PGDIS Position Statement on Chromosome Mosaicism and Preimplantation Aneuploidy Testing at The Blastocyst Stage

With the introduction of methods for comprehensive, 24 chromosome copy number analysis at the single cell level, testing embryos for abnormal copy number (aneuploidy) is now being used increasingly to avoid the transfer of abnormal, mostly non-viable, embryos following IVF. Indeed, using these methods (preimplantation genetic diagnosis for aneuploidy, PGD-A, or preimplantation genetic screening, PGS) has now been shown in numerous studies to improve implantation, pregnancy and live birth rates (per embryo transferred) and reduce miscarriage rates. Large scale randomized controlled trials are underway.

Chromosome mosaicism, the presence of two or more cell lines with different chromosome sets, is known to occur in a minority of embryos at all stages of preimplantation development through errors in the mitotic cell divisions following fertilization and various other mechanisms. The incidence of mosaicism appears to be less at the blastocyst stage, however, possibly because some types of chromosome errors may be associated with developmental arrest.

Partly for this reason, most IVF labs now culture embryos to the blastocyst stage to select for developmentally competent embryos and then biopsy small numbers of trophectoderm cells for aneuploidy testing.

Recently, some genetics labs have reported that, by careful calibration of sensitive technologies such as array CGH and NGS based copy number methods, they can distinguish simple, uniform aneuploidies (affecting all cells in the biopsy) from partial (mosaic) aneuploidies (affecting only some of the cells in the biopsy) by quantifying the extent of the copy number change (1). Further to this, pregnancies and healthy live births have been reported following transfer of blastocysts in which only mosaic aneuploidies had been detected in trophectoderm biopsies (2).

Preliminary evidence however, suggests that pregnancy rates are lower and miscarriage rates are higher (3).

How does this affect aneuploidy testing in clinical practice?

Most trophectoderm biopsy results are either normal for all chromosomes (euploid) or abnormal, with one or more aneuploidies. However, a small proportion has intermediate copy number changes for one or more chromosomes, which may indicate possible chromosome mosaicism. In many cases, these occur in conjunction with other uniform aneuploidies. However, in some cases only mosaic aneuploidies are detected, and these may be the only embryos which are available for possible transfer. Because mosaic aneuploidies detected in trophectoderm biopsies may theoretically have clinical implications for the pregnancy, including effects on placental function, and/or in live births clinically affected by mosaic aneuploidies, transfer of these embryos should only be considered when there is no alternative and preferably only after appropriate genetic counseling of the patient.

Recommendations for the laboratory (if reporting mosaic aneuploidies)

For reliable detection of mosaicism, ideally 5 cells should be biopsied, with as little cell damage as possible. If the biopsy is facilitated using a laser, the identified contact points should be minimal and preferably at cell junctions. Overly aggressive use of the laser may result in cell damage and partial destruction of cellular DNA.

Only a validated NGS platform that can quantitatively measure copy number should be used for measurement of mosaicism in the biopsy sample. Ideally, a NGS methodology that can accurately and reproducibly measure 20% mosaicism in a known sample.

For reporting embryo results, the suggested cut-off point for definition of mosaicism is >20%, so lower levels should be treated as normal (euploid), > 80% abnormal (aneuploid), and the remaining ones between 20-80% mosaic (euploid-aneuploid mosaics).

Recommendations for the clinician

Patients should continue to be advised that any genetic test based on sampling one or small number of cells biopsied from preimplantation embryos cannot be 100% accurate for a combination of technical and biological factors, including chromosome mosaicism.

The patient information and consent forms for aneuploidy testing (if used) should be modified to include the possibility of mosaic aneuploid results and any potential risks in the event of transfer and implantation. This needs to be explained to patients by the clinician recommending the aneuploidy testing.

Transfer of blastocysts with a normal euploid result should always be prioritized over those with mosaic aneuploid results.

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In the event of considering the transfer of a blastocyst with only mosaic aneuploidies, the following options should be discussed with the patient:

- A further cycle of IVF with aneuploidy testing to increase the chance of identifying a normal euploid blastocyst for transfer
- Transfer of a blastocyst with mosaic aneuploidies for low risk chromosomes only, after appropriate genetic counseling if available
- Appropriate monitoring and prenatal diagnosis of any resulting pregnancy, preferably by early amniocentesis (> 14 weeks' gestation).

Suggested guidelines to prioritize mosaic embryos for transfer

Based on our current knowledge of the reproductive outcomes of fetal and placental mosaicism from prenatal diagnosis, the following can be used as a guide by the clinician (or a genetic counselor if available) when a mosaic embryo is being considered for transfer:

- Embryos showing mosaic euploid/monosomy are preferable to euploid/trisomy, given that monosomic embryos (excepting 45, X) are not viable
- If a decision is made to transfer mosaic embryos trisomic for a single chromosome, one can prioritize selection based on the level of mosaicism and the specific chromosome involved
  - The preferable transfer category consists of mosaic embryos trisomic for chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 17, 19, 20, 22, X, Y. None of these chromosomes involve the adverse characteristics enumerated below
  - Embryos mosaic for trisomies that are associated with potential for uniparental disomy (14, 15) are of lesser priority
  - Embryos mosaic for trisomies that are associated with intrauterine growth retardation (chromosomes 2, 7, 16) are of lesser priority
  - Embryos mosaic for trisomies capable of liveborn viability (chromosomes 13, 18, 21) are of lowest priority, for obvious reasons

Clinical discussion

Developments in genomic technologies for preimplantation genetic diagnosis have revolutionized our ability to detect genetic abnormalities of various kinds at the level of single or small numbers of cells. Perhaps inevitably, the increased sensitivity and resolution of these methods has allowed a spectrum of chromosome abnormalities, including chromosome mosaicism, to be detected. Available evidence currently suggests that mosaicism (at least in the trophectoderm layer) only occurs in a small minority of embryos. Nevertheless, this can present a clinical challenge in managing patients, particularly poor prognosis patients, with no normal euploid embryos available for transfer. We are aware of many ongoing studies with different methodologies, which will clarify the extent of chromosome mosaicism and the impact on clinical outcomes over the next few years. Until then, transfer of blastocysts in which only mosaic aneuploidies have been detected should only be considered following expert advice and appropriate genetic counseling of patients. The laboratory reporting guidelines should also be understood when advising patients of the reasoning behind any concerns regarding the transfer and the appropriateness of follow up such as amniocentesis.

References

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