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REVIEW

Preimplantation genetic testing for aneuploidy: what technology should you use and what are the differences?

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Abstract

Purpose The purpose of the review was to define the various diagnostic platforms currently available to perform preimplantation genetic testing for aneuploidy and describe in a clear and balanced manner the various strengths and weaknesses of these technologies.

Methods A systematic literature review was conducted. We used the terms "preimplantation genetic testing," "preimplantation genetic diagnosis," "preimplantation genetic screening," "preimplantation genetic diagnosis for aneuploidy," "PGD," "PGS," and "PGD-A" to search through PubMed, ScienceDirect, and Google Scholar from the year 2000 to April 2016. Bibliographies of articles were also searched for relevant studies. When possible, larger randomized controlled trials were used. However, for some emerging data, only data from meeting abstracts were available.

Results PGS is emerging as one of the most valuable tools to enhance pregnancy success with assisted reproductive technologies. While all of the current diagnostic platforms currently available have various advantages and disadvantages, some platforms, such as next-generation sequencing (NGS), are capable of evaluating far more data points than has been previously possible. The emerging complexity of different technologies, especially with the utilization of more sophisticated tools such as NGS, requires an understanding by clinicians in order to request the best test for their patients..

Conclusion Ultimately, the choice of which diagnostic platform is utilized should be individualized to the needs of both the clinic and the patient. Such a decision must incorporate the risk tolerance of both the patient and provider, fiscal considerations, and other factors such as the ability to counsel patients on their testing results and how these may or may not impact clinical outcomes.

Capsule This review discusses the current technologies available for aneuploidy testing and highlights the current indications for its clinical use.

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Introduction (200)

In vitro fertilization (IVF) is a technology that has transformed the field of infertility medicine [1–3]. While many IVF-associated interventions have emerged over the past decades, few have garnered more attention, both from professionals and society at large, than the introduction of preimplantation genetic testing (PGT) [2]. The most commonly utilized application of PGT currently is through aneuploidy testing (PGS, CCS, PGD-A).

Spontaneous miscarriages in human pregnancies are documented to be highly associated with chromosomal aneuploidy [2, 4, 5]. Aneuploidy is the most common cause of reproductive failure throughout nature [4, 6]. PGS was introduced to

minimize aneuploid pregnancies in infertile patient populations undergoing IVF. PGS is a procedure in which cell(s) can be biopsied from cultured early embryos and tested for their chromosome complement prior to uterine transfer [2]. Therefore, PGS attempts to identify euploid embryos for uterine transfer and thereby both increase implantation rates and decrease miscarriage rates associated with IVF.

Data associated with PGS has been highly variable over the past 10–15 years. In general, data generated from PGS trials 5–10 years ago are markedly different than those from PGS trials performed in more recent years [5]. These differences are most likely due to the dramatic improvements in the application of PGS over time and the ability to test blastocysts [2]. This review will briefly outline both the evolution of PGS and current recommendations for its use. Additionally, the review will outline the various technologies used currently to perform PGS along with their relative advantages and disadvantages.

PGS: the early days

The first successful PGT procedure was performed by Dr. Alan Handyside in 1990 [7]. This case utilized the identification of the Y chromosome to reduce the chances of having a child with an X-linked recessive disease known to exist in the mother. Soon after this case, geneticists began attempting PGS using fluorescence in situ hybridization (FISH) for three chromosomes on either a single blastomere from cleavage stage embryos or a polar body [2, 8, 9]. This was done in an attempt to reduce the risk of a spontaneous miscarriage or the birth of a baby with a genetic syndrome. Over time, FISH was used to identify aneuploidy for 12 or more chromosomes [2, 9].

Initial data from these early techniques generated much excitement within the field of reproductive medicine. However, in 2007, a prospective and randomized study published in the New England Journal of Medicine failed to show an increase in pregnancy rates associated with PGS [10, 11]. Following this report, other studies followed failing to demonstrate a benefit of PGS in terms of pregnancy outcome. Subsequently, multiple major professional societies including the American Society for Reproductive Medicine (ASRM), the American College of Obstetrics and Gynecology (ACOG), and the European Society of Human Reproduction and Embryology (ESHRE) all released formal opinions discouraging the general use of PGS [12–14].

