The pros and cons of preimplantation genetic testing for aneuploidy: clinical and laboratory perspectives

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Disclaimer: Authors for "fertile battles" are chosen to represent the full breadth of opinions. Individual authors, even within one side of the debate, do not necessarily agree with all viewpoints expressed.

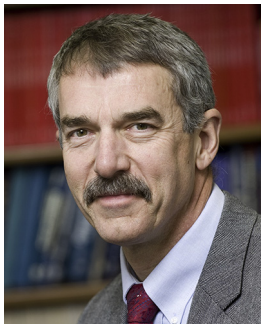
To contribute to a balanced debate, we invited leading proponents and opponents of preimplantation genetic testing for aneuploidy (PGT-A) to briefly summarize their arguments. Four contributors debate the pros and cons from a clinical perspective, two consider technical aspects related to the validation and accuracy of current methodologies, and finally two consider the implications of chromosomal mosaicism, which has recently been identified using next-generation sequencing–based chromosome copy number analysis.

PRO: PGT-A is clinically beneficial and cost effective

Pro 1. Richard T. Scott, Jr., M.D.

Aneuploidy is a major source of adverse outcomes in human reproduction. The impact is so severe, that the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine now recommend universal screening. They have concluded that there is no age group or clinical setting where the risk of an aneuploid gestation is low enough that screening is not beneficial (1). Given the trend in antepartum surveillance is to screen earlier into gestation which is safer for the patients, preimplantation genetic testing for aneuploidy (PGT-A) represents the logical limit.

Given the straightforward logic, why is there such a debate regarding the value of PGT-A? As with most technologies it relates to demonstrating safety, efficacy, and cost effectiveness. To that end, four fundamental questions must be assessed.



CON: PGT-A risks outweigh clinical benefits

Con 1. Richard J. Paulson, M.D.

The preimplantation human embryo represents an unusual life form, whose biology is still not well understood. Blastomeres divide at very high rates, particularly in the TE, increasing the risk for mitotic errors and mosaic aneuploidy, even if the inner cell mass remains euploid. This limits the accuracy of PGT-A. Genetic testing of a sample of cells obtained from the TE may lead to false-positive results: an incorrect diagnosis of aneuploidy, resulting in the potential discarding of embryos that would otherwise lead to normal births. Furthermore, the additional manipulation of embryos that is required to obtain the cells is inherently traumatic, and decreases the live birth potential of those embryos judged to be normal.

The false-positive rate of PGT-A has, at present, been addressed by only one clinical trial (4). In a non-selection study,

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PRO: PGT-A is clinically beneficial and cost effective *(continued)*

Impact of biopsy on embryonic reproductive potential. A randomized paired clinical trial provided robust evidence that trophectoderm (TE) biopsy may be done without adversely impacting the reproductive potential of the embryo (2).

Determine the positive and negative predictive values of PGT-A. A critical step in validating any diagnostic technique is determining the predictive values of the analytical results. Some authors have expressed concerns that PGT-A leads to viable embryos capable of becoming healthy euploid gestations being discarded (3). Their question, the predictive value of an abnormal result, may only be resolved by performing a non-selection study. In these studies, blastocysts are biopsied and transferred prior to performing any analysis. Once the outcome from the cycle is known, the sample is analyzed and it is determined if the analysis correctly prognosticated the clinical outcomes (aneuploidy = no euploid delivery).

A non-selection study using quantitative single nucleotide polymorphism (SNP) array demonstrated a high predictive value for aneuploid results at 96% (4). Unfortunately, this critical validation has not been done for array comparative genomic hybridization (aCGH), quantitative polymerase chain reaction (qPCR), or next-generation sequencing (NGS) based platforms. A large non-selection trial for one of the NGS-based PGT-A platforms is underway. It should be noted that each new testing platform requires a separate non-selection study. Validation cannot be attained by simply running the same samples on two platforms and showing equivalent results.

Randomized clinical trial demonstrating enhanced sustained implantation rates. Multiple randomized controlled trials and meta-analyses in different clinical settings now demonstrate enhanced outcomes when PGT-A is used (5).

