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ASSISTED REPRODUCTION TECHNOLOGIES

Euploid blastocysts implant irrespective of their morphology after NGS-(PGT-A) testing in advanced maternal age patients

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Abstract

Purpose Does blastocyst morphology following euploid elective single embryo transfer (eSET) after preimplantation genetic testing for aneuploidies (PGT-A) via next generation sequencing impact clinical outcome?

Methods Two hundred ninety-six patients underwent PGT-A. Of 1549 blastocysts, 1410 blastocysts had a conclusive result after PGT-A and were included for analysis. An eSET policy was followed in a frozen embryo replacement cycle. A total of 179 euploid blastocysts were thawed and transferred. Clinical outcomes were categorized in four different embryo quality groups: excellent, good, average and poor.

Results Euploidy rate was 19/36 (52.7%, 95% CI 37–68), 199/470 (42.3%, 95% CI 38–47), 156/676 (23.0%, 95% CI 20–26) and 39/228 (17.1%, 95% CI 13–23) in the excellent, good, average and poor quality blastocyst groups, respectively. Fitted logistic regression analysis taking into account the following covariables: female, age, embryo chromosomal status and day of blastocyst development/biopsy showed that morphology was predictive of the comprehensive chromosome screening result ($p < 0.05$). A logistic regression analysis was also performed on clinical outcomes taking into account the effect of blastocyst morphology and day of blastocyst development/biopsy. None of the parameters were shown to be significant, suggesting morphology and day of blastocyst development/biopsy do not reduce the competence of euploid embryos ($p > 0.05$).

Conclusions After eSET, implantation rate was 80–86%; live birth rate per embryo transfer was 60–73% and clinical miscarriage rate was found to be < 10% and were not significantly affected by the embryo morphology. Results are concordant with those reported when using aCGH and highlights the competence of poor-quality euploid embryos.

Keywords Embryo quality · Morphology · Euploid · PGT-A · Clinical outcomes

Introduction

The benefits of elective single embryo transfer (eSET) are widely recognized across different in vitro fertilization (IVF) units. Recent data from the Human Fertility and Embryology Authority (HFEA) shows that approximately 11% of IVF births in the UK are multiple births, which has declined over the last years as more clinics opt to offer eSET. With the advent of eSET policies, it has become imperative to emphasize on embryo evaluation criteria. Despite the exponential rise in knowledge and understanding of the dynamic process of embryo development in the laboratory, the classical evaluation methods based on morphology are still considered to be the gold standard methods to classify and select embryos.

In view of the lack of a consensus on embryo assessment using light microscopy, the European Society of Human Reproduction and Embryology (ESHRE) issued a consensus statement aiming to reduce the inevitable subjectivity of embryo evaluation [2]. In the same report, it highlighted that culture of embryos to blastocyst stage was the best policy to maximize embryo selection and subsequent embryo transfer outcome. Morphological assessment helps embryologists to detect dysmorphic or arrested embryos, but chromosomal abnormalities can still be only identified by preimplantation genetic testing for aneuploidies (PGT-A). It has been demonstrated that embryo evaluation of embryo morphology at the

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blastocyst stage cannot accurately predict aneuploidy status and that almost half of 'top quality' blastocysts are tested to be aneuploid [14].

Alfarawati et al. [1] have shown that the presence of aneuploidy in good quality embryos is not rare for patients receiving IVF treatment with PGT-A. Published data on chromosome analysis at the blastocyst stage performed with array comparative genomic hybridization (aCGH) showed aneuploidy rates between 34 and 62% in patients undergoing PGT-A [7, 14]. After identifying a euploid embryo out of a cohort of biopsied blastocysts, eSET has been implemented in many units. This has resulted in significantly increased implantation (IR) and ongoing pregnancy rates compared to transfer of non-biopsied embryos [6, 10, 18]. As PGT-A allows the increased use of eSET, multiple pregnancy rates have been minimized. In 2014, Capalbo et al. showed that poor and average quality euploid embryos screened using aCGH yielded the same ongoing IR compared with embryos classified with better qualities (45.2% versus 51.4%, respectively). In the same study, the authors found a relationship between blastocyst morphology and chromosomal status, which explains in part the good implantation potential of good quality embryos reported in younger patients during IVF cycles. Furthermore, Majumdar et al. [13] confirmed these findings following PGT-A with aCGH on 152 blastocysts and also found that the rate of development of an embryo was also highly indicative of euploidy (70% vs. 34.1% when comparing day 5 to day 6 blastocysts, respectively). Although the relationship between the ploidy status and morphology has been described with aCGH screening, it has been inconsistent as a finding. Other studies have suggested that the link between morphology and ploidy status of embryos is weak and morphological analysis cannot be relied on to ensure replacement of euploid blastocysts [1].

