Association between growth dynamics, morphological parameters, the chromosomal status of the blastocysts, and clinical outcomes in IVF PGS cycles with single embryo transfer

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Abstract

Purpose The purpose of the present study is to examine interconnection between speed of embryo development, the genetic status of the blastocysts, and clinical outcomes in IVF preimplantation genetic screening (PGS) cycles with single embryo transfer (SET).

Methods The retrospective comparative study has been performed between January 2013 and January 2016. Seven hundred thirty-seven cycles of IVF treatment with PGS, followed by 503 SETs, were included in the study. Normally fertilized oocytes were hatched on day 3, were cultured to the blastocyst stage, and were biopsied only when at least three to seven cells were herniating from zona pellucida on the morning of day 5 (≤ 118 h) or day 6 (≥ 139 h). A total of 3705 embryos were analyzed for euploidy rates and blastocyst morphology. All embryos were vitrified after the biopsy, and selected embryos were subsequently thawed for a hormone replacement frozen embryo transfer cycle.

Results The euploidy rate was significantly higher among embryos biopsied on day 5 versus day 6: 59.44 ± 4.1 and 48.19 ± 3.8 , respectively, $p < 0.05$. The difference in euploidy rates between embryos biopsied on day 5 versus day 6 in matched age groups increased from 5.83 to 25.46% with advancing maternal age. Our data demonstrated no statistically significant difference in euploidy rates between good-quality embryos biopsied on day 5 in the group of patients < 38 years old and embryos in PGS cycles using donor oocytes: 71.12%

(336/472) and 75.68% (221/292), respectively, $p = 0.174$, $\chi^2 = 1.848$. In 270 out of 503 SETs, transferred embryos were biopsied on day 5 (ongoing pregnancy rate was 64.6% in a group of patients < 38 years old, and in a group of patients ≥ 38 years old, ongoing PR was 64.2%). In 233 out of 503 cycles, transferred embryos were biopsied on day 6 (ongoing PR was 46.6% in a group of patients < 38 years old, and in a group of patients ≥ 38 years old, ongoing PR was 50.8%). In all study groups, the ongoing pregnancy rate was higher when the transferred embryo was available for biopsy on day 5.

Conclusions Good- and fair-quality embryos available for biopsy on day 5 have higher euploidy rates and have a higher chance to result in an ongoing pregnancy. Euploidy rate has significant variations within the same age group depending on the morphology of the blastocysts.

Keywords Preimplantation genetic screening · IVF · CCS · Euploidy rates · Blastocyst morphology · Blastocyst biopsy · Embryo development

Introduction

Comprehensive chromosomal screening has become a routine test in many IVF practices around the globe. Reliable information about euploidy status of the embryos has created a solid background for significant improvements in IVF treatment outcomes, especially for the older patient population [1].

Genome activation and unfolding of genetic information in the human preimplantation embryo are regulated by many internal [2–4] and external [5, 6] factors. The cumulative effect of these factors can be evaluated in the embryology laboratory by assessing embryo morphology and the dynamics of embryo development. The morphokinetic and lineage marker

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expression analyses recently presented by Noli [7] suggested that the earliest stages of human development are under strict temporal control.

It has previously been shown by FISH-based preimplantation genetic screening (PGS) that cleavage stage embryos with a faster speed of development are more likely to have various genetic abnormalities [8, 9]. While data about timing of blastocyst development and genetic status of the embryos are limited and fragmented [10, 11], several studies reported higher clinical pregnancy rates in non-PGS cases where blastocysts with faster development were selected for embryo transfer [12, 13].

In some IVF units, clinical pregnancy rates in non-PGS cases with single embryo transfer in a group of patients 35–38 years old are higher than the average euploidy rates for this age group. This fact suggests a preferential selection of euploid embryos based solely on the morphology [14]. Positive correlation between the number of cells in the blastocysts and clinical outcomes has been well documented and published previously [15, 16].

