The challenge of embryonic mosaicism in preimplantation genetic screening

The availability of reliable 24 chromosome aneuploidy screening platforms has provided clinicians and scientists with a powerful new diagnostic tool in the embryology laboratory. Class I data demonstrate increased implantation and delivery rates and reduced multiple gestation rates by empowering more effective single ET after preimplantation genetic screening (PGS) is performed on trophectoderm biopsies (1, 2). Sustained implantation rates of 60% or higher are routinely being attained in multiple programs after synchronous transfer of euploid blastocysts—even in women in their early forties. While outcomes are clearly improved, it remains disappointing that a large percentage of morphologically normal euploid blastocysts fail to implant and progress to delivery. Understanding why those euploid blastocysts fail is an area of intensive investigation.

Mosaicism has been considered as a likely explanation for some failures after the transfer of PGS-screened embryos. Mosaicism is a likely culprit since the prevalence is quite high at both the cleavage and blastocyst stages of development and is traditionally associated with impaired clinical outcomes. While estimates vary, the prevalence of mosaicism may be as high as 20%. Evaluating an embryo to detect the presence of mosaicism provides a number of significant challenges. Some of these are biological, while others are analytical. Given that the efforts to diagnose embryonic mosaicism are only beginning, it is not surprising that clinical algorithms that show how best to counsel patients and manage these embryos are not yet available.

Biological Challenges in the Diagnosis of Mosaicism

The very nature of mosaicism makes it difficult to make the diagnosis from a single trophectoderm biopsy. Before addressing the complex analytical issues, it is important to understand the biological limits that impact the ability to determine whether an embryo is mosaic. There are at least two fundamental issues: the distribution of the cells with different chromosomal compliments throughout the embryo and the problem of cells with reciprocal errors in the same biopsy specimen.

The first issue relates to sampling error—will the biopsy capture sufficient cells of different chromosomal complements to allow detection of the mosaicism? Knowledge of the distribution of mosaicism in human embryos is important and is lacking at the current time. Are the cells with different chromosomal balances spread randomly throughout the trophectoderm? Alternatively, are cells of similar genetic complement located adjacent to each other? The latter might reflect that cells proliferate and remain near each other, creating regions or "nests" of cells with a specific chromosomal complement.

If cells with different chromosomal complements are widely distributed throughout the trophectoderm, a random trophectoderm biopsy would have a good chance of capturing a representative sample. In the situation where the nature of the mosaicism results in clustered nests of cells with different genetic complements, mosaicism would only be detectable if the biopsy happens to involve the junction between two different cell lines. That would occur almost by coincidence so the results of a single biopsy would likely be poorly sensitive. Of course, these factors would also be dependent on how far the embryo has progressed to development when the error leading to mosaicism occurred. There are data to suggest that most of the errors occur at the cleavage stage before blastocyst formation.

In the case of mosaicism with a clustered nest of cells with different genetic complements, many of the biopsies would contain cells that are of a single genetic complement in spite of the presence of mosaicism in the embryo. If the cells in that area were normal, then a false normal result would occur as the mosaicism is not truly genetically normal. Similarly, if the biopsy were to sample all aneuploid cells, the embryo would be labeled as "pure aneuploid" and would be discarded in spite of the fact that it is just mosaic and that some of those embryos contain true reproductive potential to produce a healthy offspring. At the current time, there is simply no way to know with certainty.

Unfortunately, sampling error may not be the only biological challenge. There is also the potential of having cells with reciprocal errors contained within a single biopsy. Consider the case where mitotic nondysjunction creates cells with two different but reciprocal chromosomal complements—one with trisomy 15 and one with monosomy 15. As per routine, the trophectoderm biopsy is placed into a reaction tube and the cells are lysed. This frees the DNA from all the cells creating a mixture, which is then analyzed as a single sample. For this example, assume that the number of trisomic and monosomic cells in the biopsy were equal. The extra chromosome 15s from the trisomic cells would compensate for the lack of one of the chromosome 15s in the monosomic cells. The total amount of DNA from chromosome 15 in the lysate would be normal, and the result would appear normal (disomy 15); the presence of mosaicism would go undetected. The closer the ratio of monosomic and trisomic cells is to 50:50, the less likely the analysis would detect the error. The overall impact of reciprocal errors is not known at this time. Its prevalence may be impacted by the nature of the mosaic error (nondysjunction vs. anaphase lag, etc.) as well as multiple other factors, including the specific chromosome involved.

Analytical Challenges in the Diagnosis of Mosaicism

Development and validation of a testing paradigm to detect chromosomal mosaicism in trophectoderm biopsies present many analytical challenges (3–5). While overcoming the problems may be complex, understanding the issues responsible for that complexity is actually quite straightforward.