The present study aims to corroborate whether blastocyst morphology correlates with ploidy status of embryos analysed with next-generation sequencing (NGS). We also wanted to ascertain whether biopsy of poor-quality embryos yielded a euploid embryo to transfer and thus result in a favourable outcome. This is the first data set describing the correlation between blastocyst morphology and embryo ploidy status using NGS and clinical outcomes.

Materials and methods

This retrospective observational study was performed at the Centre for Reproductive and Genetic Health, London, UK. A database was generated in order to analyse embryos that were cultured to the blastocyst stage for PGT-A. All patients who underwent conventional ovarian stimulation during December 2015 to February 2018 and had PGT-A carried out on their embryos were included. Clinical outcomes were studied in patients that had a single euploid blastocyst transferred. Natural IVF cycles, cycles where the embryos were thawed on day three for blastocyst culture and frozen oocyte cycles, were excluded from this study.

Study cohort

A cohort study of 296 patients was analysed. Indication for PGT-A included advanced maternal age (AMA, > 37 years of age), recurrent miscarriage (RM, loss of ≥ 3 consecutive pregnancies), recurrent implantation failure (RIF, repetitive failed embryo transfers) and patient request. All couples included in the study have normal karyotype. The mean female age was 38.6 ± 5.2 years. A total of 3849 oocytes was retrieved from which 2542 zygotes were obtained. After 5–6 days of culture, 1549 blastocysts were considered suitable for biopsy. A total of 179 single euploid blastocyst transfers were performed in a subsequent frozen cycle. Only euploid embryos were considered for transfer.

Embryo culture and morphology evaluation

Intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) were used as the insemination methods together with culturing the embryo to blastocyst stage (day 5 or 6). Assisted zona hatching (AH) was performed on day 3 of embryo development in preparation for blastocyst biopsy. Blastocyst evaluation was performed prior to embryo biopsy at high magnification (× 400) using an inverted microscope with Hoffman modulation contrast microscopy. Embryos were graded according to modified Gardner and Cornell's group scoring system [8, 19]. For the inner cell mass (ICM) and trophectoderm (TE) cells, grade assignment depended on cellularity and cell clustering and was classified in different categories: A, B^+, B^-, C and D (Table 1). In the event ICM was graded as 'C' or 'D', a trophectoderm biopsy was not carried. The grading C or D referred to absent or degenerating ICM, respectively. Blastocyst expansion was graded correspondingly equal for all screened blastocysts given that AH was performed.

For the purpose of the study, embryo qualities were categorized into four main groups in order to generate comparable data to previous literature: excellent (AA), good (B+B+, B+A, AB+), average (B−A, B−B+, B−B−, B+B−, AB−, AC) and poor (B−C, B+C). All embryologists were subjected to an external quality assessment service (UK NEQUAS) in order to minimize inter-operator variability.

Blastocyst biopsy and NGS

Embryo biopsy was performed on days five and six of development using standard biopsy methods. All screened embryos were biopsied only once, and a minimum of 5 cells were

Table 1 Modified Gardner and Cornell's group scoring system

	Grade	Rating	Description	Equivalent to consensus
Degree of expansion	1		Early (blastocoel < 50% of the volume of the embryo)	1
	2		Blastocyst (blastocoel is half of the volume of the embryo)	2
	3		Expanded (blastocoel > 50% of the volume of the embryo)	3
	4		Herniating blastocyst	4
	5		Hatched blastocyst (natural)	Not described
	6		Hatched blastocyst (artificial assisted zona hatching)	Not described
ICM	A	Excellent	Tightly packed and compacted cells	1
	B+	Good	Larger cells, not tightly packed/cells making up a cellular bridge	1
	B−	Fair	ICM visible but loose and/or fragmented cells	2
	C	Poor	No ICM distinguishable	3
	D			3
TE	A	Excellent	Many healthy cells forming a cohesive epithelium	1
	B+	Good	Moderate levels of cells, but healthy and larger in size	1
	B−	Fair	Few large but healthy cells	2
	C	Poor	Unevenly distributed large cells (few cells squeezed to the side)	3
	D		Cells appear degenerative	3