Some studies have indicated that embryos able to proceed through cell cycle checkpoints without any delays are more likely to complete mitotic divisions promptly and become expanded or hatching blastocysts on the morning of day 5 (~116 h) [17–19]. In many PGS cases, patients have euploid embryos with similar morphological characteristics, but embryos become available for biopsy (and are subsequently frozen) at a different time: most frequently on day 5 or day 6 or even on day 7 [20–22]. Analysis of our data suggests that in order to optimize clinical outcomes in PGS cycles, another variable should be considered in addition to the embryo morphology. This variable is the time when a particular euploid embryo became available for the biopsy. The correlation between dynamics of embryo development, the chromosomal status of the blastocysts, and clinical outcomes in the PGS cycle was a primary focus of this paper.

Materials and methods

A retrospective comparative study was performed between January 2013 and January 2016. Seven hundred thirty-seven cycles of IVF treatment with PGS (638 patients) were included in the study. After comprehensive chromosomal testing, 212 patients had only aneuploid embryos available. Four hundred twenty-six patients had at least one euploid embryo and had a total of 503 single embryo transfers.

Preimplantation genetic diagnosis cases for single gene disorder, translocation (balanced and unbalanced), and gender selection were excluded from the study.

Embryos were cultured in MINC benchtop incubators at 37 °C in low-oxygen culture system in a humidified atmosphere (7% CO₂ and 5% O₂, 88% N₂). Quinn's Advantage

sequential culture media supplemented with 10% Serum Protein Substitute under mineral oil was used according to the manufacturer's recommendations (Sage In Vitro Fertilization, Inc., USA).

All embryos were hatched on day 3 (~70 h post insemination) by a series of three to five laser pulses (200 µs at 100% power) in order to facilitate herniation of the trophectoderm. Only good- and fair-quality blastocysts that had at least three to seven herniating cells were considered for biopsy. Embryos that did not develop at least three to seven herniating cells by 10 a.m. on day 5 were cultured to day 6 and biopsied on day 6 if the criteria were met. The biopsy procedure was performed in Washing mHTF w/ HEPES (Life Global, USA) in 25 µl drops under oil in Falcon 351006 dishes (50 × 9 mm) on the heated stage of Olympus IX 71 microscope equipped with Narishige micromanipulator (MM-91) and 1460 nm, 300 mW Class 1 laser (Hamilton Thorne, Lykos).

A total of 3720 embryos were analyzed for euploidy rates and blastocyst morphology: 1522 embryos were biopsied on day 5, and 2198 embryos were biopsied on day 6. After genetic analysis, 15 embryos (0.04%) were reported as “no result” and were excluded from the study.

Morphology of blastocysts was evaluated independently by two embryologists using Gardner classification [23] before trophectoderm biopsy. Embryos were divided into six groups based on blastocyst expansion/blastocoel size and into four groups based on blastocyst quality: good (AA/AB/BA, a total of 1487 embryos)-, fair (BB, a total of 1331 embryos)-, borderline fair (B-/B-, a total of 815 embryos)-, and poor-quality embryos (C/C-, a total of 72 embryos). Poor-quality blastocysts were biopsied as exceptions only per MD or patient request and were also included in the study (19 of them had a blastocoel cavity of less than half of total blastocyst volume and were biopsied without consideration for subsequent clinical use). Poor-quality blastocysts were included in the analysis of the association between embryo morphology and euploidy rates and were excluded from the analysis of the association between embryo morphology and ongoing pregnancy rates due to small sample size (only 2 SETs).

All embryos were vitrified after the biopsy on day 5 or day 6 of embryo development (Irvine Scientific Vitrification kit), and selected embryos were subsequently thawed for a hormone replacement frozen embryo transfer cycle. Embryos that did not meet previously mentioned criteria for biopsy on day 6 of embryo development were discarded.

Euploidy rates were assessed in each study group using SNP (Illumina HumanCytoSNP-12 DNA BeadChips in combination with an informatics-based algorithm, Parental Support™, Natera Inc.).

Chi-squared analysis was used to assess the difference in categorical values. Univariate logistic regression analysis was

used to evaluate the association between pregnancy rates and maternal age. Continuous values were presented as means with standard deviation. The statistical analysis was performed using R statistical software version 3.3.1, The R Foundation for Statistical Computing. A p value of <0.05 was considered as statistically significant.

