Erratum

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DOI: 10.4103/jhrs.JHRS_98_17

Correction to: J Hum Reprod Sci, January-March 2017; DOI: 10.4103/jhrs.JHRS_103_16

Due to the editorial office's error, a number of corrections were not made to the article prior to its publication; the publisher wishes to apologize to all concerned. The corrected version of the article appears in full below.

Original Article

Relationship between morphology, euploidy and implantation potential of cleavage and blastocyst stage embryos

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ABSTRACT

Aim: The aim of this study was to investigate the relationship between morphology, euploidy and implantation rate of cleavage stage and blastocyst stage embryos. **Setting:** Institution-based, tertiary care *in-vitro* fertilization centre. **Study Design:** This study included a retrospective data analysis of 306 embryos: 154 cleavage stage embryos and 152 blastocysts that underwent biopsy on day 3 and day 5/6, respectively, which were subsequently screened for aneuploidy by array comparative genomic hybridization. **Materials and Methods:** Both cleavage stage and blastocyst stage embryos were categorized according to their morphology into the following three groups: good, average and poor. In addition, blastocysts were categorized into day 5 and day 6 embryos on the basis of their developmental rate.

Results: The euploidy rate was found to be significantly higher for blastocysts with good morphology as compared to those with poor morphology, with 73.2, 50 and 40.5% euploid embryos in the good, average and poor morphology groups, respectively ($P = 0.001$). No significant association was found between day 3 embryo morphology and euploidy rates with 40.6, 29.3 and 25.8% euploid embryos in the three groups, respectively ($P = 0.254$). The implantation rates, as per morphology, for the transferred euploid cleavage stage and blastocyst stage embryos were 43.8, 37.5 and 0% ($P = 0.354$) and 51.7, 71.4 and 66.7% ($P = 0.562$) in the good, average and poor morphology groups, respectively. The euploidy rate for day 5 blastocysts was significantly higher (70% vs. 34.1%, $P < 0.001$) than that of day

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Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/0974-1208.204013

How to cite this article: Majumdar G, Majumdar A, Verma IC, Upadhyaya KC. Relationship Between Morphology, Euploidy and Implantation Potential of Cleavage and Blastocyst Stage Embryos. J Hum Reprod Sci 2017;10:49-57.

6 blastocysts, but the implantation rate was similar in both groups (58.8 and 50%, respectively). The miscarriage rates for the euploid cleavage stage and blastocyst stage embryos were 18.2 and 8.3% ($P = 0.575$), respectively. **Conclusion:** Blastocyst morphology and rate of development were found to be significantly associated with euploidy, whereas cleavage stage morphology was not. Implantation rates of good quality euploid cleavage stage embryos were higher than that of poor quality embryos, while implantation rates were similar for all transferred euploid blastocysts, irrespective of their morphology or rate of development.

Keywords: *Aneuploidy, blastocyst, cleavage stage embryo, implantation rate, morphology, preimplantation genetic screening, PGS*

Introduction

Embryos have been routinely selected for transfer during *in-vitro* fertilization (IVF) cycles on the basis of morphology and developmental rate *in vitro*.[1] However, morphological evaluation alone has not proved to be a very efficient tool for embryo selection, as implantation rates continue to be very low. This is mainly because morphology does not necessarily correlate with the chromosomal status of the selected embryos.[2,3] Numerical chromosomal abnormalities or aneuploidies are highly prevalent in embryos generated *in vitro*, and the rate of aneuploidy rises with increasing maternal age.[4-7] Hence, a significant proportion of morphologically normal embryos selected for transfer may result in failed implantation, or further more, result in a spontaneous miscarriage, on account of being aneuploid.

Preimplantation genetic screening (PGS) involving biopsy of one blastomere from a day 3 embryo followed by fluorescence *in-situ* hybridization (FISH) analysis of only a limited number of chromosomes failed to show any improvement in IVF success rates.[8-12] Contrastingly, the advent of PGS in the form of comprehensive chromosomal screening (CCS) for all 24 chromosomes using newly validated platforms[13-18] is proving to be a promising approach for improving clinical outcomes in IVF, as evidenced by recent studies that showed that biopsy at the cleavage stage or the blastocyst stage followed by CCS resulted in improved embryo selection as compared to traditional morphology-based selection alone.[19-25] Furthermore, the combination of trophectoderm (TE) biopsy followed by blastocyst vitrification and CCS has not only resulted in an improvement in clinical outcomes, but is also allowing infertile women, even of advanced maternal age (AMA),[24] to adopt single-embryo transfers (SETs) as a viable option without lowering success rates.