Equivalences with Istanbul Consensus on embryo assessment

taken. Two different outcomes were considered after the PGT-A testing: euploid and aneuploid. At the time of the study, mosaicism was not clinically reported as per unit's policy. Embryos with more than 20% level of mosaicism were classified as aneuploid. Genetic testing was performed externally at a dedicated genetic testing provider. The NGS technology for aneuploidy detection was based on single read end sequencing of 36 base-pairs conducted on an Illumina MiSeq instrument. Whole genome amplification of the biopsied cells was performed using the SurePlex kit (Illumina, USA). Library preparation was performed by using the VeriSeq PGS kit from Illumina and following the manufacturer's instructions. In certain cases of 'good quality biopsies', the assay could detect chromosomal abnormalities as small as 5 megabase pairs. However, this high resolution also strongly depended on the genomic region and therefore can slightly vary for each genomic location. An embryo with segmental aberrations was classified as aneuploid.

Vitrification, thawing and blastocyst transfer

Blastocysts were cryopreserved individually and immediately after embryo biopsy using Blastocyst vitrification media (CooK IVF, Brisbane, Australia) as described by Stojanov (2009). Euploid blastocysts were thawed using Cook Blastocyst Thawing kit (CooK IVF, Brisbane, Australia) as described by Stojanov (2009) and transferred individually in a hormonal replacement treatment. Blastocyst transfer was performed on day 6 from the start of progesterone therapy using a Wallace embryo replacement catheter (Smiths Medical International, Kent, UK). The medicated frozen embryo transfer regimen followed the protocol previously described [3]. In the defined time period, all patients proceeded with eSET.

Clinical outcomes

Clinical outcomes were defined according to the Vienna consensus established by the ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine [5]. Implantation rate (IR) was determined as the number of gestational sacs divided by the total number of embryos transferred. Live birth rate (LBR) was calculated per embryo transferred as the number of live births divided by the sum of embryos transferred for treatment cycles included in the cohort. Clinical miscarriage was defined as clinically recognized pregnancy loss before 24-week gestation.

Statistical analysis

Statistical calculations were performed with Statistical Package for the Social Sciences version 23 (SPSS Inc., Chicago, IL). The main outcome was to observe any correlation between blastocyst morphology and clinical outcomes after euploid embryo replacement in single embryo transfer cycles. Analysis of variance (ANOVA) was required for continuous data when comparing all quality categories. Normal distribution was tested using Shapiro-Wilk test, and continuous variables were compared using the Student's t test. The differences of IR and LBR were evaluated using Pearson's χ^2

test with Yates' continuity correction. To confirm the findings, euploidy rate, IR and LBR were then independently analysed following a logistic regression fitted to age considering the subsequent covariates: day of blastocyst development/biopsy (day 5 or 6) and embryo morphology (excellent, good, average, or poor). Significance for statistical analysis was fixed at a 5% level. Moreover, a post hoc power analysis was conducted using the software package where SE is the size effect and B the observed power for IR ($B = 0.89$, SE = 0 .211, $p = 0.010$), LBR (B = 0.92, SE = 0.312, $p = 0.002$) and miscarriage rates ($B = 0.80$, SE = 0.296, $p = 0.01$).

Results

In this study, 296 patients were included for analysis. Of the 296 patients who underwent PGT-A, 187 (63.2%) had advanced AMA, 36 (12.2%) had AMA and RM, 28 (9.5%) had RM, 20 (6.7%) had RIF and 15 (5%) had AMA and RIF. Ten (3.4%) couples requested PGT-A as it was their preference and had none of the aforementioned indications.