Analysis of cost burden to patients based on actual clinical care and outcomes. Neal recently completed a detailed analysis of more than 11,000 actual PGT-A cycles with all ensuing cryopreserved embryo transfer cycles and assessed the total costs of infertility care defined as total costs to either attain a delivery or exhaust the available cohort of embryos and compared them to costs of cycles without PGT-A (6). Cycles with PGT-A have the additional burden of biopsy, aneuploidy analytical testing, and a cryopreserved embryo transfer cycle if any embryos are euploid. Patients without screening have the burden of futile transfers and more pregnancy losses. These data clearly demonstrate that the total cost of infertility care is lower when using PGT-A when two or more blastocysts are available to be screened. In summary, PGT-A provides many benefits and is cheaper than free for all patients except those with only a single blastocyst.

There are other important benefits to PGT-A. Fewer clinical losses, less time to achieve an ongoing pregnancy, and reduced transfer order resulting in the near elimination of multiple gestation (6–8). The latter data are highly credible

CON: PGT-A risks outweigh clinical benefits *(continued)*

Scott et al. (4) transferred biopsied embryos, whose ploidy, as determined by PGT-A, was known only after the transfer was accomplished. In their series, 41.4% (55 of 133) of euploid embryos resulted in live births, whereas only 4% (4 of 99) of aneuploid embryos produced live births. To accurately estimate the false-positive rate, it should be noted that since 41.4% of the euploid group implanted, the 4 implantations in the aneuploid group resulted, logically, from the transfer of 10 euploid embryos. Thus, the false-positive rate of the test in this study was 10/99, or about 10%. This error rate would have resulted in 6.8% (4 of 59) of embryos leading to live births being discarded if the results of the genetic testing had been known prior to transfer and the aneuploid group had not been transferred.

Most PGT-A proponents believe that this is the only source of loss of potential live births with PGT-A. Their reasoning is based on the one study (2) that showed that TE biopsy did not decrease implantation rates. However, that study evaluated only excellent quality blastocysts (grade 4AA or 4BB), all transferred within 3 hours of biopsy, in patients under 35 years of age with at least 2 such blastocysts available for paired analysis. It may not be valid to extrapolate these results to blastocysts with lower morphological grades, in older patients, or to embryos which are cryopreserved prior to transfer.

Clinical outcome data strongly suggest that far more potential live births are lost due to PGT-A, most likely due to the additional manipulation that biopsied embryos undergo. When embryos are discarded as a result of aneuploid designation, the pregnancy success of the remaining embryos should increase proportionately. However, this has not been observed clinically. For example, in a prior analysis (3), implantation rates from the Society for Assisted Reproductive Technology registry (59) were analyzed for women under 35 years of age undergoing elective SET with or without prior PGT-A. Both groups experienced a live birth rate of 50%; no increase in implantation rates with PGT-A was found. These results were similar to those of Kang et al. (60), whose retrospective analysis of elective SET cycles in women $\leq$37 years old also found similar live birth rates of about 50% in women with and without PGT-A. Using an estimated aneuploidy rate of 40% in this age group (2, 4, 61), the implantation rate of the remaining embryos should instead have increased to 83% (3). The difference between the calculated rate of 83% and the actual rate of 50% represents an unexpected 40% loss rate of potential live births.

PGT-A proponents may argue that implantation rates after PGT-A are, in their hands, increased, and that 60% is a better estimate of implantation rates after PGT-A. However, a similar analysis can be used to show that even with this very good, clinically significant improvement in pregnancy rates, 28% of potential live births would nevertheless be lost (3).

Technology doesn't stand still, and accuracy of PGT-A is likely not at its biological limit. With increased accuracy of

PRO: PGT-A is clinically beneficial and cost effective *(continued)*

as evidenced by the Society for Assisted Reproductive Technology recently reducing the recommended transfer order to one when using PGT-A (9).

PGT-A is valuable and cost-effective. Further enhancements will evolve as additional non-selection data become available and experience with the higher resolution NGS-based platforms is gained. In the meantime, the data are overwhelmingly positive and PGT-A is already a meaningful adjunct to assisted reproductive technology.

Pro 2. Michael C. Summers, M.D., Ph.D.