However, despite mounting evidence in favour of CCS coupled with blastocyst stage biopsy, there are concerns[26,27] regarding the limitations associated with this approach when applied to patients with poor prognosis. While day 5 biopsies may provide for more safety[28] and accuracy by providing more cells for genetic analysis as opposed to only a single cell in day 3 biopsies, TE biopsies that mandate culture till day 5/6 in patients with poor prognosis may be fraught with an inherent danger of high cycle cancellation rates on account of embryos failing to reach the blastocyst stage. Consequently, it has been suggested that day 3 biopsies coupled with the new diagnostic platforms for determining aneuploidies in all chromosomes may, therefore, be more effective in improving clinical outcomes in PGS cycles[27] in women with poor prognosis.

Currently, there is a relative paucity of studies that have attempted to find reliable links between morphology and aneuploidy for both cleavage stage embryos as well as blastocysts using 24-chromosome screening. Phan *et al.*[29] reported that day 3 embryos with <6 cells and >15% fragmentation had a higher aneuploidy rate. Kroener *et al.*[30] evaluated the relationship between blastomere number and aneuploidy and found that embryos with >9 cells on day 3 were associated with a significantly high aneuploidy rate. In 2011, Alfarawati *et al.*,[2] the first group to correlate blastocyst morphology with CCS results using microarray comparative genomic hybridization (array CGH) analysis, found a weak association between the two, stating that a significant proportion of aneuploid embryos were capable of attaining the highest morphologic scores. Recently, Capalbo *et al.*[3] found that only morphology but not the developmental rate correlated with aneuploidy and that neither parameter correlated with the implantation potential of the transferred euploid blastocysts.

No studies have assessed the impact of morphology on the implantation potential of a euploid cleavage stage embryo compared to that of a euploid blastocyst. Only one study, which attempted to investigate if implantation rates were affected by maternal age after PGS with cleavage or blastocyst stage biopsy, showed that implantation rates were the same across all the maternal ages, irrespective of day of biopsy.[31] The goal of this study was to determine the relationship between morphology and implantation potential of euploid embryos, when euploidy assessment was performed either at the cleavage stage or at the blastocyst stage. This knowledge could be helpful in the

identification of morphological parameters associated with poor implantation of euploid embryos, if any, to avoid unnecessary biopsies.

The inherent difference in the two approaches of PGS, one in which euploidy assessment is carried out on day 3 followed by fresh transfer versus the other where the screening is undertaken on day 5/6 followed by blastocyst vitrification and transfer in a subsequent frozen thawed embryo transfer (FET) cycle, makes it difficult to compare the implantation rates between the two groups because of the inherent difference in the uterine receptivity between a fresh and frozen cycle, since the endometrium in ovarian stimulation cycles has been shown to have impaired receptivity in comparison to that of FET cycles.[32] Accordingly, the purpose of this study was not to compare the clinical outcomes/implantation rates between day 3 and day 5 PGS cycles. Rather this study was undertaken to assess the efficacy of the day 3 and day 5 approaches separately, in terms of prediction of euploidy and the subsequent implantation potential of such euploid embryos.

Materials and Methods

In this study, data was analyzed retrospectively from PGS cycles performed between June 2014 and June 2016, which was approved by the institutional ethics committee. All patients who were enrolled for data retrieval and retrospective analysis gave written informed consents before undergoing the IVF–PGS cycles and had the following primary indications: AMA with age >37 years, recurrent implantation failure (RIF) with ≥2 IVF failed cycles, recurrent miscarriages (RM) with ≥2 pregnancy losses and secondary indications including previous aneuploid conceptions and severe male factor. Based on patient request, PGS was also offered to RIF or RM patients opting for an oocyte donation cycle, on having failed PGS with their own gametes. These patients who underwent PGS with donor oocytes were included in the good prognosis group. Some patients included in the analysis had more than one indication.