The mean female age was 38.6 ± 5.2 years. A total of 3849 oocytes was retrieved. Intracytoplasmic sperm injection was performed in 3694 mature oocytes, and 2542 zygotes were obtained. Of the 2542 2PN embryos, 1549 (60%, 95% CI 59–63) that developed into blastocysts were considered suitable for the biopsy on day 5 or 6 of embryo development. A total of 1410/1549 (91%) blastocysts with conclusive results were included for analysis, whilst 139/1549 (8.9%) embryos failed to produce a result. The number of embryos for each morphology category was 36 (2.5%, 95% CI 1.9–3.5), 470 (33.3%, 95% CI 31–36), 676 (47.9%, 95% CI 45–51) and 228 (16.2%, 95% CI 14–18) for excellent, good, average and poor blastocysts, respectively. Global analysis for the PGT-A program with the present data revealed an overall euploidy rate of 452/1410 (32.1%, 95% CI), whilst 958/1410 (67.9%) were aneuploid. A total of 179 single euploid blastocyst transfers were performed in a subsequent frozen cycle. Only euploid embryos were considered for transfer. IR and LBR per embryo replacement were 148/179 (82.7%, 95% CI 76–88) and 121/179 (67.6%, 95% CI 60–74), respectively.

Embryo biopsy day and euploidy

Embryo development rate allowed biopsy to be predominantly performed on day 5 (844/1410; 59.8%, 95% CI 57–62) compared to day 6 (566/1410; 40.2%, 95% CI 38–43). The mean female age was not statistically significant between day 5 and day 6 blastocyst development (37.8 and 38.5 years; $p = 0.089$). The euploidy rate for day 5 blastocysts (256/844; 30.3%, 95% 27–34) was also not statistically significant compared to the euploidy rate of day 6 blastocysts (196/566; 34.6%, 95% CI 31–39) (p value = 0.108).

Embryo quality and chromosomal status

For each morphological category based on combined trophectoderm and inner cell mass scores, euploidy rate was calculated. Embryos with higher morphological combination scores displayed higher euploidy rates (Tables 2 and 3). In order to compare the different categories, four groups were differentiated as described in 'Materials and methods'. Euploidy rate was 19/36 (52.7%, 95% CI 37–68), 199/470 (42.3%, 95% CI 38–47), 156/676 (23.0%, 95% CI 20–26) and 39/228 (17.1%, 95% CI 13–23) in the excellent, good, average and poor blastocyst groups, respectively. Differences were noted among all groups except between excellent and good blastocysts (p value = 0.223) or average and poor blastocysts (p value = 0.058) (Table 2).

Fitted logistic regression analysis taking into account the co-variables female age, embryo chromosomal status and day of blastocyst development/biopsy showed that morphology was predictive of the comprehensive chromosome screening/PGT-A (Table 4).

Table 2 PGT-A and clinical outcomes considering different embryo quality categories. [In brackets, number of embryos in the category. *P1* p value comparing excellent versus good groups, *P2* p value comparing excellent versus average groups, *P3* p value comparing excellent versus poor groups, *P4* p value comparing good versus average groups, *P5* p value comparing good versus poor groups, *P6* p value comparing average versus poor groups]

Variable	Embryo quality				*p* value					
	Excellent ($n = 36$)	Good ($n = 470$)	Average ($n = 676$)	Poor ($n = 228$)	P1	P2	P3	P4	P5	P6
Age	36.92 ± 3.2	37.13 ± 4.5	37.87 ± 3.5	37.81 ± 4.4	0.782	0.238	0.687	0.164	0.475	0.896
Euploidy	52.7% (19/36)	42.3% (199/470)	23.0% (156/676)	17.1% (39/228)	0.223	<0.001	<0.001	<0.001	<0.001	0.058
Implantation rate	85.7% (12/14)	84.0% (84/100)	80.0% (44/55)	80.0% (8/10)	0.869	0.625	0.711	0.529	0.744	0.999
Clinical miscarriage rate	7.1% (1/14)	6% (6/100)	5.4% (3/55)	10% (1/10)	0.867	0.809	0.802	0.889	0.621	0.582
Live birth rate	64.2% (9/14)	66.0% (66/100)	72.7% (40/55)	60.0% (6/10)	0.899	0.534	0.831	0.388	0.703	0.415

Table 3 PGT-A and clinical outcomes considering different inner cell mass and trophectoderm combinations. [In bold, euploidy rate for each of the possible combination. Embryos with 'C' inner cell mass (ICM) were not biopsied. *IR* implantation rate, *LBR* live birth rate, *TE* trophectoderm]

