REVIEW ARTICLE

PREIMPLANTATION GENETIC SCREENING

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One of the biggest challenges in assisted reproduction technology (ART) is the selection of embryos for transfer with the highest implantation potential. Apart from morphological scoring criteria[1–3], and allowing culture to the blastocyst stage for transfer[4], the role of genetic analysis for embryo selection has been highlighted[5,6] The technique of preimplantation genetic diagnosis (PGD) has currently expanded to perform PGD for aneuploidy screening (PGD-AS). This genetic screening can improve the selection and transfer of chromosomally normal embryos in infertile patients with poor prognosis and thereby have an immediate impact on implantation rate.[7]

The procedure involves three stages, IVF, embryo biopsy and single cell diagnosis. It is possible to remove cells from the embryos at three stages; polar body, cleavage stage biopsy and blasocyst biopsy. The majority of the centres perform cleavage stage aspiration using acid tyrode's solution for zona drilling. The use of Ca^{2+}/Mg^{2+}-free medium has been found to facilitate the blastomere biopsy.

Concerns have been raised that the biopsy could possibly be detrimental to the embryos. In a study by Hardy *et al*, however, the embryos were followed up after blastomere biopsy till the blastocyst stage with no adverse effects. In this study however, the embryo transfer procedures were not taken into account[10]. Another available study looking into safety of embryo biopsy, did not compare infertile controls to infertile PGD cases.[11]

Single cell diagnosis in PGD-AS has been made possible by using Fluorescent in situ hybridization (FISH) technique and availability of commercially available probes labelled with multiple fluorochromosomes.[8,9] FISH analysis could allow selection of embryos which possess full potential for development till term. In addition, a reduced number of transferred embryos reduces the risk of multiple pregnancy and a higher number is available for cryopreservation.[12] This is especially important in countries where there is strict regulation on the number of embryos to be transferred.

With *blastomere biopsy*, PGD can screen 95% of the chromosomal abnormalities which can potentially develop till term with 90% efficiency. FISH errors and mosaicism account for the inefficiencies. With *first polar body biopsy* abnormalities of maternal MII and paternal origin cannot be picked and detection of FISH is reduced to 80%. Results can be improved further by discovering better methods of embryo biopsy, using more probes and/or improved image analysis system.[13]

According to the report of ESCHRE PGD Consortium[14], the indications for PGD-AS include the following groups:

i) maternal age more than 35 years
ii) recurrent IVF failures (at least three failed IVF treatment cycles), where a higher percentage of chromosomally abnormal embryos leads to reduced implantation rate
iii) more than two miscarriages, with the parents having a normal karyotype
iv) others, including a combination of the above indications, or an indication which does not fit into one of the above categories

The pregnancy rates after PGD-AS have been reported to be 20% per oocyte recovered (OR) and 26% per embryo transfer procedure. However in patients with recurrent IVF failures the rates were much lower being 7% per OR and 11% per embryo transfer procedure. In 8% of the cases the original results could not be confirmed which may either be due to chromosomal mosaicism or FISH failures.[14]

With morphologically and developmentally normal human embryos, cleavage stage aneuploidy significantly increases with maternal age.[15] It has been demonstrated that PGD-AS can positively affect the embryo outcome by reducing embryo wastage and improving implantation rates in patients with *maternal age* 35 years or more.[9,16] A recent multicentre retrospective controlled study demonstrates that PGD-AS significantly reduces the risk of spontaneous abortions in women undergoing IVF particularly over 40 years of age. In addition it may also reduce the risk of trisomic offspring.[17] However the argument for PGD-AS has been questioned in situations if there are no restrictions on the number of embryos to be transferred[18], or if blastocysts are used for embryo transfer.[19]

It has been recommended that the screening should be offered to patients with *recurrent implantation failure*. The chromosomal abnormality has been found in 41.3% cases with 72.8% abnormalities being aneuploidies. The use of Ca^{2+}/Mg^{2+} free medium has been found to facilitate the blastomere biopsy procedure without compromising embryo cleavage, ongoing pregnancies and healthy births[20]. The clinical benefits however have not been convincingly demonstrated.[13]