In case of cleavage stage PGS, blastomere biopsy was performed on day 3 following which the biopsied embryos were cultured till day 5. Once the array CGH results were available on the morning of day 5, only euploid embryos that had progressed to the blastocyst stage were chosen for transfer. A maximum of two euploid embryos were transferred after an evaluation of morphology and discussion with the patient. All supernumerary euploid blastocysts were vitrified on both day 5 and day 6. Only fresh transfers were included in the analysis for cleavage stage PGS cycles. For blastocyst stage PGS, TE biopsy was performed on day 5 or day 6, following which the biopsied blastocysts were immediately cryopreserved by vitrification, and the biopsy sent for aneuploidy screening for all 24 chromosomes by array CGH. Once genetic analysis results were in, euploid blastocysts were transferred subsequently in frozen–thawed transfer cycles. Embryos with no results were excluded from the analysis. 80% of all embryo transfers performed in the cleavage stage PGS cycles were SETs, whereas 100% of the transfers in the blastocyst stage PGS cycles were SETs. Implantation outcomes of only those double-embryo transfer cycles were correlated with morphology, which resulted either in twin implantation or no implantation. Biochemical pregnancy was defined as a rise in beta human chorionic gonadotropin (β-hCG) without any clinical evidence on ultrasound. Blighted ovum was defined as a pregnancy with a sac but no foetal pole. Those pregnancies with a sac and a foetal pole but no cardiac activity after 7 weeks were termed as missed abortions. Ongoing pregnancy rate per embryo transfer was defined as the ratio of the number of foetal hearts at 20 weeks of gestation to the total number of embryo transfer cycles performed. Implantation rate was defined as the ratio of the number of gestational sacs to the total number of transferred embryos.

Controlled ovarian stimulation, oocyte retrieval and intracytoplasmic sperm injection (ICSI) were performed as previously reported.[25] Fertilization was checked at 16–18 h post ICSI. Embryo culture was undertaken in sequential culture media (Vitrolife, Sweden) to blastocyst stage, wherein 2pn embryos were cultured in groups of up to 6–8 embryos in 0.7 ml of G1P under oil (Vitrolife, Sweden) from day 1 to day 3 and G2P under oil (Vitrolife, Sweden) from day 3 to day 5.

Cleavage stage embryo biopsy and morphology scoring

On the morning of day 3, embryos were graded based on their morphology and cleavage rates, and biopsy was performed on embryos with 6–12 cells and 0–30% fragmentation. Prior to biopsy, the embryos were placed in calcium–magnesium-free buffer (PGD Biopsy Media, Vitrolife, Sweden) to loosen cell–cell adhesions that facilitate blastomere removal. For embryos that had already commenced compaction, it was preferred to incubate the compacting embryos in the Ca–Mg-free media for 2–3 min before proceeding for biopsy. Biopsy was performed on the heated stage of an inverted microscope (Nikon TE 300, Japan) using a non-contact diode laser (Hamilton Thorne, UK) to make a hole in the zona that allowed a single blastomere with a visible nucleus to be gently sucked out using a biopsy micropipette (TPC, Australia) without damaging the cell. If cell lysis occurred during biopsy and the nucleus got lost, then a second cell was biopsied. The biopsied embryos were then immediately washed and placed back

into individual 10 µl droplets of culture media under oil for traceability. The aspirated cell was washed and then transferred to a polymerase chain reaction (PCR) tube containing 2 µl of phosphate buffer saline under a laminar flow in strictly aseptic conditions to avoid contamination, which was then taken for array CGH analysis.

Embryos selected for biopsy, were categorized into the following three groups based on morphology:

(1) Good: 7-, 8- and 9-cell embryos with stage-specific cell sizes and no fragmentation.
(2) Average: 7, 8- and 9-cell embryos with non-stage-specific cell sizes and/or ≈15% fragmentation.
(3) Poor: 6-cell embryos, >9-cell embryos and/or grossly unequal cell sizes, and/or 15–30% fragmentation.