	↓TE ICM→	A	B+	B-	C
Excellent	A	**52.7%** IR 85.7% LBR 64.2%	**47.2%** IR 88.9% LBR 68.6%	**25%** IR 90% LBR 66.6%	*Not biopsied*
Good	B+	**47.8%** IR 59.2% LBR 68.1%	**39.1%** IR 64.5% LBR 64.2%	**25.0%** IR 85.7% LBR 63.8%	*Not biopsied*
	B-	**22.7%** IR 66.6% LBR 65.2%	**23.3%** IR 80.0% LBR 76.8%	**20.6%** IR 50.0% LBR 55.3%	*Not biopsied*
Average	C	**10.0%** IR 50.0% LBR 49%	**16.6%** IR 84.3% LBR 61.2%	**17.1%** IR 77.7% LBR 59.1%	*Not biopsied*
			Poor		

Pregnancy and birth outcomes following vitrified-warmed single euploid transfer

Table 2 summarizes the IR and LBR after euploid eSET, which were similar across different category groups. IR was 12/14 (85.7%, 95% CI 60–96), 84/100 (84%, 95% CI 76–90), 44/55 (80%, 95% CI 68–88) and 8/10 (80%, 95% CI 49–94) in the excellent, good, average and poor blastocyst morphology categories, respectively. For the aforementioned categories, LBR was 9/14 (64.2%, 95% CI 39–84), 66/100 (66.0%, 95% CI 56–75), 40/55 (72.7%, 95% CI 60–83) and 6/10 (60.0%, 95% CI 31–83). Similarly, clinical miscarriage rate was found to be between 7 and 10 %. There was no statistical significance in the IR, LBR and clinical miscarriage rates across all four embryo quality categories ($p > 0.05$). Illustrated in Table 3, clinical outcomes are reported for all possible combinations of ICM and TE after single euploid blastocyst transfer.

A logistic regression analysis was performed on IR and LBR to account for the effect of blastocyst morphology and day of blastocyst development/biopsy. Table 4 shows that none of the covariates was predictive of the clinical outcome.

Discussion

PGT-A aims for the detection of the chromosomally normal embryo prior to embryo replacement. Our experience with NGS reproduces findings reported in literature using previous gold standard techniques such as a-CGH. To assess the effect of the different group analysis in our dataset, ploidy status was fitted to a logistic regression adjusted for female age with

Table 4 Logistic regression analysis comparing embryo quality and ploidy status. Clinical outcomes were compared on 179 euploid transfered blastocysts

Variable	Values	Ploidy status		Implantation rate		Live birth rate	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age		0.69 (0.4–1.3)	0.687				
Biopsy day	Day 5	[a]	0.541	[a]	0.122	[a]	0.148
	Day 6	1.4 (0.9–2.1)	0.840	0.6 (0.4–1.2)	0.096	0.3 (0.1–1.3)	0.121
Quality	Excellent	[a]	0.000	[a]	0.552	[a]	0.589
	Good	0.6 (0.4–0.8)	0.001	0.6 (0.1–2.2)	0.638	0.7 (0.4–1.5)	0.349
	Average	0.5 (0.4–0.7)	0.001	2.2 (0.4–1.9)	0.348	1.2 (0.8–1.4)	0.293
	Poor	0.2 (0.1–0.5)	0.005	0.9 (0.2–2.1)	0.944	1.4 (0.9–1.7	0.784

[a] Reference class

blastocyst developmental/biopsy day and quality as covariates. Our results have shown that embryos reaching excellent and good quality blastocysts were more likely to be euploid, and the percentage varies from 42 to 53% in our cohort of blastocyst screened via NGS. Interestingly, for the same categories, previous literature reported rates of 39.1% up to 56.4% in blastocysts screened via aCGH [1, 4, 13]. Similarly, euploidy rate after NGS was found to be the lowest among poorer quality embryos reaching values close to 17% with NGS (reported between 25.5 and 37% with aCGH). It is noteworthy that, for all categories, euploidy rates were slightly lower that the ones reported previously using aCGH. The high resolution of NGS in detecting smaller genomic imbalances may explain these differences. The high resolution of NGS can now classify 'aneuploid' embryos that could have been categorized as 'euploid' by aCGH. The ability to report mosaicism using NGS is one of the advantages when compared to aCGH. Mosaicism was not reported in the current study which implies that false positives could have been included in the dataset. It would be interesting to investigate how embryo quality could be mirroring different blastocyst qualities including mosaic embryos.