Abnormalities in chromosome number, or aneuploidies, are a common feature of human in vitro fertilization (IVF), and arise primarily due to the inheritance of maternally-derived aneuploidies. The incidence of aneuploid conceptions is strongly correlated with maternal age rising exponentially during the decade before the onset of menopause. This is well illustrated in a recently published summary of all assisted reproductive technology outcomes in Japan in 2015 (10). For women aged 40-45 years, approximately 87,000 embryo transfers (mostly single-embryo transfer [SET]) were performed resulting in about 8,700 live births and 6,900 early pregnancy losses (10). This represents a staggering level of embryo loss in this patient group, when 90% of embryo transfers do not result in a pregnancy and, of those who conceive, 44% suffer a miscarriage. As clinical professionals in reproductive medicine, we need to ask if this is an effective use of time and resources. Perhaps it is time to reassess the current practice of transferring embryos based solely on morphological criteria. We owe this much to our patients, since it is now generally accepted that embryo aneuploidy is the most common reason for failed IVF treatment.

Recent advances in embryo vitrification provide the option for freeze-all embryos at the blastocyst stage, "freeze-only," without compromising pregnancy rates (11). This has led to the view that PGT-A will never improve live birth rates per cycle start, but may lower them if the accuracy is <100% and viable embryos are discarded (12, 13). Freeze-only without PGT-A, however, presents its own challenges: first, the cost and time spent performing multiple transfers of embryos that are genetically unbalanced; and second, the morphological criteria used for embryo cryopreservation. Blastocysts with a poorer morphology score can be euploid, and following transfer result in normal pregnancies and deliveries. It therefore seems likely that clinics performing standard IVF are routinely discarding some normal embryos and worldwide the numbers may be huge.

CON: PGT-A risks outweigh clinical benefits *(continued)*

genetic analysis, the apparent aneuploidy rate may decrease. Embryo biopsy techniques may improve. Both factors would be expected to decrease the loss of potential live births from the cohort of available embryos, thus resulting in an increase in the overall efficiency of PGT-A.

Con 2. Norbert Gleicher, M.D.

Since it was proposed almost 30 years ago (62), the preimplantation genetic screening (PGS) hypothesis has not only remained unconfirmed but has been mostly refuted. Even many of its proponents no longer claim that it improves pregnancy and live birth rates. After initially declaring in 2008 that an earlier version of PGS was ineffective (63), the Practice Committee of the American Society for Reproductive Medicine, now 10 years later, has once again noted that the clinical efficacy of the test, now renamed PGT-A, has still not been established (64). Yet, in a practice likely unprecedented in modern medicine, this ineffective screening test is used to determine whether an embryo can be transferred or should be discarded.

Paulson recently calculated an approximately 40% false-positive rate in PGS-PGT-A (3), which in practical terms means that approximately 40% of embryos recommended for disposal are really chromosomally normal. In other words, PGS/PGT-A not only does not improve IVF outcomes but, actually, adversely affects some IVF cycles by leading to the disposal of large numbers of perfectly normal embryos. Early in 2012, we became convinced that patients most negatively affected by this loss of embryos are poorer prognosis patients with few embryos, who are often prematurely pushed into egg donation under the false narrative that they no longer produce euploid embryos.

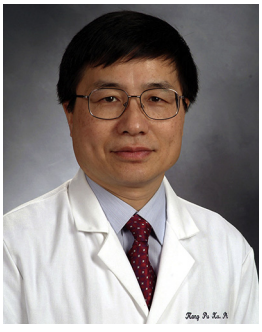
With informed consents in 2012, we began offering such patients transfers of selected "aneuploid" embryos. For the first time at the 2015 American Society for Reproductive Medicine meeting, we reported on five healthy births (65). Shortly thereafter, Greco et al. (14) published six live births after transfers of "mosaic" embryos. Bernabeau's group published a larger series the following year at the European Society of Human Reproduction and Embryology meeting (66), while a 2017 multicenter study (47) added the so-far largest series of normal ongoing pregnancies after transfer of "mosaic" embryos. To date, over 100 healthy births have been reported worldwide following transfers of chromosomally "abnormal" embryos.