Results

A total of 12,603 oocytes (17.1 ± 8.53 per cycle) were retrieved, and 9728 MII oocytes (13.2 ± 6.74 per cycle) were inseminated by intracytoplasmic sperm injection (ICSI). A total of 7989 (10.84 ± 4.82 per cycle) zygotes were obtained and cultured to the blastocyst stage.

The average maternal age of patients included in the study was 37.48 ± 4.26 . Four hundred one cases out of 737 (54.4%) represent an older patient population (≥ 38 years old). We observed a strong correlation between euploidy rates and maternal age. Euploidy rates decreased gradually with advancing maternal age: 62.57% in a group of patients <35 years old, 55.29% in a group of patients 35–37 years old, 41.84% in a group of patients 38–40, and 28.99% in a group of patients ≥ 41 years old.

There were 70 patients (average age 38.23 ± 4.12) who had two or more IVF PGS cycles during the study period (a total of 144 cycles). Only 22 out of these 70 patients (31.4%) had different embryo development patterns in subsequent IVF cycle.

PGS cycles with donor eggs represent 4.89% (36/737) of PGS cycles in this study. At the same time, these cycles may be considered as an ideal benchmark to assess embryo morphology, blastulation, and euploidy rates of young, presumably fertile women and to compare these parameters with analogous parameters of patients going through IVF treatment using autologous eggs.

The blastulation rate (number of embryos available for biopsy divided by number of zygotes) in PGS cycles with donor eggs was 55.95%, while in autologous cycles with PGS, it decreased with advancing maternal age from 59.42% in a group of patients <35 years old to 30.36% in a group of patients ≥ 45 years old.

PGS cases with donor eggs have not only the highest euploidy rate—75.68% (221/292)—but also highest proportion of embryos available for the biopsy on day 5. In PGS cases with donor eggs, 49.65% of the embryos become available for the biopsy on day 5. In PGS cases with autologous eggs in the younger patient group (<35 years old), the ratio of embryos biopsied on day 5 was similar to cycles with donor eggs. The majority of the patients under 35 years old (115/168) had primary infertility diagnosis not related to embryo development or genetic aberrations: ovulation disorder, endometriosis, uterine factor, and tubal ligation.

In our study, in a group of patients <35 years old, 44.9% of all embryos were biopsied on day 5 and 55.1% of embryos were biopsied on day 6. With advancing maternal age, this proportion gradually changed and the majority of embryos become available for the biopsy on day 6. In a group of patients ≥ 45 years old, 85.1% of all embryos were delayed and become available for the biopsy on day 6 (Fig. 1).

Overall, the euploidy rate was significantly higher for embryos biopsied on day 5 than on day 6—59.44 and 48.19%, respectively, $p < 0.05$. Moreover, the difference in euploidy rates between embryos biopsied on day 5 vs day 6 was detected in all matched age groups and had the tendency to increase with advancing maternal age from 5.83% in a group of patients <35 years old to 25.46% in a group of patients ≥ 45 years old. The difference in euploidy rates was statistically significant in all SART age groups ($\chi^2 \geq 4.6463$, $p < 0.05$), except IVF cycles where donor eggs were used ($\chi^2 = 1.345$, $p = 0.2455$).

Biological aging of the patient was reflected not only in decreasing euploidy rates, but also in the timing of embryo development. With advancing maternal age, embryo development slowed: blastulation rates and proportion of embryos available for the biopsy on day 5 decreased. The combined effect of mentioned earlier factors is manifested in a significant increase in the proportion of cycles in which no embryos were available for the biopsy on day 5: from 19.59% in a group of patients <35 years old to 60.34% in a group of patients ≥ 41 years old. Nevertheless, this study demonstrated that patients <38 years old with the current stimulation protocols can expect to have on average at least one euploid embryo biopsied on day 5 in each IVF cycle.