Literature on PGT-A using fluorescent in situ hybridization (FISH) in patients with recurrent pregnancy loss has shown a reduction from 26 to 39 to 10–13% on miscarriage rates after euploid embryo transfer considering all age groups [9, 15, 17]. Later, studies reported a reduction in the miscarriage rate to 8.6% using aCGH technology in patients above 35 years of age [11]. Focusing on patients above 35 years of age, the current study reports a clinical miscarriage rate of 5.3% (9/168) following PGT-A via NGS.

The main indication for PGT-A testing was advanced maternal age (63%), which generates a bias and makes these results not comparable to general IVF population. Furthermore, 3.4% of couples requested PGT-A without having an indication for such testing (age range 35–36), which had negligible impact to the overall result. Analysis of blastocyst quality groups and female age showed lack of significant difference. It is widely recognized that the effect of female age is eliminated following PGT-A which results in pregnancy and live birth rates for this cohort of patients that exceed the expected livebirth for the age-matched patient population [10]. In 2016, Wirleitner et al. analysed the pregnancy and birth outcomes following vitrified embryo transfer according to blastocyst morphology before vitrification and maternal age. Aneuploid screening was not performed prior to embryo transfer nor eSET policy. For female age between 36 and 40 years, they reported IR of 4.5–29.5%, 9.3–21.6% and 0–23.3% for top-quality, intermediate and non-top-quality embryos, respectively. Similarly, LBR was 5.4–34.3%, 12.5–27.2% and 0–39.4% for the same categories. In contrast, Irani et al. [12] analysed ongoing pregnancy rate of 477 euploid embryos reporting 84.2%, 61.8%, 55.8% and 35.8% for excellent, good, average and poor blastocysts.

Our data reports IRs of 50–90% independent of female age and embryo quality once blastocyst biopsy and PGT-A has been carried out. When embryo quality was taken into account in our study, a logistic regression analysis concluded that euploid blastocysts implant irrespective of their morphology. Our data further explored LBR as a result of the follow-up of all single euploid embryo transfers reporting the same findings. LBR was 64%, 66%, 73% and 60% in the excellent, good, average and poor blastocyst morphology categories, respectively, which were not statistically significant. Our data reports higher IRs and LBRs after NGS-euploid single embryo transfer than with PGT-A via aCGH or when conventional morphological assessment is implemented. This emphasizes the eSET policy, which should be recommended in this group of patients. We selected blastocysts to transfer according to a modified Gardner and Cornell's group scoring system score if more than one euploid embryo is available. Thus, this study may have introduced bias since transfer of euploid blastocysts with the higher morphological score was prioritized over euploid embryos with the lower morphological score, if more than one euploid was available for transfer.

Studies have shown that untested poor embryos yield low clinical outcomes, and many units often discard them instead of allowing them to be cryopreserved. Our data shows that the lowest quality euploid embryos (B-C) yielded an IR and LBR of 78% and 59%, respectively. This supports the practice of performing biopsy on poor quality embryos (B-C) and transfer in the event of a euploid result. Our data can be used to reassure and counsel couples that a poor quality euploid embryo has a similar IR and LBR to top quality euploid embryo transfer. It can also provide reassurance for both patients and clinicians that the outcome of such embryos is similar to the outcomes of transferring euploid high-quality embryos. However, we acknowledge the limited number of cases available for each embryo morphology category in the current study, our findings ought to be strengthened with increased sample size to confirm then non-significant differences on clinical outcomes among the embryo morphology groups.

Aiming to identify the embryo with highest potential for implantation and subsequently term delivery is still one of the main drawbacks in IVF. Literature has been published with potential markers for embryo potential, but PGT-A still offers the 'ultimate' answer when it comes to reveal the ploidy status and competence of the embryo. IR and LBR described in the present study are superior to those achieved without the use of PGT-A [12, 20]. Therefore, we suggest that embryo selection via PGT-A with NGS reveals embryonic potential irrespective of embryo morphology, which in return can significantly improve IVF outcome reducing the time to pregnancy and minimizing the risk of multiple pregnancies if eSET is performed [16]. Further prospective multi-centre studies with increased sample size are required to confirm or refute these findings.

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