The underlying biology of early-stage human embryos explains why PGS/PGT-A simply cannot work, for the following reasons: [1] A 5-7 cell trophectoderm (TE) biopsy,

PRO: PGT-A is clinically beneficial and cost effective *(continued)*

It is axiomatic that the use of PGT-A with freeze-only followed by SET will not improve cumulative live birth rates per cycle start. PGT-A using NGS-based analysis is quite robust for detecting euploid and full copy aneuploid blastocysts. On the other hand, mosaicism and segmental aneuploidies are a challenge, particularly distinguishing a true positive from a technical artefact. Indeed, live births have been reported following the transfer of mosaic embryos (14) even though mosaicism detected in established pregnancies has been associated with early pregnancy loss and intrauterine growth restriction (15, 16). In our own clinic, some TE biopsies reported as chaotic aneuploid/mosaic have been shown to be euploid on further analysis and resulted in successful pregnancies (unpublished data). Furthermore, we do not discard any embryos based on the findings of PGT-A, but simply shift the rank order of embryos for transfer after taking note of the pattern of early embryo cleavage (17, 18) and blastocyst morphology. In our clinic using only SET implantation rates of 80% to 82% and ongoing pregnancy/delivery rates of 66% to 70% depending on the patient group have been maintained for the last two years. To achieve these levels requires attention to detail on every aspect of the IVF/PGT-A process including, but not limited to, clinical protocols, embryo culture, biopsy and cryopreservation, along with a careful review of the PGT-A report and rigorous analysis, when indicated, of the raw data and NGS plots before embryo selection.

PGT-A is potentially a powerful adjunct to standard IVF practice. Preliminary clinical data have demonstrated shorter time to pregnancy, lower clinical losses, and a decrease in higher order pregnancy in selected patient groups. The challenge is identifying the most appropriate patient group(s) where PGT-A shows an unequivocal clinical gain.



Pro 3. Kangpu Xu, Ph.D.

The goal of successful IVF is to select and transfer the single embryo that is likely to develop into a heathy baby (19). Even with contemporary, sophisticated technologies to assess embryo morphology, this goal has yet to be achieved. For patients who have many morphologically high-grade embryos available for transfer, selecting embryos based on genetics is an effective option. Data published by us and others clearly show that transferring euploid embryos into young, as well as older, women results in similar implantation rates (20). One of the key questions is how to identify euploidy?

Recent research on early human preimplantation development has revealed a few specific phenomena. The two most critical issues related to PGT-A are chromosome instability post-fertilization (21) and mosaicism in preimplanta-

CON: PGT-A risks outweigh clinical benefits *(continued)*

even under best assumptions of even distribution of aneuploidy in TE, cannot reliably reflect the whole embryo. A TE biopsy would, indeed, require 27 TE cells to be diagnostic for the TE alone (67). [2] Aneuploidy in the inner cell mass and TE lineages do not perfectly correlate. For example, TE lineage -derived mature placentas frequently demonstrate aneuploid cell islands in the presence of perfectly normal euploid fetuses. [3] Moreover, it appears increasingly likely that abnormal embryos at the blastocyst stage downstream not only self-correct in the mouse (68) but also in humans. What, then, is the purpose of testing embryos on day 5, when aneuploidies disappear on days 6 and 7 further downstream?

In July of 2016, without explanation, the Preimplantation Genetic Diagnosis International Society changed not only the nomenclature to PGT-A but also the guidelines for interpretation and reporting of PGS results (69). A new "threshold concept," based on alleged aneuploid DNA load in a single TE biopsy, now designated embryos as "normal-euploid," "mosaic," or "aneuploid-abnormal." The chosen thresholds of 20% and 80% to demark "normal" from "mosaic" and "mosaic" from "aneuploid," however, have absolutely no scientific basis, and neither do new claims by proponents of PGS/PGT-A that 40% to 50% aneuploid DNA load among mosaic embryos differentiates between better and poorer pregnancy chances (70).

Con 3. Nathan R. Treff, Ph.D.

While it is convenient to classify all methods of aneuploidy screening into a single category (i.e., PGS 2.0 or PGT-A), the many possible methods available for use may provide significantly different results, due to different levels of accuracy. Despite widespread use, validation of a specific method, involving WGA and NGS (i.e., high resolution [HR]-NGS) for PGT-A, remains limited. Concordance levels using blinded positive controls, such as cell lines with known chromosomal imbalance, have not been published, while examples of this study design have been published for several other methodologies (28, 71, 72). Most groups have claimed validity using concordance between aCGH and HR-NGS, using leftover WGA DNA from embryo biopsies (31, 73). Results have suggested near 100% concordance between the two platforms. Interestingly, one group subsequently found that HR-NGS was superior to aCGH, after demonstrating that aCGH euploid embryos which miscarried were found to be mosaic when using HR-NGS on the original WGA sample (57). Results from these studies suggested 38.6%

PRO: PGT-A is clinically beneficial and cost effective *(continued)*

tion development (22). An optimal PGT-A procedure should, therefore, meet at least the following criteria: screen all 24 chromosomes; detect mosaicism in the specimens; identify segmental gains/losses on all chromosomal regions; and be performed at a low cost. Ideally, the platform should also be able to provide other specific genetic information, such as uniparental disomy, triploid, origin of meiotic/mitotic errors, etc.