PGS: current state

Following the discouraging results associated with FISH utilizing cleavage stage embryo and polar body biopsies, geneticists, physicians, and embryologists began developing and utilizing new tools and techniques that dramatically changed the PGS process. This paradigm shift has been facilitated by several significant advances in the manner PGS is currently performed and includes the following:

1. The ability to simultaneously evaluate the ploidy status of all 23 chromosome pairs
2. The ability to perform trophectoderm biopsy
3. The ability to vitrify embryos following biopsy

A clear benefit to PGS utilizing 23 chromosome pair evaluation at the trophectoderm stage has been demonstrated in multiple prospective randomized trials [15–20]. Additionally, the use of embryo vitrification following PGS biopsy has allowed the process of PGS to be performed with much more flexibility, leading to increased utilization of the technology, particularly in the USA. Patient populations evaluated in studies demonstrating a benefit of PGS are many and include recurrent pregnancy loss and unexplained infertility [2, 19]. The clinical utilization rate of PGS has dramatically increased in recent years, and this trend is likely to continue for the foreseeable future.

The ASRM Practice Committee in March of 2016 issued a Brief Communication on Preimplantation Genetic Screening for Aneuploidy [21]. This stated that "The use of PGS as a universal screening test for IVF may demonstrate higher live birth rates with increased elective single embryo transfer utilization." This is the first international professional committee recommendation stating that PGS demonstrates higher live birth rates and should be considered to reduce multiple gestations.

Why is the data different now?

As previously mentioned, there are several key differences in the way in which PGS is recommended to be performed currently, using 23 chromosome pair evaluation with trophectoderm biopsy, as compared to FISH evaluation with cleavage stage or polar body biopsy.

Perhaps most significant of these differences is the ability of more recent technologies to reliably identify aneuploidy on all 23 chromosome pairs. In FISH evaluations, only a discrete number of chromosomes could be evaluated, typically 9–12 chromosomes at a time [8, 22]. Early miscarriage data from failed pregnancies suggested that certain chromosomes were much more likely than others to be involved with failed aneuploid pregnancies [2]. Therefore, in the early days of PGS, many experts believed that focusing on these "problem chromosomes" would enable them to identify the major chromosomes associated with aneuploidy. However, recent data from 23 chromosome evaluation of embryonic DNA shows a relatively equal distribution of aneuploidy among all 23

chromosome pairs [23]. Therefore, technologies that cannot detect aneuploidy in all 23 chromosome pairs, such as FISH, are at a significant disadvantage to identify aneuploidy as many aneuploid errors will be on chromosomes simply not evaluated utilizing this technology.

Another significant advance is the utilization of trophectoderm biopsy as opposed to biopsy performed at the cleavage stage or with polar body biopsy [2]. There are several reasons why this change resulted in such an improvement in outcome success. One of the most relevant of these reasons is that of embryo mosaicism. In many embryos, there are multiple different cell lines—a condition known as mosaicism [24–27]. This means that that the concept of all cells being derived from a common initial division cannot be wholly accurate. Therefore, not all aneuploid errors stem from meiotic non-disjunction and some ploidy status changes must occur after fertilization (mitotic non-disjunction). Data report the rate of mosaicism at the cleavage stage of development to be as high as 50 % [25]. While mosaicism does persist at the trophectoderm stage, the rate of mosaicism is markedly lower, approximating 3-5 %, than that at earlier stages of development [28–30]. Therefore, it is possible that the cell biopsied and tested during PGS may not be representative of the ploidy status of the other cells comprising the embryo. Thus, mosaicism makes it possible to have a clinical misdiagnosis, a result that does not match the clinical outcome (placenta or fetus) even in cases where an accurate cellular genetic diagnosis has been performed [29]. Logically then, a trophectoderm biopsy at the blastocyst stage, where lower rates of mosaicism exist, would be more accurate in predicting the preponderance of cells within the embryo compared to earlier stages of development, where higher rates of mosaicism exist.

In addition to an inherently higher clinical misdiagnosis rate, PGS biopsy at the cleavage stage confers a deleterious effect on embryo development as compared to trophectoderm biopsy [31]. Data evaluating the impact of cleavage stage embryo biopsy shows a significant developmental lag that is associated with the biopsy process itself [31]. Therefore, cleavage stage biopsy is inferior to trophectoderm biopsy in terms of both diagnostic accuracy and embryo trauma.

What are some current and future challenges for PGS?

Even when embryo biopsy performed at the trophectoderm stage with evaluation of all 23 chromosome pairs is evaluated, the likelihood of achieving and maintaining a pregnancy is not certain [2]. Other factors, such as an altered ratio of mitochondrial copy number to nuclear DNA suggesting embryonic stress, de novo clinically significant deletions (dels) or duplications (dups), autoimmune factors, endometrial receptivity,

endocrinologic abnormalities, anatomic abnormalities, or other factors that are currently unknown or undefined, likely play a role in maintaining a pregnancy [32].

However, despite the fact that there are multiple variables involved in the processes surrounding implantation and early fetal development, ploidy status remains a significant and requisite component of a successful pregnancy. PGS data clearly shows that, while the incidence of fetal aneuploidy can be reduced with PGS, it cannot be eliminated entirely using current technology in large part due to embryo mosaicism [2, 29]. Current diagnostic technologies test cells destined to become the placenta and not cells from the inner cell mass which differentiates into the fetus. Biopsy of the inner cell mass is discouraged because of concerns regarding future differentiation of the fetus.