Trophectoderm biopsy and morphology scoring

On day 5 or day 6, only full blastocysts of expansion grade 3[1] or higher, were selected for biopsy. First, a small hole of ≈10 µm was introduced in the zona with the help of the laser to initiate herniation on the morning of day 5 or day 6, after which the blastocysts were put back into individual culture. After 3–4 h, once an adequate portion of the TE had undergone herniation, the blastocysts were placed in the biopsy dish in 6 µl drops of MOPS buffer (GMOPS Plus, Vitrolife, Sweden) under equilibrated oil. The clump of herniated TE cells (3–5 cells) was gently sucked with a biopsy pipette (TPC, Australia) and detached from the blastocyst with the help of several laser shots. For embryos in which herniation did not occur even after 3–4 h, the biopsy pipette was gently pushed beyond the hole in the zona and the TE cells were aspirated and biopsied, as described in an earlier publication.[3] Post biopsy, the embryo was placed back in culture, and the TE clump of cells underwent washing and tubing as described above for day 3 biopsies.

At the time of biopsy, blastocysts were graded on the basis of morphology and categorized into the following three groups: good, average and poor. This overall quality was assigned based on individual scores that were assigned to each blastocyst in terms of degree of expansion, inner cell mass (ICM) and TE cells using the Gardner and Schoolcraft[1] scoring system. The degree of expansion was denoted by grades 3–6 as follows: 3 = full blastocyst, 4 = expanding blastocyst wherein the zona starts thinning, 5 = hatching blastocyst and 6 = hatched blastocyst. The ICM and TE were graded into the following three categories: A = good, B = average and C = poor. The blastocysts were grouped as follows:

(1) Good: blastocyst with grades ≥3 and AA, AB and BA.
(2) Average: blastocyst with grades ≥3 and BB.
(3) Poor: blastocyst with grades ≥3 and either ICM or TE with grade C.

In addition, the blastocysts were categorized on the basis of their developmental rate into day 5 and day 6 blastocysts.

Microarray comparative genomic hybridization protocol

At the genetics laboratory, the biopsied cell(s) were subjected to whole genome amplification using SurePlex™ kit (Bluegnome, Cambridge, UK) in accordance with the manufacturer's guidelines to obtain sufficient quantities of sample deoxyribonucleic acid (DNA) for array hybridization. 24sure array kit (Bluegnome, Cambridge, UK) was used to perform array CGH according to the 24sure protocol. Briefly, sample and control DNAs were labelled with Cy3 and Cy5 fluorophores using random primers. The labelled DNAs were mixed and co-precipitated with COT human DNA. The labelled DNA was then hybridized under coverslips to V3 slides overnight. The slides were then washed to remove the unbound labelled DNA and scanned using a laser scanner. The data were extracted and analyzed using BlueFuse Multi software (Bluegnome, Cambridge, UK) for detection of gains and losses across all 24 chromosomes using detection criteria defined by the 24sure platform.

Statistical analysis

Statistical testing was conducted with the Statistical Package for the Social Sciences system version 17.0 (SPSS Inc., Chicago, IL, USA) software. Results were expressed as mean ± standard deviation or numbers or percentages. The comparison of normally distributed continuous variables between the groups was performed using Student's *t* test. Nominal categorical data between the groups were compared using Chi-squared test or Fisher's exact test as appropriate. $P < 0.05$ was considered statistically significant.

Results

Day 3 PGS

Three hundred and six embryos were included in this analysis, out of which 154 embryos were analyzed at the cleavage stage and 152 were analyzed at the blastocyst stage. The 154 cleavage stage embryos from 30 patients underwent blastomere biopsy on day 3 in 31 cycles of IVF–PGS. One embryo was reported with no result, which was excluded from the analysis. A euploidy rate of 33.3% (51/153) was reported after array CGH analysis for the cleavage stage embryos. The mean female age of this patient population was 35.7 years. Out of 51 day 3 embryos that were reported as euploid, 88.2% (45/51) progressed to the blastocyst stage on day 5, out of which 29 euploid embryos underwent transfer in 24 fresh embryo transfer cycles, resulting in a positive β-hCG rate of 45.8% (11/24), two missed abortions, an ongoing pregnancy rate of 37.5% (9/24) and an implantation rate of 41.4% (12/29).