Early attempts by fluorescence in situ hybridization for chromosomal ploidy were limited to a small number of chromosomes. Comprehensive chromosomal evaluation, which began with comparative genomic hybridization (CGH), opened a new chapter in chromosome copy number assessment (23, 24). However, it took nearly 10 years of intensified research before efficient 24-chromosome tests such as CGH and SNP arrays were commercially produced and validated in PGT-A clinics (25, 26). Although arrays (aCGH and SNP) are still in use, they have clear limitations. In addition, qPCR and digital PCR are platforms used to obtain rapid results (within hours), allowing a same-day biopsy and transfer for patients (27, 28). However, effective cryopreservation renders this fast turnaround time unnecessary for most patients.

NGS (or massively parallel sequencing) technologies aim to interrogate 3 billion base pairs of the entire human genome. Currently, two major NGS platforms are widely used in the field of PGT-A (29, 30). Both platforms have been clinically validated (31, 32). Other new but less well-known platforms have also emerged (33, 34), although their utility in PGT-A is still under evaluation. One study indicated that at very low resolution, results can be obtained within a few hours, allowing same-day biopsy and transfer (35).

Indeed, current NGS-based methods can recognize and quantify mosaicism and segmental alterations in much more detail than other PGT platforms. Moreover, the main advantage for adopting NGS is its enormous potential. Although many laboratories call current NGS-based PGT "high resolution," it should be called "low-coverage whole-genome sequencing" (30). As the cost of NGS decreases, higher resolution can easily be clinically achieved. The steps involved in NGS procedures may also be further optimized, leading to improvements in their performance and results. Chromosome copy number analysis and monogenic disease detection will certainly be clinically feasible in one combined test (35).

It is clear that current NGS platforms are best for meeting the challenge of selecting for optimal embryo viability. It also will have tremendous potential for predicting embryo viability based on, for example, gene expression profiles (36), mitochondria copy numbers (37), parental origin (37), structural changes and segmental alterations (38), and epigenetic information (39). As advancements are made in NGS-based PGT, it may soon become the primary platform globally.

CON: PGT-A risks outweigh clinical benefits *(continued)*

discordance. In contrast, the second group claimed that aCGH could accurately detect mosaicism, and that these so-called mosaic embryos could lead to healthy births (14). These clear discrepancies illustrate the impact of both a lack of sufficient validation prior to clinical utilization, and elective interpretation of results to fit with commercial interest.

The claim that HR-NGS provides "the greatest power to detect mosaic samples" (53) could be similarly characterized, given the lack of supportive evidence. Prediction of mosaicism in blastocysts is marred by technical limitations (49). Performance on aneuploid/euploid cell line mixtures suggests equivalent performance of quantitative real time PCR and HR-NGS (56). This same study illustrates that altering thresholds to improve sensitivity concomitantly increases false-positive predictions from 0 to 1 in 3 (56). In fact, when reanalyzing embryos predicted to be mosaic by HR-NGS, discordance rates approach 50% (53). False-positive mosaicism prediction is an obvious but unacknowledged contribution to both observations made by Greco et al. (14), wherein embryos which resulted in babies were likely uniformly euploid to begin with, and the arguments against the validity of PGT-A made by Gleicher at al. (52), where use of inaccurate methods, rather than true mosaicism, was likely to have led to discrepancies.

In addition to false-positive predictions contributing to overestimation of mosaicism in human embryos, false-negative uniform aneuploidy may also result in significant inaccuracy. In one of the most comprehensive studies performed to date, Katz-Jaffe et al. (74) recently suggested that as many as one third of embryos predicted to be mosaic by HR-NGS were actually uniformly aneuploid. This observation may be relevant to explaining the reduced reproductive potential of so-called mosaic embryos, which has often been used as evidence of validity (47). That is, many embryos predicted to be mosaic are actually uniformly aneuploid, which is a status that is well established to possess little-to-no reproductive potential (4).

