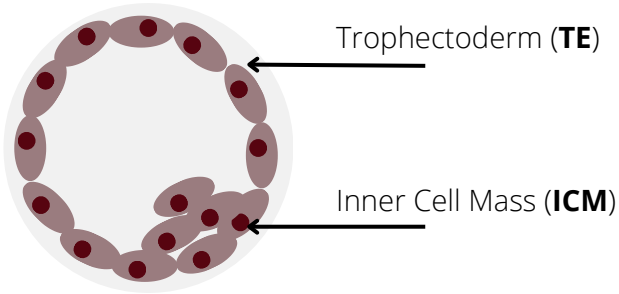
Can we rely on the genetic testing results of one biopsy to inform us of what is going on for the rest of the embryo?

Preimplantation genetic testing for aneuploidies (PGT-A) analysis is performed on cells biopsied from the trophectoderm (TE) of a blastocyst (embryo at about day 5 or 6 of development). Using these results, it is inferred what the chromosome composition is of the entire embryo, particularly the inner cell mass (ICM). TE cells give rise to the trophoblast cells of placenta whereas the ICM gives rise to the fetus.



How have studies determined the concordance of the TE and ICM?

Studies utilize a reference TE biopsy and take subsequent TE or ICM biopsies to compare the results and determine concordance (1, 2, 3, 4).

What did they find out?

The initial TE result matters to how concordant it will be to the ICM. Typically, there are three main category of PGT-A results: euploid, aneuploid, and mosaic. The specific thresholds of how each get categorized varies by lab and by study. For example, some labs use a cut off of <20% for their euploid results whereas another lab may use <30%.

Studies overall found concordance rates or probabilities over 90% for whole chromosome aneuploid and euploid TE results to be concordance to the ICM results. For mosaic and segmental aneuploidies, the concordance rate are less, and it is difficult to predict the chromosome composition using the one TE biopsy result.

What does this mean practically?

For a whole chromosome aneuploid result or euploid result, then it is reasonable to infer that the rest of the embryo is also either aneuploid or euploid, keeping in mind that the studies did not find the concordance to be 100%. Testing the trophectoderm is a limitation of PGT-A analysis. Given this, whole chromosome aneuploids may be deprioritized for transfer whereas euploid may be prioritized.

For mosaic and segmental aneuploidies, the concordance rate are less and more uncertainty is introduced for the rest of the embryo's composition. Rebiopsying of those embryos have not yet become clinical practice, and labs may require director approval if retesting is desired with a new sample. Outcome studies for these kinds of results could be used in determining if transferring embryos with these results is suitable for an individual given their values, goals, and comfort with current data.

Study 1:

Capalbo et al. partitioned 73 embryos into a reference TE sample, three other sets of TE samples, and the ICM. They used the calculation of 73 embryos x 22 autosomal chromosomes x 4 permutations of reference biopsies for a total of 6424 comparisons.

Reference TE result	probability that the other 4 sets would be euploid	probability that the other 4 sets would be aneuploid
euploid (<20%)	99.6%	0%
low grade mosaic (20-30%)	99.3%	0%
medium grade mosaic (30-50%)	95%	1.8%
high grade mosaic (50-70%)	15%	65%
aneuploid (>70%)	1.9%	98%

Study 2:

Victor et al. isolated an average of 7.3 cells from the ICM and in total 15 cells from the TE biopsies of 100 embryos; therefore, there were cells left unanalyzed from the total embryo.

Original TE biopsy result	Aneuploid ICM
Whole Chromosome aneuploidy	90/93 (96.8%)
Segmental Aneuploidy	3/7 (42.9%)

Karyotypic concordance was also evaluated. Karyotypic concordance is when the chromosome abnormality identified in one sample is the same abnormality in the other sample. For example, if Trisomy 21 is identified in the TE biopsy and in the ICM, then the results would be karyotypically concordant. There are times that the ICM or other samples may be classified as aneuploid, yet not necessarily the same abnormality. Of the 93 samples that had aneuploid ICM, 79 had karyotypic concordance. The details can be found on Table 1 from the study. Of note, 3/14 ICMs had mosaic deletions of 1p36 - an established deletion syndrome. When discussing possible outcomes of an aneuploid transfer, it could be considered to discuss the possibility of other persisting aneuploidies, not limited to the one detected on the TE biopsy.