The role of PGS has also been supported for patients with *recurrent miscarriage* (RM) in various studies.[21,22] Recurrent miscarriage is associated with a significantly higher incidence of embryos with chromosomal abnormalities. Many of these abnormal embryos especially those with monosomy X and mosaics are able to develop to the blastocyst stage, hence the importance of PGS in the diagnosis and treatment of these patients.[23] PGD-AS has been reported to reduce the risk of miscarriage in RM patients, especially those in which the women is aged 35 years or more, to baseline levels.[24] Again this is more relevant if there is a legal restriction in the embryos that can be transferred, as replacement of three or four embryos to increase the chance of becoming pregnant with chromosomally normal embryos has also been supported as a treatment option in this group of RM patients.[25] Platteau *et al*, on the other hand have suggested that there is no therapeutic evidence to prescribe PGD-AS in patients with unexplained RM, as younger patients have a future pregnancy rate of more than 60% even without any treatment and the outcome of older patients is not encouraging despite treatment.[26]

The technique has also been recommended in high risk groups like patients with diabetes, hypertension, fibromas and myomas in which multiple pregnancy can be particularly dangerous.[9] A case report of aneuploidy 12 in a Robertsonian (13;14) carrier suggests a combination of PGD (preimplantation genetic diagnosis) for translocations with aneuploidy screening(AS) to possibly reduce the chance of replacing chromosomally abnormal embryos.[27] The contradictory reports, saying that aneuploidy screen does not improve implantation rates or lower miscarriage rates, have been found to have removed an excess number of cells for biopsy, inadequate choice of probes and suboptimal fixation technology.[28]

In a study conducted to determine if the outcomes of PGD-AS at the 8-cell stage have a predictive value for new genetic diagnosis cycles, correlation between euploidy rates and pregnancy rates could not be objectively assessed between cycles.[29]

The best chance of eliminating chromosomal abnormalities could be by analyzing all the chromosomes which is not so far possible with FISH. The technique which can analyze full karyotypes of polar bodies or blastmeres is spectral imaging.[30] A combination of FISH and spectral imaging analysis has been used for eight probe chromosome enumeration scheme.[31] Comparative genomic hybridization (CGH) allows comprehensive cytogenetic analysis but needs further development before wider clinical application.[32]

A high rate of mosaic embryos after both day 3 (50%) and day 5 analysis (45% for blastocysts and 65% for arrested embryos) has been reported, questioning the impact of mosaicism on the reliability of the PGD diagnosis[19]. The chromosomal constitution of mosaic embryos is subject to changes during further development of the embryo. Therefore it has been reported that analysis of two blastomeres on day 3 yields a better prediction of the chromosome constitution on day 5 and the development potential of the embryo.[33] Cytogenetic changes keep on taking place until the embryonic genome becomes fully active, probably at the blastocyst stage.[34] The mechanisms responsible for mosaic aneuploidies are post-zygotic chromosome loss, chromosome gain, mitotic non-disjunction[35] and anaphase lagging.[36] The developmental potential of the mosaic embryos is dependent on the type and proportion of non-diploid cells and the stage at which it is detected.[37]

Blastocysts have a lower degree of mosaicism than cleavage stage embryos possibly due to natural selection against chromosomes with high frequency of mosaicism.[38] However, despite a strong natural selection against chromosomally abnormal embryos, reanalysis of chromosomal complement on day 5 suggests that extended culture to day 5 or 6 cannot be used as a reliable tool to select against clinically important chromosomal anomalies.[39] Moreover, the data at present does not suggest that there is any preferential allocation of euploid cells to the inner cell mass and aneuploid cells to the trophectoderm.[40]

This genetic screening can improve the selection and transfer of chromosomally normal embryos in infertile patients with poor prognosis and thereby have an immediate impact on implantation rate. In addition, a reduced number of transferred embryos reduces the risk of multiple pregnancy and a higher number is available for cryopreservation.

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