: gonadotropin; MII: metaphase-II stage.

[a]: comparison between PGT-A cycles which had biopsy on both day 5 and day 6 blastocyst and which had only day 5 blastocyst; [b]: comparison between PGT-A cycles which had biopsy on both day 5 and day 6 blastocyst and which had only day 6 blastocyst; [c]: comparison between PGT-A cycles which had biopsy on only day 5 blastocyst and which had only day 6 blastocyst.

Table 3 Comparisons of day 5 and day 6 blastocyst regarding PGT-A outcomes and clinical outcomes

	Day 5 blastocyst	Day 6 blastocyst	*P* value	Day 5 blastocyst	Day 6 blastocyst	*P* value
In the same OS cycle	Yes	Yes		No	No	
PGT-A results			< 0.001			< 0.001
Euploidy, % (n)	49.9 (357/716)	35.7 (158/443)		48.2 (561/1163)	27.8 (58/209)	
Aneuploidy, % (n)	31.1 (223/716)	41.3 (183/443)		37.6 (437/1163)	51.2 (107/209)	
Mosaicism, % (n)	19.0 (136/716)	23.0 (102/443)		14.2 (165/1163)	21.0 (44/209)	
Clinical outcomes			NS			NS
Clinical pregnancy rate per transfer, % (n)	59.0 (115/195)	44.2 (19/43)		62.0 (160/258)	43.9 (18/41)	
Biochemical pregnancy rate per transfer, % (n)	7.7 (15/195)	7.0 (3/43)		8.1 (21/258)	7.3 (3/41)	
Miscarriage rate per transfer, % (n)	10.4 (12/115)	21.0 (4/19)		14.4 (23/160)	11.1 (2/18)	
Ongoing pregnancy rate per transfer, % (n)	73.9 (85/115)	63.2 (12/19)		72.5 (116/160)	66.7 (12/18)	
Live birth rate per transfer, % (n)	15.7 (18/115)	15.8 (3/19)		13.1 (21/160)	22.2 (4/18)	

OS: ovarian stimulation; *NS*: not statistically significantly different.

Table 4 Multivariate model for predictors of blastocyst formation by day 5 and euploid blastocyst

Variables	Blastocyst formation by day 5		Euploidy blastocyst	
	Adjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Female age	0.978 (0.949-1.008)	NS	0.917 (0.892-0.944)	<0.001
AMH	1.130 (1.080-1.182)	<0.001	1.024 (0.989-1.060)	NS
Male age	0.992 (0.968-1.016)	NS	0.990 (0.968-1.012)	NS
Day 5 stage	NA	NA	1.735 (1.415-2.127)	<0.001
Day 6 stage	NA	NA	Reference	Reference

NS, not statistically significantly different.; *NA,* not applicable.