Comparison of blastocyst morphology and euploidy rates of embryos biopsied on day 5 versus day 6

We observed that regardless of patient age, the proportion of good-, fair-, borderline fair-, and poor-quality embryos remained stable with a slight decrease in the number of good-quality embryos with advancing maternal age (linear regression coefficients: $y = -0.008x + 0.4315$). In all age groups, over half of all embryos biopsied on day 5 met the criteria for good-quality embryos. On the other hand, the significantly lower number of embryos that became available for the biopsy on day 6 was graded as good-quality embryos (Fig. 2).

Our data demonstrated a statistically significant difference ($\chi^2 = 9.78$, $p < 0.05$) in euploidy rates between good-, fair-, borderline fair-, and poor-quality embryos in all age groups. The biggest difference in euploidy rates between

Fig. 1 Distribution of embryos for biopsy (left) and comparison of euploidy rates (right) between embryos biopsied on day 5 and day 6 in matched age groups

embryos with different morphology was identified among young (<38 years old) patients: the euploidy rate for good-quality embryos was 69.92% (544/778); for fair-quality embryos, 57.23% (380/664); for borderline fair, 47.48% (198/417); and for poor-quality embryos, 35.29% (12/34) (Fig. 3).

Despite the fact that the proportion of good-quality embryos remained stable with advancing maternal age, euploidy rates among good-quality embryos decreased from 80.30% in donor cycles to 22.22% for patients ≥ 45 years old. The significant decrease in euploidy rates with advancing maternal age considerably reduces the predictive value of embryo morphology in a group of patients ≥ 38 years old.

The difference in euploidy rates between embryos with different morphological characteristics had a tendency to decrease with advancing maternal age. The difference in euploidy rates between good and poor embryos decreased from 47.0% in a group of patients <35 years old to 22.2% in a group of patients ≥ 45 years old.

Our study demonstrated an association of euploidy rates with morphological characteristics of blastocysts. Embryos with an advanced degree of blastocyst expansion, well-formed inner cell mass/trophoblast had higher chances of being euploid ($\chi^2 = 10.73$, $p < 0.05$ and $\chi^2 = 4.34$, $p < 0.05$, respectively) (Table 1).

This association was consistent for embryos that were available for the biopsy on day 5 as well as for embryos that were available for the biopsy on day 6. In a group of embryos biopsied on day 6, the decrease in euploidy rates is

significantly higher for embryos of borderline fair (B-/B) and poor (C-/C) quality.

As described earlier in this paper, embryos available for the biopsy on day 5 had higher euploidy rates than embryos available for the biopsy on day 6 in all age groups. In addition, good-quality embryos had higher euploidy rates in all age groups. The combination of these two factors resulted in significantly higher euploidy rates among good-quality embryos available for the biopsy on day 5 of embryo development. This study offers evidence that good-quality embryos from patients <38 years old have euploidy rates similar to euploidy rates in IVF cycles with donor eggs. Our data demonstrated no statistically significant difference in euploidy rates between good-quality embryos biopsied on day 5 in the group of patients <38 years old (mean age 33.32 ± 2.63) and embryos in IVF cycles using donor oocytes (mean age 25.75 ± 3.65): 71.19% (336/472) and 75.68% (221/292), respectively, $\chi^2 = 1.848$, $p = 0.174$ (Fig. 3).

Clinical pregnancy rates after SET of day 5 versus day 6 euploid embryo

Analysis of the data shows a statistically significant difference in ongoing pregnancy rates after single embryo transfer (SET) between embryos biopsied on day 5 versus day 6: 64.4% (174/270) and 48.9% (114/233), respectively, $\chi^2 = 12.305$, $p < 0.05$.

No statistically significant difference between the two study groups was found in miscarriage rates: 4.8% (13/270)

Fig. 2 Distribution of embryos in PGS cycles by embryo quality biopsied on day 5 (left) and on day 6 (right) in matched age groups

Fig. 3 Comparison of euploidy rates of good-, fair-, borderline fair-, and poor-quality blastocysts in matched age group

Table 1 Comparison of euploidy rates of 3705 embryos with different degrees of expansion and ICM/trophectoderm quality biopsied on day 5 (top) and day 6 (bottom)

