Aim: The aim of this study was to investigate the relationship between the 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 analysis. 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 6 blastocysts, but the implantation rate was similar in both the groups (58.8 and 50%, respectively). The miscarriage rates for the euploid cleavage stage and the blastocysts stage embryos were 18.2 and 8.3% ($P=0.575$), respectively. Conclusion: Blastocyst morphology and the rate of development were found to be significantly associated with euploidy, whereas cleavage stage morphology was not. The implantation rates of the good quality, euploid cleavage stage embryos were higher than that of the poor quality embryos. The implantation rates were similar for all transferred euploid blastocysts, irrespective of their morphology or the rate of development. Keywords: Aneuploidy, blastocyst, cleavage stage embryo, implantation rate, morphology, preimplantation genetic screening
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 the success rates.

However, despite mounting evidence in the 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 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 the clinical outcome 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.[31] 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 the aneuploid embryos were capable of attaining the highest morphologic scores. Recently, Capalbo et al.[32] 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 stage or blastocyst stage biopsy, showed that the implantation rates were the same across all the maternal ages, irrespective of the day of biopsy.[31] The goal of this study was to determine the relationship between the morphology and the implantation potential of euploid embryos, when euploidy assessment was conducted either at the cleavage stage or at the blastocyst stage. This knowledge could be helpful in the identification of the morphological parameters associated with poor implantation of the 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 in 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 were 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. On the patient’s 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 group with good prognosis. 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 analysis 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 the morphology and a 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, trophectoderm (TE) biopsy was performed on day 5 or day 6, following which the biopsied blastocysts were immediately cryopreserved by vitrification, and the biopsy was sent for aneuploidy screening for all the 24 chromosomes by array CGH analysis. Once genetic analysis results were in, euploid embryos were transferred to a polymerase chain reaction (PCR) tube containing 2 μl droplets of culture media under oil for traceability. The aspirated cell was washed and then transferred to a PCR tube containing 2 μl of phosphate buffer saline under a laminar flow in strictly aseptic conditions to avoid contamination, which was then sent for array CGH analysis.

The embryos selected for biopsy were categorized into the following three groups based on their 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.

Cleavage stage embryo biopsy and morphology scoring
On the morning of day 3, the 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 the 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 sent for array CGH analysis.
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. 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, the blastocysts were graded on the basis of their morphology and categorized into the following three groups: good, average and poor. This overall quality was assigned based on the individual scores that were assigned to each blastocyst in terms of the degree of expansion, the inner cell mass (ICM) and the TE cells using the Gardner and Schoolcraft 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 cells were graded into the following three categories: A = good, B = average and C = poor. The blastocysts were grouped as follows:

1. Good: blastocysts 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 analysis 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 the detection of gains and losses across all the 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. The 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 preimplantation genetic screening**

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).

The relationship between euploidy rate and implantation rate with age, indication and the morphology of 153 cleavage stage embryos analyzed by array CGH analysis is given in Table 1. First, the embryos were assessed in relation to the female age. As expected, the euploidy rate for maternal age ≤37 years was found to be statistically higher than that for the patients with age >37 years (43% vs. 18.3%, P = 0.002). The embryos were also assessed on the basis of indication. Specifically, the embryos were categorized into different groups based on the indications of the patients who generated them. The patients who were included in the group with good prognosis did not qualify to have any of the following indications of AMA, RIF or RM. No significant difference was observed in the euploidy rates between different indications when compared with the patients with good prognosis. When the embryos were classified on the basis of their 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 $\leq 37$ years were higher as compared to those with age $\geq 37$ years (47.4% vs. 30%, respectively); however, the difference was not statistically significant. Similarly, the euploid embryos from patients with good prognosis had no statistical difference in the implantation rates when compared to that of the patients with any of the primary indications. According to morphology, the euploid cleavage stage embryos with good morphology showed a higher implantation rate compared with that of the 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 preimplantation genetic screening

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 SET 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 and morphology of 151 blastocysts analyzed by array CGH analysis is given in Table 2. Maternal age $>37$ years resulted in a significantly higher aneuploidy rate (65.5% vs. 40.6%, $P = 0.011$). Contrary to our day 3 PGS findings, our day 5 PGS data suggest that the patients with any one or more of the following indications including RM, RIF and AMA had a significantly higher aneuploidy rate as compared to the 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 a rate of development showed a significant association with euploidy rate. The euploidy rate was found to be significantly higher for the embryos 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 ($P = 0.001$). 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.