Mosaicism can exist within the cells comprising the trophectoderm [24]. The ability to identify aneuploid/euploid mosaicism within the trophectoderm presents a significant challenge, and strategies have been implemented to overcome this issue. One strategy is to biopsy approximately 5–10 cells and to use a testing technology capable of identifying mosaicism at this level. However, embryo trauma induced from the biopsy process must be weighed in making the decision of how aggressively to perform the trophectoderm biopsy.

All aneuploidy testing technologies are not equal

Another significant factor to improve the clinical outcome of IVF/PGS is to understand that all genetic testing for aneuploidy is not the same. When validated properly, all genetic technologies will equally identify "whole chromosome" aneuploidy [33, 34]. However, some genetic technologies in addition to providing whole chromosome aneuploidy are also capable of identifying large (>50 Mb) segmental chromosome duplications (dups) or deletions (dels) [35, 36].

New and enhanced genetic technologies will provide whole chromosome aneuploidy and the identification of large segmental dels or dups (>50 Mb) and mitochondrial copy number and can also simultaneously identify de novo clinically significant dels and dups (~800kb - 2Mb) [37, 38]. Hence, the ordering physician must understand that "*all aneuploidy testing technologies are not equal.*" Physicians should command a clear understanding of the pros and cons of each technology in order to optimize a patients' chance of achieving a viable pregnancy and the birth of a healthy baby.

What is the best platform currently available to perform PGS?

A common challenge facing many reproductive medicine physicians is which genetic diagnostic platform and which

company to use in performing PGS testing. There are certain diagnostic technologies, such as FISH, which are not recommended according to current medical literature. All of the diagnostic testing platforms that are capable of testing for all 23 chromosome pairs have comparable efficacy in identifying whole chromosome aneuploidy but differ widely in their ability to simultaneously identify other structural chromosome abnormalities or mitochondrion copy number. Furthermore, some diagnostic testing platforms can also simultaneously test for chromosome aberrations and single gene mutations.

The following summary provides an overview of each platform commonly utilized for PGS genetic diagnostic technologies.

Microarrays

In the early to mid-2000s, several laboratories began developing new technologies offering the ability to test for all 23 pairs of chromosomes for aneuploidy, simultaneously testing for structural chromosome aberrations [22, 39–42]. There are

two main types of microarrays available for genetic testing. These are single-nucleotide polymorphism (SNP) and comparative genomic hybridization (CGH) arrays. The differences between SNP and CGH are extensive.

For both microarray platforms, the trophectoderm cells must be lysed and amplified by some type of DNA amplification protocol that provides whole genome coverage. As with any genetic test, the quality of the diagnostic result begins with the quality of the amplified DNA sample.

SNP microarrays

SNPs (pronounced *snips*) are pairs of single nucleotides (A, T, C, or G) in genomic DNA that are highly variable within a given species (Fig. 1). In the context of PGS, the SNPs evaluated are generally in non-exon coding segments of the genome. SNP microarrays in PGS typically evaluate approximately 300,000 SNPs spaced throughout the genome [2, 42, 43]. SNP arrays provide a genotype (AA, AB, or BB) for each sample analyzed and compare these results to a human hap map reference genome.