Table 1: The relationship between euploidy rates and implantation rates with age, indication and morphology of 153 cleavage stage embryos

Variables	Euploidy rate % (*N*) 153	*P* value	Implantation rate % (*N*)	*P* value
Age (years)				
≤37	43.0% (40/93)	0.002*	47.4% (9/19)	0.450
>37	18.3% (11/60)		30.0% (3/10)	
Indications				
Good prognosis	39.1% (9/23)	0.053	100% (3/3)	0.145
AMA	18.3% (11/60)		30.0% (3/10)	
RIF	35.1% (20/57)		33.3% (5/15)	
RM	41.5% (17/41)		33.3% (3/9)	
Morphology				
Good	40.6% (26/64)	0.254	43.8% (7/16)	0.354
Average	29.3% (17/58)		37.5% (3/8)	
Poor	25.8% (8/31)		0% (0/3)	

AMA = advanced maternal age, RIF = repetitive implantation failure, RM = repeated miscarriages. *Difference was considered significant when $P < 0.05$.

The relationship between euploidy rate and implantation rate with age, indication and morphology of 153 cleavage stage embryos analyzed by array CGH is given in Table 1. First, the embryos were assessed in relation to female age. As expected, the euploidy rate for maternal age ≤37 years was found to be statistically higher than that for patients with age >37 years (43% vs. 18.3%, $P = 0.002$). Embryos were also assessed on the basis of indication. Specifically, embryos were categorized into different groups based on the indications of the patients who generated them. Patients who were included in the good prognosis group did not qualify to have any of the following indications of AMA, RIF or RM. No significant difference was observed in euploidy rates between different indications when compared with the patients with good prognosis. When embryos were classified on the basis of morphology, our day 3 PGS data showed that no significant association was present between day 3 embryo morphology and euploidy rates, with 40.6, 29.3 and 25.8% euploid embryos in the good, average and poor morphology groups, respectively ($P = 0.254$).

The implantation rates of the day 3 euploid embryos with maternal age ≤37 years were higher as compared to those with age >37 (47.4% vs. 30%, respectively); however, the difference was not statistically significant. Similarly, euploid embryos from patients with good prognosis had no statistical difference in implantation rates when compared to that of patients with any of the primary indications. According to morphology, euploid cleavage stage embryos with good morphology showed a higher implantation rate compared with that of morphologically poor quality embryos, with implantation rates of 43.8, 37.5 and 0% in the good, average and poor morphology groups. However, this difference was not statistically significant owing to small numbers in the poor morphology group.

Blastocyst stage PGS

One hundred and fifty-two blastocysts from 46 patients underwent TE biopsy on day 5 or day 6 in 49 cycles of IVF–PGS. One embryo was reported with no result, which was excluded from the analysis. The percentage of euploid embryos was found to be 60.3% (91/151). The mean female age of this patient population was 34.4 years. Out of the 91 euploid embryos, 42 euploid blastocysts were transferred in single embryo transfer FET cycles, resulting in a positive β-hCG of 64.3% (27/42), three biochemical pregnancies, one blighted ovum, one missed abortion, an ongoing pregnancy rate of 52.4% (22/42) and an implantation rate of 57.1% (24/42).

The relationship between euploidy rate and implantation rate with age, indication, morphology and develop- mental rate of 151 blastocysts analyzed by array CGH is given in Table 2. Maternal age >37 resulted in a significantly higher aneuploidy rate (65.5% vs. 40.6%, $P = 0.011$). Contrary to our day 3 PGS findings, the day 5 PGS data suggests that patients with any one or more of the following indications including RM, RIF and AMA had a significantly higher aneuploidy rate as compared to patients with good prognosis who underwent PGS without any of those indications ($P < 0.001$).

For blastocyst stage PGS, all variables assessed including morphology, ICM, TE and rate of development showed a significant association with euploidy rate. The euploidy rate was found to be significantly higher for embryos with good morphology as compared to those with poor morphology, with 73.2, 50 and 40.5% euploid embryos in the good,

Table 2: The relationship between euploidy rates and implantation rates with age, indication, morphology and developmental rate of 151 blastocysts