First and foremost, as currently practiced, mosaicism screening is an exercise in bioinformatics. No additional laboratory procedures or processes are done. The fundamental

analytical testing protocols for single nucleotide polymorphism array, quantitative polymerase chain reaction, array comparative genomic hybridization, or NextGen sequencing are wholly unchanged. In fact, the fundamental statistical smoothing and copy number assignment algorithms are also unchanged—at least at the current time. What does vary is the way the results of those analyses are categorized when diagnoses are assigned.

In understanding how a diagnosis of mosaicism is assigned, it is helpful to review how the assays were initially calibrated to distinguish normal and abnormal results. Investigators commonly begin validating an assay by analyzing samples containing a minimal number of cells (typically 5) from fully characterized cell lines. This is done first for pure euploid cell lines and then repeated with aneuploid cells. Significantly, the actual log2 ratios for the individual data points vary over wide ranges for all of the samples. It is common for the raw data points from euploid and aneuploid samples to overlap extensively (3). However, once statistical smoothing algorithms are applied to the results for a given chromosome, a weighted average is attained that allows discrimination between the samples.

Ideally, the distribution of statistically smoothed results for normal and abnormal results would be widely separated. However, even after optimal smoothing, there is typically very little gap between the outer limits of the distributions (Fig. 1) (3, 4). A threshold value discriminating disomy from the monosomic samples is selected. Typically, this value is 3 standard deviations from the mean of the smoothed averages from multiple replicates of the disomic samples. The values from the abnormal samples are then confirmed to fall outside that threshold value. Note that there is typically no "middle ground" and that a single threshold value is used to discriminate disomic from either monosomic or trisomic samples. For those chromosomes where stable monosomic or trisomic cells lines are unavailable, the smoothed disomic ranges are computed and levels outside those ranges are typically considered abnormal.

Mosaic biopsies should produce assay results that reflect a blended average of chromosomal complements of the cells contained in the actual biopsy. For example, an analysis of a sample that is a mix of disomic or trisomic cells for a given chromosome will have a statistically smoothed mean that will fall somewhere between the mean of the pure disomic and the pure trisomic samples. Recent studies have demonstrated this to be the case. The authors have taken these data and created a new "middle category," which they have labeled as mosaic. The range of results for these mosaic samples (mean ±3 SD) is quite wide and spans the threshold value previously used to discriminate between normal and abnormal results (Fig. 1).

It should be emphasized that prior analyses of pure disomic samples did fall into the lower portion of this new mosaic range. Thus the mosaic range would not be expected to contain only those samples that are mosaic. It might be expected to be enriched with embryos whose biopsies were mosaic, but the fact that the ranges overlap with the disomic samples means that the results do not provide clean discrimination. The pregnancies that ensue, particularly from results in the lower portion of this newly defined mosaic range, may certainly be from mosaic embryos but may also be from normal embryos whose analytical result simply fell into the upper part of the normal range. Clinical confirmation of mosaicism from the fetuses and placentas of ongoing gestations or from the products of conceptions of clinical losses would be most helpful in confirming that these embryos were in fact mosaic in at least some of the cases.

While already challenging, the reality is that interpreting results is even more complicated. If a result appears to be 20% mosaic, it is entirely possible with reciprocal errors that the sample is completely abnormal, with a mix of 40% monosomic and 60% trisomic cells. Such a result would be indistinguishable at the current time from a sample that was 80% disomic and 20% trisomic. In each case, the chromosome would be overrepresented by approximately 20%. This is significant since an embryo whose results suggest low-level mosaicism may in fact be fully aneuploid with all the attendant clinical risks.

FIGURE 1

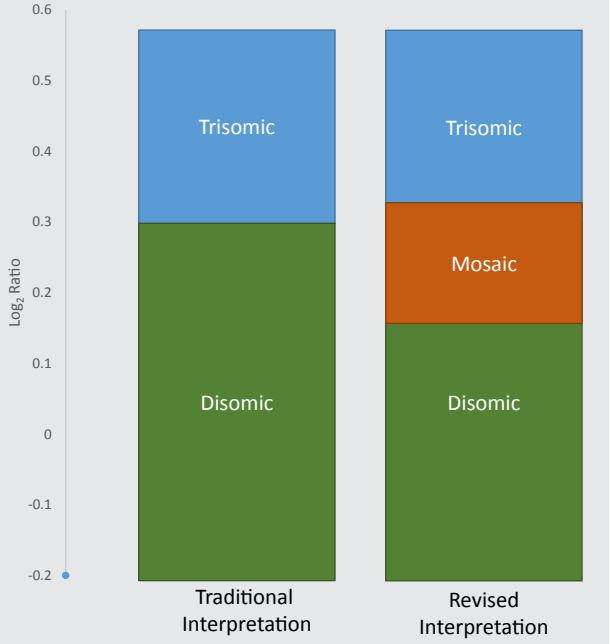

Illustration of the change in interpretation of aCGH results with contemporary PGS screening versus those defining a new midrange designation for biopsies that may contain mosaicism. Note that the actual analysis of the trophectoderm would remain unchanged. The traditional interpretation seeks only to separate disomy from trisomy. The revised interpretation considers values near the prior threshold value separating disomy from trisomy to be considered mosaic.