Day 5 biopsy		AA	AB/BA	BB	B-/B	C/C	Total
Degree of blastocyst expansion	1	n/a	0.00	n/a	n/a	n/a	1
	2	0.71	0.51	0.38	0.29	0.00	149
	3	0.63	0.54	0.51	0.47	0.33	692
	4	0.63	0.76	0.64	0.71	1.00	126
	5	0.72	0.71	0.62	0.53	n/a	497
	6	0.89	0.87	0.79	0.50	n/a	51
Total		325	558	465	160	8	

Euploidy rate

Day 6 biopsy		AA	AB/BA	BB	B-/B	C/C	Total
Degree of blastocyst expansion	1	0.00	1.00	0.00	n/a	0.08	18
	2	0.50	0.35	0.31	0.31	0.33	158
	3	0.59	0.45	0.44	0.25	0.19	468
	4	0.50	0.43	0.47	0.32	0.50	95
	5	0.70	0.60	0.49	0.46	0.22	993
	6	0.80	0.68	0.62	0.46	0.60	457
Total		230	374	866	655	64	

Euploidy rate

for day 5 SETs and 6.9% (16/233) for day 6 SETs. The biochemical pregnancy rate after transferring embryos biopsied on day 5 was 10.7% (29/270), and it was considerably higher after transferring embryos biopsied on day 6—17.6% (41/233), $\chi^2 = 4.907$, $p < 0.05$. In all age groups, higher pregnancy rates were achieved with embryos that became available for the biopsy on day 5 versus day 6 (Table 2).

The ongoing pregnancy rate in a group of patients <38 years old where transferred embryos were available for biopsy on day 5 was 64.6% (95/147). In the same age group, the ongoing PR was lower if the transferred embryo was available for the biopsy on day 6—46.6% (48/103), $\chi^2 = 8.037$, $p < 0.05$. Differences in pregnancy rates tended to remain with advancing maternal age. In a group of patients ≥ 38 years old, ongoing pregnancy rate after transferring a single embryo that was available for the biopsy on day 5 was 64.2% (79/123). In the same age group, the ongoing pregnancy rate was significantly lower if the transferred embryo was available for the biopsy on day 6—49.2% (64/130), $\chi^2 = 23.83$, $p < 0.05$ (Fig. 4).

In many cases, several euploid embryos were available for the transfer and in some cases embryos biopsied on day 6 had better morphological characteristics than embryos biopsied on day 5. So, we evaluated the impact of morphological characteristics of the embryo on the ability to establish viable ongoing pregnancy in IVF cycles.

In the group of patients with good-quality embryos for SET, the ongoing pregnancy rate was statistically not different after transferring embryos biopsied on day 5 versus day 6: 66.0% (136/206) and 62.9% (61/97) (Fig. 5).

In the group of patients with fair-quality embryos for SET, our data demonstrated considerable difference in ongoing pregnancy rate after transferring embryos biopsied on day 5 versus day 6: 56.0% (28/50) and 41.0% (34/83), respectively, although this did not reach statistical significance ($\chi^2 = 2.835$, $p = 0.09$). Similar data were obtained for borderline fair-quality embryos: the ongoing pregnancy rate after transferring

embryos biopsied on day 5 was 71.43% (10/14) and only 35.3% (18/51) for embryos biopsied on day 6, $\chi^2 = 5.849$, $p < 0.05$.

Discussion

Evidence of a correlation between inner cell mass/trophectoderm morphology and ongoing pregnancy rates in IVF programs has been reported worldwide for a long time [24–26]. Morphological assessment is a reliable and cost-effective method of embryo evaluation. Nevertheless, it does not present a definite answer about implantation potential of a particular embryo. In this regard, comprehensive chromosomal screening offers an unequivocal statement about particular embryo reproductive potential and allows reproductive specialists to deselect embryos with minor or major chromosomal aberrations [27, 28] (thus this statement was challenged recently by embryo mosaicism) [29, 30].

Recent reports have suggested that euploidy rates vary significantly between different IVF clinics [31]. Clinic-specific information regarding euploidy rates may become a foundation for evidence-based clinical decision making and proper patient consultation. Comprehensive chromosome screening provides an opportunity to re-evaluate the correlation between embryo euploidy and embryo morphology in order to create the best treatment strategy for the infertile couple undergoing IVF treatment.