Variables	Euploidy rate % (*N*)	*P* value	Implantation rate % (*N*)	*P* value
Age (years)				
≤37	65.5% (78/119)	0.011*	60.6% (20/33)	0.462
>37	40.6% (13/32)		44.4% (4/9)	
Primary indications				
Good prognosis	85.4% (35/41)	<0.001*	55.6% (5/9)	0.745
AMA	42.4% (14/33)		50.0% (5/10)	
RIF	53.3% (33/60)		60% (9/15)	
RM	35.1% (13/37)		75.0% (6/8)	
Morphology				
Good	73.2% (60/82)	0.001*	51.7% (15/29)	0.562
Average	50% (16/32)		71.4% (5/7)	
Poor	40.5% (15/37)		66.7% (4/6)	
Day of biopsy				
Day 5	70% (77/110)	<0.001*	58.8% (20/34)	0.706
Day 6	34.1% (14/41)		50% (4/8)	
ICM				
A	75.9% (63/83)	<0.001*	57.6% (19/33)	0.489
B	41.9% (26/62)		62.5% (5/8)	
C	33.3% (2/6)		– (0/1)	
TE				
A	73.6% (53/72)	0.005*	48.0% (12/25)	0.307
B	51.0% (25/49)		66.7% (8/12)	
C	43.3% (13/30)		80% (4/5)	

AMA = advanced maternal age, RIF = repetitive implantation failure, RM = repeated miscarriages, ICM = inner cell mass, TE = trophectoderm. *Difference was considered significant when $P < 0.05$.

average and poor morphology groups, respectively ($P = 0.001$). According to the developmental rate, day 5 blastocysts were associated with a significantly higher euploidy rate (70% vs. 34.1%, $P < 0.001$) as compared to the day 6 blastocysts. Individually, both, grades of ICM and TE, also showed a strong association with euploidy rate.

Implantation rates were found to be similar, when euploid blastocysts of different ages, indications or morphologies or developmental rates, were transferred [Table 2]. The implantation rates, as per morphology, for the transferred euploid blastocysts were 51.7, 71.4 and 66.7% in the good, average and poor morphology groups ($P = 0.562$), respectively. Quality of ICM and TE were also not associated with the developmental potential of euploid blastocysts. Similarly, the implantation rate was similar when day 5 or day 6 euploid blastocysts were transferred (58.8 and 50%, respectively).

Discussion

This study sought to investigate the relationship between morphology and chromosomal complement for cleavage and blastocyst stage embryos analyzed by array CGH. Our PGS data confirmed a significant association of blastocyst morphology and developmental rate with the euploidy rate. On the other hand, we found that day 3 embryo morphology had little association with euploidy.

The euploidy rate was found to be significantly higher for blastocyst stage embryos as compared to that of cleavage stage embryos (60.3 and 33.4%, respectively, $P = 0.001$). In addition, a much higher euploidy rate was observed for patients with good prognosis on day 5 than on day 3 (85.4% vs. 39.1%, $P < 0.001$). This higher euploidy rate on day 5 could be a result of the phenomenon of self-correction, as has been suggested by a few authors,[33,34] or just natural selection that allows only euploid embryos to grow further to the blastocyst stage.[35] Additionally, for cleavage stage PGS cycles, euploidy rates were found to be similar between patients with poor prognosis and those with good prognosis, whereas in case of blastocyst stage PGS, the type of indication was found to be strongly related to the euploidy rate [Table 2]. This suggests that the type of indication may come in handy as an additional factor in predicting euploidy, but only when the embryos are allowed to grow till blastocyst stage.

There have been numerous studies that have examined day 3 morphologic parameters such as number of cells, fragmentation and cell size/symmetry that are commonly used to select embryos for transfer.[36-39] However, most of these studies used FISH, which may have, therefore, resulted in an inaccurate classification of these embryos as euploid. Consequently, no morphological parameter has been found to be a strong predictor of chromosomal status for the cleavage stage embryo. More recently, few authors[29,30,40] have searched for a link between cleavage stage morphology and aneuploidy using 24-chromosome screening. While some studies[29,30] have revealed an association between chromosomal aneuploidies and number of cells at the cleavage stage and presence of fragmentation, our study evaluated the overall morphologic score given to cleavage stage embryos on the basis of multiple parameters such as number of cells, stage-specific cell sizes and degree of fragmentation and found poor association between morphology and euploidy. As reported in an earlier study by Fragouli *et al.*,[40] our analysis was able to confirm that overall day 3 embryo morphology is a poor indicator of euploidy status, because majority of good quality embryos on day 3 (~60%) were reported as abnormal whereas 29.3% of average morphology and 25.8% of poor morphology cleavage stage embryos were reported as normal [Table 1].