At a minimum, studies utilizing HR-NGS for the prediction of mosaicism and aneuploidy should acknowledge the potential contribution of false positives and false-negative uniform aneuploidy when interpreting results, and include critical analysis on the specificity of the methodology. While there appears to be a trend towards avoiding the use of mosaicism prediction altogether, transferring embryos with a result "consistent with possible mosaicism" should coincide with extensive characterization of products of conception, in order to determine the true positive rate of prediction. To date, there is not a single true positive mosaicism prediction reported in the literature, drawing more attention to inadequate demonstration of validity prior to clinical utilization of HR-NGS based PGT-A.

PRO: PGT-A is clinically beneficial and cost effective *(continued)*

Pro 4. Francesco Fiorentino, Ph.D.

Embryonic mosaicism, the presence of a mixture of diploid and aneuploid cell lines within a single embryo, could represent a likely explanation for failed euploid embryo transfer. Its prevalence has been noted to be high throughout embryonic development, with an incidence of 15% to 90% on embryos at cleavage-stage and 30% to 40% at blastocyst-stage (40–45).

Mosaic embryos are not usually transferred because, similar to aneuploidy embryos, they were considered abnormal. However, recent studies demonstrated that diploid/aneuploid mosaic embryos hold the potential to implant and result in the birth of healthy babies (14, 46–48). Since then, many reproductive genetics laboratories are now routinely including embryonic mosaicism information on their diagnostic reports.

These findings have generated an extensive debate on the diagnostic accuracy of mosaicism detection. Opponents to chromosomal mosaicism reporting have concerns on its clinical utility, claiming that false positives may arise as a consequence of technical artifacts that are expected to be introduced by the whole genome amplification (WGA) technique, used to amplify embryonic DNA as the first step of the comprehensive chromosome screening (CCS) process (49–51), or by CCS on low input DNA samples (49). These artefacts might be misinterpreted as true mosaic aneuploidy and potentially result in discarding euploid embryos, thus causing a decrease in the cumulative live birth rate (52).

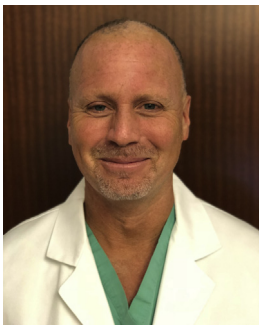
The detection of chromosomal mosaicism in the preimplantation embryos is technically challenging and the accuracy of mosaicism predictions is strictly related with the validation of the CCS assay used in PGT-A.

Validation experiments are based upon determination of threshold values set to discriminate between statistically smoothed data points based upon standard deviations from calibration standards composed of euploid cell lines, as well as mixture models of euploid/aneuploid cell lines simulating chromosomal mosaicism.

When setting these thresholds, euploid/aneuploid cell mixing experiments are performed that allow for established cutoffs. For instance, the first step of the validation process involves analysis of a wide number of euploid samples (including 6-8 cells from euploid cell lines), in order to determine the standard deviation from the euploidy baseline value (2 copies value) and thus define the "euploidy threshold values."

Mixture models of a mosaic TE biopsy are also useful to establish the detection limits of different CCS platforms for whole chromosome mosaic aneuploidy. For instance, mixing experiments with euploid/aneuploid cell lines at different

CON: PGT-A risks outweigh clinical benefits *(continued)*



Con 4. Glenn Schattman, M.D.