Several authors tried to assess the link between blastocyst morphology and chromosomal abnormality [10, 11, 32, 33], build a time-lapse deselection model [34], and create generally applicable morphokinetic algorithm [35]. Our findings are supported by those of Alfarawati and collaborators, who also reported a link between morphological characteristics of the embryo and aneuploidy. The main strength of this study is in its significant sample size, so the association between

Table 2 Cycle outcomes after single embryo transfer of day 5 versus day 6 euploid embryo

Parameter	Day 5	Day 6	<i>p</i> value
No. of patients	231	195	
No of cycles	270	233	
Maternal age at retrieval $\pm$ SD (years)	36.7 $\pm$ 4.3	37.5 $\pm$ 3.8	NS
Peak E ₂ (pg/ml)	3359.2 $\pm$ 1511	2998.6 $\pm$ 1265	NS
Gonadotropin dosage $\pm$ SD (IU)	3701.7 $\pm$ 1440	4081.6 $\pm$ 1504	NS
Stimulation duration (days)	11.3 $\pm$ 1.6	11.3 $\pm$ 1.6	NS
Average number of eggs	18.6 $\pm$ 9.8	16.7 $\pm$ 8.9	NS
Positive hCG (%)	80 (216/270)	73.4 (171/233)	$p = 0.0793$
Biochemical PR (%)	10.7 (29/270)	17.6 (41/233)	$p < 0.05$
Spontaneous abortion rate (%)	4.8 (13/270)	6.9 (16/233)	$p = 0.3248$
Ongoing PR (%)	64.4 (174/270)	48.9 (114/233)	$p < 0.05$
Live birth rate (%)	61.5 (166/270)	46.8 (109/233)	$p < 0.05$

Fig. 4 Comparison of ongoing pregnancy rates after single embryo transfer of day 5 versus day 6 euploid embryo

blastocyst morphology and chromosomal abnormality was evaluated not only based on inner cell mass/trophoblast morphology, but also by the degree of blastocyst expansion and the dynamics of embryo development.

Analysis of our data showed statistically significant difference in ongoing pregnancy rate between embryos that were available for a biopsy on day 5 versus day 6. Other studies have reported similar results in non-PGS cycles [12, 13]. When comparing day 5 and day 6 frozen embryos in IVF PGS cycles, timing of embryo biopsy is crucial. Many embryos that are not available for biopsy by 10 a.m. on day 5 will become available after noon on day 5 or on day 6. Early cutoff time on day 5 in this study (10 a.m.) emphasizes the difference in reproductive potential between embryos. This may explain why some researchers did not detect a statistically difference in clinical outcomes between day 5 and day 6 frozen embryos, especially in multicenter settings [11].

We cannot conclude from here presented results that there is something intrinsically better about day 5 euploid embryos versus day 6 euploid embryos; rather, this study provides objective evidence that patients who have cycles with an early

day 5 blastocyst available have a better prognosis for live birth in outcome.

The small, but statistically significant difference in euploidy rates between embryos that become available for the biopsy on day 5 versus day 6 reported in this study can also explain high implantation rates for good-quality embryos transferred on day 5 in non-PGS cycles.

Is it not clear what can cause statistically significant difference in ongoing pregnancy rates in IVF PGS cycles where embryos were biopsied on day 5 versus day 6. Intracellular factors such as anaphase lag [36], spindle assembly checkpoints [37], microtubule kinetics, mitochondrial function, or depletion of critical nutrients can cause developmental delay in preimplantation embryo, and at the same time, an embryo still may have a normal chromosomal number and be considered by reference genetic lab as euploid. The impact of intracellular factors in the euploid embryos can also be a reason for high biochemical pregnancy rate reported in this study if selected for embryo transfer embryo was biopsied on day 6.

The gradual decrease in euploidy rates suggests that several factors affect and define chromosomal constitution of human

Fig. 5 Comparison of ongoing pregnancy rates after transferring good-, fair-, and borderline fair-quality embryos available for the biopsy on day 5 versus day 6

preimplantation embryos. In addition, the wide range of euploidy rate values throughout all morphological groups provides systematic evidence of an association between chromosomal status and morphological characteristics of the embryos. Automatization of morphological assessment of preimplantation embryos in vitro via computer software algorithms, coupled with comprehensive chromosomal screening results, would facilitate the determination of the strength of correlation between the morphology and genetics of the embryos.