The implantation rates were found to be similar when the 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. The quality of ICM and TE cells 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).

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

| Variables      | Euploidy rate | Implantation rate | $P$ value | $P$ value |
|----------------|---------------|-------------------|-----------|-----------|
|                | % (N)         |                   |           |           |
| Age (years)    |               |                   |           |           |
| $\leq 37$      | 43.0% (40/93) | 47.4% (9/19)      | 0.002     | 0.450     |
| $>37$          | 18.3% (11/60) | 30.0% (3/10)      |           |           |
| Indications    |               |                   |           |           |
| Good prognosis | 39.1% (9/23)  | 100% (3/3)        | 0.053     | 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) | 43.8% (7/16)      | 0.254     | 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 $P < 0.05$. 

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Majumdar, et al.: Morphology, euploidy and implantation potential of PGS embryos

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**DISCUSSION**

This study sought to investigate the relationship between morphology and chromosomal complement for cleavage and blastocyst stage embryos analyzed by array CGH analysis. Our PGS data confirmed a significant association of the blastocyst morphology and the 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 the blastocyst stage embryos as compared to that of the 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 the euploid embryos to grow further to the blastocyst stage.\(^{35}\) Additionally, for the cleavage stage PGS cycles, the euploidy rates were found to be similar between the patients with poor prognosis and those with good prognosis, whereas in case of the blastocyst stage PGS cycles, 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 the 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 the embryos for transfer.\(^{36-39}\) However, most of these studies used FISH, which may have, therefore, resulted in an inaccurate classification of the embryos as euploid. Consequently, no morphological parameter has been found to be a strong predictor of the 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 the number of cells at the cleavage stage and the presence of fragmentation, our study evaluated the overall morphologic score given to the cleavage stage embryos on the basis of the

| Variables                  | Euploidy rate | Implantation rate |
|----------------------------|---------------|-------------------|
|                            | \% (N)        | \% (N)            |
| **Age (years)**            |               |                   |
| ≤37                       | 65.5% (78/119) | 60.6% (20/33)     | 0.011*          | 0.462          |
| >37                       | 40.6% (13/32)  | 44.4% (4/9)       |                  |               |
| **Primary indications**    |               |                   |
| Good prognosis            | 85.4% (35/41)  | 55.6% (5/9)       | <0.001*         | 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)  | 51.7% (15/29)     | 0.001*          | 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)   | 58.8% (20/34)     | <0.001*         | 0.706          |
| Day 6                     | 34.1% (14/41)  | 50% (4/8)         |                  |               |
| **ICM**                   |               |                   |
| A                         | 75.9% (63/83)  | 57.6% (19/33)     | <0.001*         | 0.489          |
| B                         | 41.9% (26/62)  | 62.5% (5/8)       |                  |               |
| C                         | 33.3% (2/6)    | – (0/1)           |                  |               |
| **TE**                    |               |                   |
| A                         | 73.6% (53/72)  | 48.0% (12/25)     | 0.005*          | 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 = trophoderm. *Difference was considered significant when \( P < 0.05 \).
multiple parameters such as the number of cells, stage-specific cell sizes and the degree of fragmentation and found poor association between morphology and euploidy. As reported in an earlier study by Fragouli et al.,\textsuperscript{[40]} our analysis was able to confirm that overall day 3 embryo morphology is a poor indicator of euploid status, because majority of the 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,\textsuperscript{[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. The 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.,\textsuperscript{[40]} who recently reported that the 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 because 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 the developmental stage of the embryo.\textsuperscript{[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 the assessment of the individual morphological parameters such as fragmentation, the number of cells or the scoring of ICM or TE may be highly variable on the 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 while selecting euploid embryos for transfer. A prospective study design, in which euploid embryos could be selected for transfer randomly, irrespective of the 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, because 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, which 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 the 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 a implantation potential similar to the good quality, euploid embryos.

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There are no conflicts of interest.

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