Efforts to improve IVF pregnancy rates have been thwarted by the biology of the human preimplantation embryo. As a zygote undergoes mitotic divisions, errors occur at a relatively low but significant rate. Identifying abnormal cells in embryos to improve embryo selection by performing day-3 blastomere biopsies where a single pluripotent cell is removed and analyzed has been shown not to improve pregnancy rates (75). Embryo mosaicism, defined by the simultaneous presence of both normal and abnormal cells within an embryo, has been hypothesized to be one of the possible reasons for these findings. With the advent of extended embryo culture to the blastocyst stage, sampling of multiple TE cells allows for the identification of mosaicism, a phenomenon now known to be common in preimplantation embryos. In fact, in over 29,000 human embryos biopsied at a large, well-established center using NGS, 21.8% of embryos showed evidence of mosaicism (47). These abnormalities ranged from complex mosaic abnormalities with multiple chromosomes involved (4.9%) to simple mosaic (9.6%) and mosaic segmental abnormalities (7.3%). Mosaicism even appears to persist throughout pregnancy, as it is identified in 1% to 2% of chorionic villus samples (45). There is a growing body of evidence that the abnormal cells in a mosaic embryo divide at a slower rate and are preferentially excluded from the embryo during development, suggesting that the few identified abnormal cells at the blastocyst stage may not doom the embryo to certain death (68). In this animal experiment, embryos composed of a 50:50 mix of normal and abnormal cells made viable offspring as often as embryos that were uniformly made up of normal cells. Embryos composed of only 25% normal cells also made viable offspring but at a significantly lower rate. Not surprisingly, this finding has been replicated in human studies where the only available embryos for transfer were those in which the TE biopsy showed some percentage of abnormal cells. In these instances, mosaic embryos were transferred only after extensive counseling. The transfer of embryos with $\leq$50% abnormal cells resulted in normal babies with implantation and delivery rates comparable to embryos that were found to be "euploid." Even with embryos in which the biopsy revealed a >50% aneuploidy rate, there were some implantations and healthy children born, albeit at lower rates than seen with euploid embryos (48). This is of particular concern, as arbitrary thresholds used by many centers for identifying embryos as either normal or abnormal are artificial and lack scientific merit. When there are no euploid embryos available, it has been suggested that selection of mosaic embryos for transfer should be based on

PRO: PGT-A is clinically beneficial and cost effective *(continued)*

percentages (e.g. from 10% to 100%) of aneuploid vs euploid cells would accurately determine the threshold of mosaicism detection, i.e. the lower aneuploidy percentage detectable.

In this cell lines mixture model, any chromosome copy number value deriving from technical artefacts will fall inside the euploidy range, while chromosome copy number values that fall outside this range can confidentially be classified as mosaic aneuploidy.

If the validation of the CCS platform is performed as described above, it is very unlikely that a potential artefact can be misinterpreted as mosaicism (53). Similar validation studies have been performed for aCGH (14, 54, 55), qPCR (56), and more recently for NGS (46–48, 57, 58), demonstrating that these CCS methods are able to reliably detect mosaicism on embryos, with no false-positive predictions reported.

Considering clinical outcomes rather than technical evaluations, multiple studies reported that embryos diagnosed as mosaic have a substantially reduced reproductive competence compared with euploid embryos (46–48), thus demonstrating that mosaicism predictions are not artefacts but real findings. In this view, priority for transfer should be given to euploid embryos, while the transfer of mosaic embryos should be considered an option only for women who undergo IVF resulting in mosaic embryos but no euploid embryos (48).

CON: PGT-A risks outweigh clinical benefits *(continued)*

the percentage and type of mosaicism. However, it should be noted that most patients do not have a large number of mosaic embryos to choose from. In fact, even in the largest programs promoting PGT-A for all patients, most patients have ≤ 3 embryos available to biopsy (76).

Conversely, if mosaicism is a common phenomenon during embryo development, then embryo biopsies that are read as normal may be missing the unsampled aneuploid areas. Certainly, every clinician performing PGT-A recognizes the limitations of this technology and universally encourages all patients to have confirmatory testing once pregnant. Fortunately, false-negative errors are rarely problematic since the overwhelming majority of abnormal embryos do not implant or progress to clinical pregnancies.

Mosaicism of a single chromosome or segment is a common event that unfortunately limits the predictive ability of PGT-A. The definitive test of embryo viability is still lacking. While PGT-A may improve the pregnancy rate or implantation rate for the benefit of the clinic, it may do so at the expense of reducing the probability of pregnancy for the patient by erroneously discarding viable embryos. Even expanded blastocysts deemed normal by PGT-A testing have only an approximately 60% implantation rate associated with a biochemical/miscarriage rate of up to 26% (77).

Before starting stimulation in patients planning on embryo biopsy for PGT-A, clinicians must address the limitations of these technologies with their patients. Patients must be informed that due to misidentification both with false-positive and -negative errors, normal embryos may be discarded while abnormal embryos may be replaced due to mosaicism. Overall probabilities of having a child per oocyte retrieval will not be improved by genetic screening of embryos.

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