Several recent papers suggested that in some cases, extended culture up to day 7 may be necessary to assess genetic status of the embryos [38, 39]. At the same time, reported pregnancy rates after transferring embryos biopsied on day 7 are relatively low and long-term outcomes are unknown.

Our study also supports previously published data by Harton and collaborators [40] who postulated diminished effect of maternal age on implantation in IVF PGS cycles. Our data demonstrated that ongoing pregnancy rates were statistically not different between younger (≤ 37 years old) and older (≥ 38 years old) patient groups if embryos frozen on day 5 were transferred; similar results were obtained for embryos frozen on day 6.

A better understanding of the impact of embryo morphology on clinical outcomes in IVF PGS cycles may facilitate the improvement of clinical efficiency especially among patients with multiple euploid embryos available for the transfer, subsequently, stimulate predominant single embryo transfer, and almost entirely eliminate the possibility of multiple gestations [41].

In many ART programs in the USA, PGS cycles represent the majority of the IVF cycles. For this reason, knowledge regarding the interrelation embryo morphology and euploidy rates for all age groups can also be applied to non-PGS cycles within the same ART program to generate evidence-based clinical decisions and optimize the number of transferred embryos, especially in a young patient group as advised by ASRM Practice Committee [42, 43] and possibly will allow young patients to avoid PGS. The high euploidy rate among good-quality embryos provides support for the wider implementation of eSETs and limited benefits of invasive methods of embryo selection in a younger patient population. While the relative benefit of PGS may be lower for younger patients, there is still about 20% risk of having aneuploid embryo even with good-quality embryos; thus, genetic counseling should be considered.

The study has several limitations. Due to the retrospective nature of the study, some key statistical parameters cannot be calculated. Possible bias of morphology assessment also can be limited in further studies by applying automated image recognition techniques and machine learning algorithms.

Previous studies did not find any correlation between cleavage stage embryos and chromosomal status of the embryos [44, 45] most likely because of embryo development until genome activation is determined by oocyte's proteins

and RNA. Therefore, in our study, prolonged cultivation of embryos until blastocyst stage reveals a strong correlation between embryo morphology and genetic status of the embryo. Sizable sample size in here presented data enabled assessment of the difference in euploidy rates between good-, fair-, and poor-quality embryos by exact maternal age. The effect of maternal age was particularly evident among patient over 38 years old.

Here, the presented data are a part of growing evidence that supports an idea of creating a non-invasive model for ploidy classification based on morphological characteristics of preimplantation embryos [46–48]. Due to the high variance in euploidy rates between IVF centers, universal precise prediction model will likely remain out of the reach in the nearest future. However, general principles derived from this model can be used anywhere.

Conclusion

The presence of a correlation between embryo morphology and genetic status of the embryo for a long time was suspected, but still not a proven phenomenon. In this study, we analyzed 3705 individual embryos and proved an association between morphological characteristics, clinical outcomes, and genetics of the embryos in PGS cycles. Regardless of how we look at the data, the patient's age remains the main factor responsible for euploidy rates, embryo quality, and dynamics of embryo development in vitro. A gradual change in these parameters with advancing maternal age suggests the presence of general age-dependent mechanisms that may control unfolding and translation of genetic information during early human embryo development in vitro.

Author contributions Conceived and designed the study: O.B. and K.I.; coordinated data collection: S.W. and O.B.; analyzed the data: O.B., S.W., E.R., D.W., M.H., and S.R.; and drafted the manuscript: O.B., K.I., M.H., and L.W. All authors interpreted the data.

Compliance with ethical standards The study protocol was approved by the Ethics Committee (Institutional Review Board) of the Reproductive Science Center of the San Francisco Bay Area and was performed according to the Declaration of Helsinki for Medical Research.

Conflict of interest The authors declare that they have no conflict of interest.

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