The association was found to be strong when blastocyst stage morphology was correlated with aneuploidy rate. As reported earlier,[2,3] we found that all morphological parameters of a blastocyst stage embryo were predictive of euploidy. Any blastocyst that was expanding and having a high scoring ICM and TE had a greater chance of being euploid than those with poorer grades. Individually also, the TE or ICM score was positively associated with the euploidy status of the embryo. However, contrary to Capalbo *et al.*, our data showed that the rate of development of a blastocyst was also highly indicative of euploidy. Embryos that have a slower rate of development and reach the blastocyst stage on day 6 had a significantly higher aneuploidy rate as compared to the faster growing embryos that achieved full expansion on day 5. This finding though was in agreement with Fragouli *et al.*,[40] who recently reported that embryos that showed a faster progression to the fully expanded stage on day 5, tended to have a significantly higher euploidy rate as compared to the early blastocysts on day 5.

Given the highly invasive nature of embryo biopsy and the high cost of genetic analysis that presents the main challenge in the widespread and universal application of the PGS technology, another objective of the study was to identify reliable morphological parameters, if any, associated with poor implantation of euploid embryos to avoid unnecessary biopsies. Our data showed that poor morphology of euploid cleavage stage embryos was associated with poor implantation potential. This finding suggests that there may be less value attached to performing biopsy on poor quality cleavage stage embryos since they may have poor viability. Contrary to the cleavage stage, our blastocyst stage PGS data showed that neither age nor morphology had an impact on the implantation potential of euploid blastocysts, once transferred. A previous study has already demonstrated that age does not impact the implantation potential of euploid embryos irrespective of developmental stage of embryo.[31] Our study showed that euploid blastocysts with poor or average morphology as well as a slower rate of development yielded equivalent implantation rates to those having good morphology and a faster rate of development. Therefore, it is evident from our data, that at the blastocyst stage, morphology has a significant impact on the likelihood of euploidy in an embryo. However, once euploidy has been confirmed, then morphology may no longer affect the subsequent implantation of that embryo.

One of the main limitations of any study attempting to correlate morphology with an embryo's chromosomal status is, that assessment of individual morphological parameters such as fragmentation, number of cells or scoring of ICM or TE, may be highly variable on account of an inherent subjectivity of visual assessment or variation in the cut-offs being used. Another limitation of this study was the bias associated towards good morphology or faster growing embryos during selection of euploid embryos for transfer. A prospective study design, in which euploid embryos could be selected for transfer randomly, irrespective of morphology or developmental rate, would allow larger numbers of euploid embryos with poor morphology or slower developmental rates, to be transferred. In this study, the potential of poor quality euploid embryos could not be assessed accurately owing to the fact that poor quality euploid embryos were never the first choice for transfer.

To conclude, our analysis showed that only blastocyst stage morphology, and not cleavage stage morphology, was predictive of euploidy. A significantly high percentage of aneuploid good quality cleavage stage embryos suggests that traditional morphology assessment based on established parameters for cleavage stage embryos cannot be relied upon to select euploid embryos. On the other hand, blastocyst morphology could be relied upon to some extent while selecting embryos, since a large proportion of good quality blastocysts tended to be euploid. It appeared from our data that, despite the small numbers, poor quality euploid cleavage stage embryos were associated with poor implantation outcomes and, hence, performing a biopsy

in such embryos could be of less value. However, larger numbers would be required to confirm this finding. Nevertheless, the most crucial piece of information that emanates from our data is that cytogenetic analysis may help in identifying a new subset of blastocyst stage embryos, those with poor morphology and a slower rate of development, that are capable of viable implantation. Even though this subset of poor quality, but euploid embryos, may be small in proportion, these embryos may prove to be of critical importance in cases in which all embryos available are of poor quality. Thus, PGS would not only result in improving the efficiency of traditional embryo selection by avoiding transfer of good quality aneuploid embryos, but it will also perhaps help in improving the cumulative pregnancy rates by transferring this new subset of poor quality euploid embryos that have an implantation potential similar to the good quality euploid embryos.

Acknowledgements

The authors express their gratitude to Parul Chugh, Department of Research, for providing statistical support for the study, the clinical and embryology staff at the Center of IVF and Human Reproduction, Sir Ganga Ram Hospital, New Delhi and all patients who participated in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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