Scott. Conceptions. Fertil Steril 2016.

Complexities in Managing Mosaic Results

The opportunity to designate some embryos as being at high risk for mosaicism may further enhance the selection process when determining which embryos are suitable for transfer (5).

Discrimination of reproductive potential is unlikely to be as accurate as prior designations. For the reasons stated

previously, a definite diagnosis of mosaicism will not be possible from a single trophectoderm in which all the cells are lysed and the DNA analyzed in aggregate. Rather than calling these embryos mosaic, it is probably better to consider the new range to be "at risk for mosaicism." Results interpreted as mosaic are likely to be a mix of mosaic and pure euploids (lower part of the mosaic range) or pure aneuploids (upper part of the mosaic range). Even when mosaicism is present, it is likely that some reproductive potential remains.

Even if the actual diagnosis is not wholly accurate or precise, there is still a meaningful opportunity to improve clinical outcomes. If mosaic blastocysts have reduced implantation potential, then deselecting those embryos would remove some of the less competent embryos from the pool of transferrable embryos. This should improve implantation rates and reduce loss rates. However, given the reduced accuracy of this result, the gain in implantation rates and pregnancy rates may come at the expense of deprioritizing or even discarding competent embryos. In patients with fewer blastocysts, deselecting reproductively competent embryos may result in a decrease in ongoing pregnancy rates per retrieval. Initial data on the transfer of at risk embryos demonstrates these risks. Implantation rates are reduced by approximately half demonstrating an opportunity to improve per transfer rates at the risk of deselecting or discarding embryos capable of producing healthy children.

It remains to be determined how patients will react to the information that their embryo is at increased risk for an abnormality that may reduce implantation rates, increase loss risk (which is particularly problematic for patients and should not be taken lightly), and even diminish obstetrical and neonatal outcomes. There will be no single answer. When a pregnancy occurs after the transfer of an embryo at risk for mosaicism, the patients' obstetrical team should be informed. The impact on antenatal screening and obstetrical management also remains to be determined.

As with almost all questions that relate to diagnostics assessing embryonic reproductive competence, the key to resolving these complex issues is additional research that will provide better resolution as to which of these embryos are capable of producing healthy children and which are not. Initial steps to be taken include determining the nature of the mosaicism found in human embryos and evaluating the loss rates and range of obstetrical and neonatal outcomes after the transfer of embryos that are at risk for mosaicism. Comparison of different testing platforms to compare accuracy and precision and for the specific bioinformatic algorithms used for statistical smoothing also needs to be done. While one small series has been reported, a much larger nonselection study, where embryos are transferred without regard to their risk of mosaicism, should also be performed (5). Such information would allow more accurate and effective counseling and clinical decision making.

At the current time, patients and clinicians are left to make decisions based not only on the analytical result but on many other factors as well. These include the availability of other euploid blastocysts that do not appear to be mosaic, the patients' prior reproductive history, and their individual perspective on balancing the risks of discarding a competent embryo versus transferring an embryo that may ultimately have a lower implantation potential and possible adverse obstetrical and neonatal outcomes. Ongoing clinical and analytical research are likely to provide greater resolution in the near future and will hopefully enhance efficiency and safety while improving clinical outcomes.

Richard T. ScottJr.M.D.[a]
Daniela Galliano, M.D.[b]
[a] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey; and [b] Instituto Valenciano de Infertilidad, Rome, Italy

http://dx.doi.org/10.1016/j.fertnstert.2016.01.007

You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/scottr-embryonic-mosaicism-pgs/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

REFERENCES

1. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril 2013;100:697–703.
2. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013;100:100–7.
3. Mamas T, Gordon A, Brown A, Harper J, SenGupta S. Detection of aneuploidy by array comparative genomic hybridization using cell lines to mimic a mosaic trophectoderm biopsy. Fertil Steril 2012;97:943–7.
4. Novik V, Moulton EB, Sisson ME, Tran KD, Stern HJ, Stanley WS, et al. The accuracy of chromosomal microarray testing for identification of embryonic mosaicism in human blastocysts. Mol Cytogen 2014;7:18.
5. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med 2015;373:2089–90.