Figures

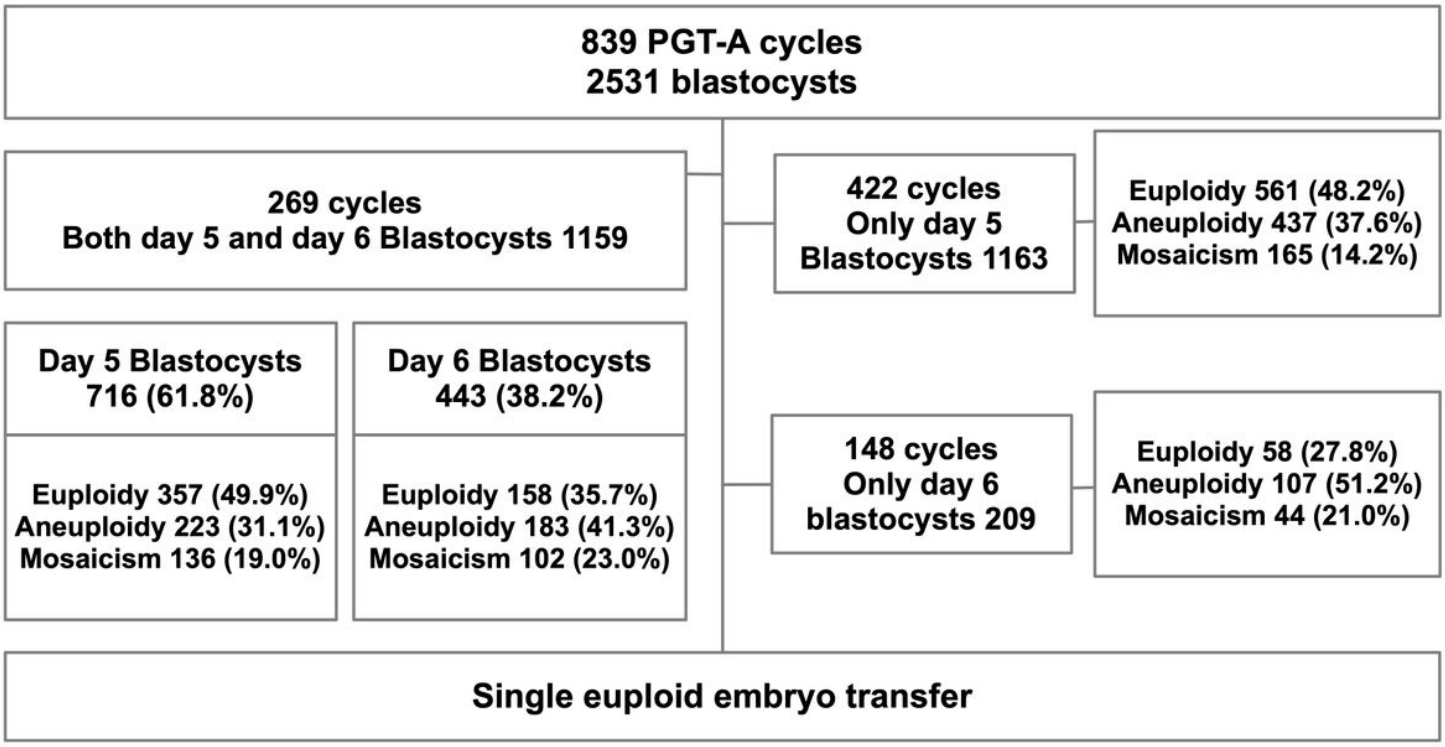

Figure 1

Diagram of study design. PGT-A cycles were grouped based on the blastocyst stage and the OS cycles.

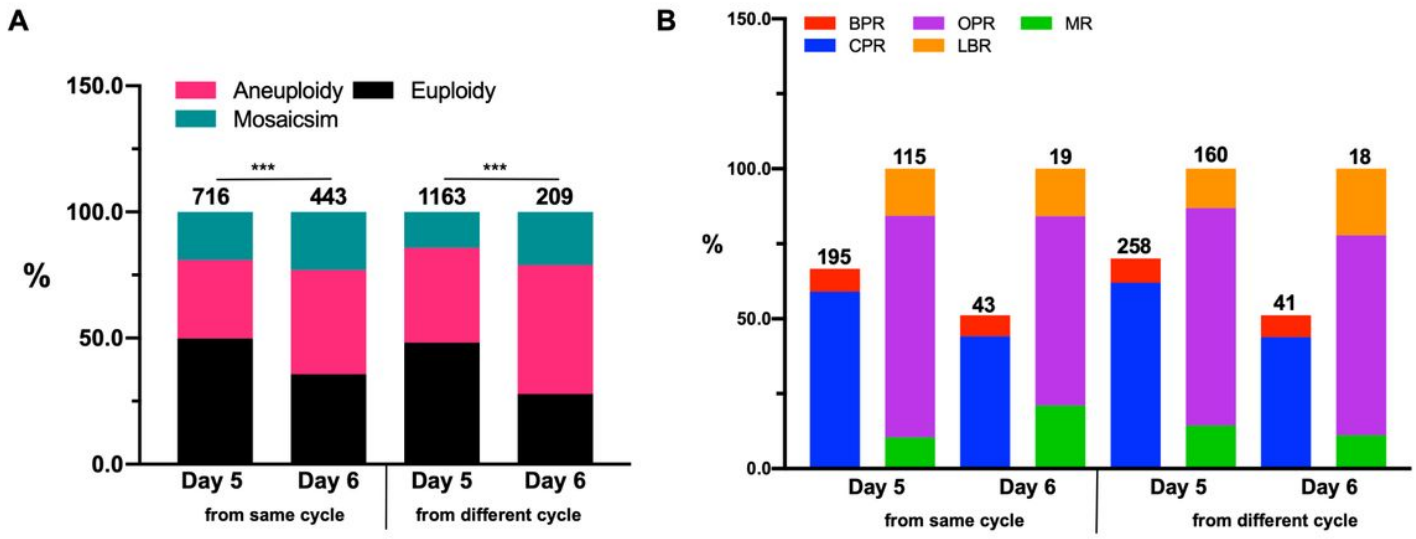

Figure 2

Distribution of PGT-A and clinical outcomes of human blastocysts according to the blastocyst stage and OS cycles. (A) Ploidy status of blastocysts: The number of blastocysts tested by NGS is displayed on the top of the bars. ***, P < 0.001. (B) Clinical outcomes of blastocyst transfers: CPR, clinical pregnancy rate per transfer; BPR, biochemical pregnancy rate per transfer; MR, miscarriage rate per transfer; OPR, ongoing pregnancy rate per transfer; LBR, live birth rate per transfer. The numbers of single euploid blastocyst transfer cycles and clinical pregnancies are presented on the top of the bars.

Supplementary Files

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

- IRBtranslationcopy.docx
- IRB.pdf