Study 3:

Kim et al. did not separate the ICM as an independent sample. Instead the embryo was partitioned into 4 biopsies and a reference biopsy. The analysis was in regard to the *whole* cohort of samples biopsied. Secondary findings = whole chromosome mosaics (WCM), segmental aneuploids (SegA), and segmental mosaics (SegM).

Result		Concordance
113 euploid 24 secondary findings	137 x 4 (biopsies) = 548 biopsies in total	545/548 (**99.45%**) were concordant, meaning they had the same sex result and did not have whole chromosome aneuploidy
163 aneuploid (some of which had a secondary finding)	241 independent aneuploid chromosomes. A total of 964 (241 x 4) aneuploid chromosomes were expected to be identified in the biopsies	944/964 (**97.9%**) were concordant, meaning they had the same abnormality and sex result

Result	Concordance Rate
segmental aneuploid	180/304 concordance (**59.21%**)
segmental mosaic	69/372 concordance (**18.55%**)
whole chromosome mosaics	57/352 concordance (**16.19%**)

Study 4:

Grkovic et al. re-biopsied embryos that initially received an abnormal segmental result (>80% as the cut off). 111 embryos were initially eligible for re-biopsy, but 1 did not make thaw, 14 degenerated during cell culture, 11 did not meet min criteria for biopsy, and 3 had no results on the second biopsy. The two biopsied results were averaged together.

The study also explored different cut offs for aneuploidy (100% versus 80-95%).

Initial Abnormal Classification	Reclassified as mosaic
deletion	7/21 (**33%**)
complex deletion	6/24 (**25%**)
abnormal duplication	16/19 (**84%**)
complex duplication	3/10 (**30%**)
del and dup	1/8 (**12.5%**)

complex = additional mosaic changes invl same chr, or a seg abnormal of the same type on a different chr.

Study 5:

Result	Concordance rate
euploidy (across all categories)	89-97%
whole chr aneuploidy	94%
all aneuploidy (with seg)	76%
mosaic	42%

Marin et al. performed a systematic analysis of 26 studies evaluating concordance of 1271 embryos. Date was categorized based on what the studies incorporated such as mosaic reporting or segmental aneuploids. The table is a snippet of the overall findings.

What are the possible explanations of concordance and discordance?

The explanation may be due to the testing methodology or because of biological mechanisms. Most of the time, the explanations center on the biological mechanisms such as the knowledge that whole chromosomal aneuploidy is most likely due to meiotic errors originating from the egg cell, thus the abnormality would be expected to persist throughout the embryo. Mosaic results may be the result of mitotic errors of nondisjunction or anaphase lag as the embryo grows and develops. Segmental aneuploidy results may also be due to mitotic errors such as double stranded DNA breaks (6).

What does this review leave out?

Some studies addressed wider questions whereas this summary narrowly focused on concordance of the TE and ICM of particular results and did not focus on PGT-A methodologies or that the biopsy is performed at a single point of development. This review is meant to be a quick reference and not as medical advice. It does not encompass all studies that discuss this topic. This review is not guaranteed to be without inaccuracies. Please reach out to info@modernreproduction.org for further discussion.

Resources:

1. Capalbo, Antonio et al. "Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial." American journal of human genetics vol. 108,12 (2021): 2238-2247. doi:10.1016/j.ajhg.2021.11.002
2. Victor, Andrea R et al. "Assessment of aneuploidy concordance between clinical trophectoderm biopsy and blastocyst." Human reproduction (Oxford, England) vol. 34,1 (2019): 181-192. doi:10.1093/humrep/dey327
3. Kim, Julia et al. "The concordance rates of an initial trophectoderm biopsy with the rest of the embryo using PGTseq, a targeted next-generation sequencing platform for preimplantation genetic testing-aneuploidy." Fertility and sterility vol. 117,2 (2022): 315-323. doi:10.1016/j.fertnstert.2021.10.011
4. Grkovic, Steve et al. "Clinical re-biopsy of segmental gains-the primary source of preimplantation genetic testing false positives." Journal of assisted reproduction and genetics vol. 39,6 (2022): 1313-1322. doi:10.1007/s10815-022-02487-z
5. Marin, Diego et al. "Preimplantation genetic testing for aneuploidy: A review of published blastocyst reanalysis concordance data." Prenatal diagnosis vol. 41,5 (2021): 545-553. doi:10.1002/pd.5828
6. Vanneste, Evelyne et al. "Chromosome instability is common in human cleavage-stage embryos." Nature medicine vol. 15,5 (2009): 577-83. doi:10.1038/nm.1924