Fig. 1 Single-nucleotide polymorphism (SNP) arrays. Figure description: In this *figure*, a diploid chromosome (chromosome 21 on *left*) and a trisomy chromosome (chromosome 21 on *right*) can be seen on SNP array. Note presence of the A, B, and heterozygote AB band in the diploid sample and the two heterozygote bands (AAB and ABB) associated with the trisomy sample
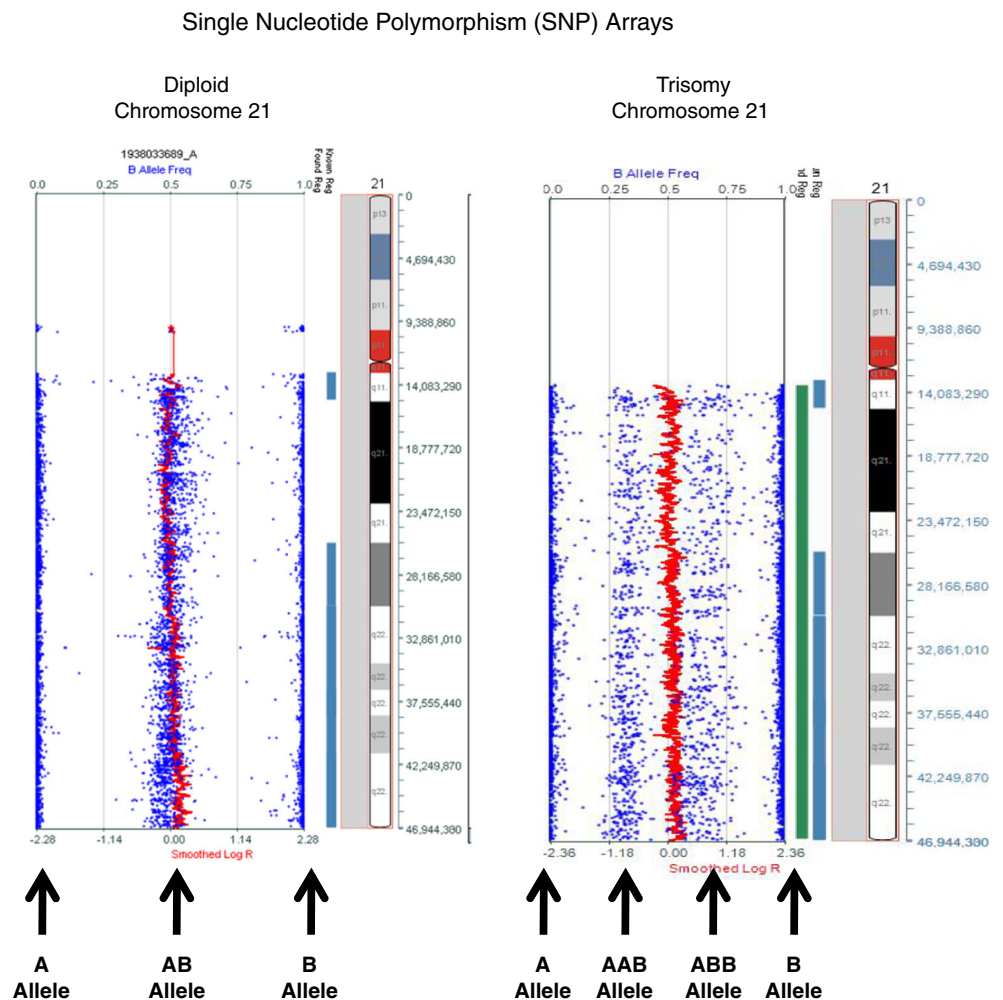
These arrays can identify whole chromosome aneuploidy and can also identify approximately 250 common structural chromosome aberrations throughout the genome. However, there are hundreds more de novo structural chromosome abnormalities that are below the resolution of the 300,000 SNP arrays used for PGS that could play a significant role in implantation, miscarriages, or giving birth to a baby with a serious genetic syndrome. Since genotype information is provided, these SNP arrays have a limited ability to identify triploidy but can identify uniparental disomy. SNP arrays can also identify mosaicism if enough trophectoderm cells are analyzed. One limitation of the SNP arrays used for PGS by some reference laboratories is the inability of their algorithm to identify copy number when husband and wife are related (consanguinity). Hence, no aneuploidy result will be reported if any consanguinity exists between the two partners.

CGH microarrays

CGH microarrays (aCGH) are less dense than SNP microarrays. aCGH chips used for PGS have approximately 4000 markers (run in duplicate) spaced throughout the genome (Fig. 2). aCGH is a ratio labeling protocol where the clinical sample is compared to normal 46, XY and 46, XX DNA samples [2, 34, 39, 40, 44]. CGH arrays are capable of being completed in a shorter timeframe as compared to SNP arrays. CGH array platforms are able to amplify DNA and complete

the entire analysis in as short as 12–15 h. This is a significant advantage over SNP arrays which take approximately 30 to 40 h to complete the analysis. There are no genotypes produced as are generated in SNP arrays, and thus, aCGH cannot differentiate between 46, XX from 69, XXX or 46, XY from 69, XXY. Additionally, aCGH cannot identify uniparental disomy. The aCGH used for PGS by all commercial laboratories can only identify whole chromosome aneuploidy and is not designed or validated to identify structural chromosome aberrations. If aCGH chips are validated against mosaic cell samples, aCGH does have a limited ability to identify mosaicism within a trophectoderm sample.

Some have suggested that aCGH has an approximate 15–30 % error rate [45]. In the validation studies conducted in our laboratory comparing DNA by next generation sequencing to aCGH, on over 400 trophectoderm samples, a greater than 99 % concordance rate was demonstrated. In this validation study, there was a single discordant diagnosis between a borderline trisomy 19 by aCGH that was diagnosed as normal by next generation sequencing [46].

Quantitative PCR or real-time PCR

Quantitative PCR (qPCR) or real-time PCR (RT-PCR) is a polymerase chain reaction assay that can identify whole chromosome aneuploidy by detecting the copy number of each
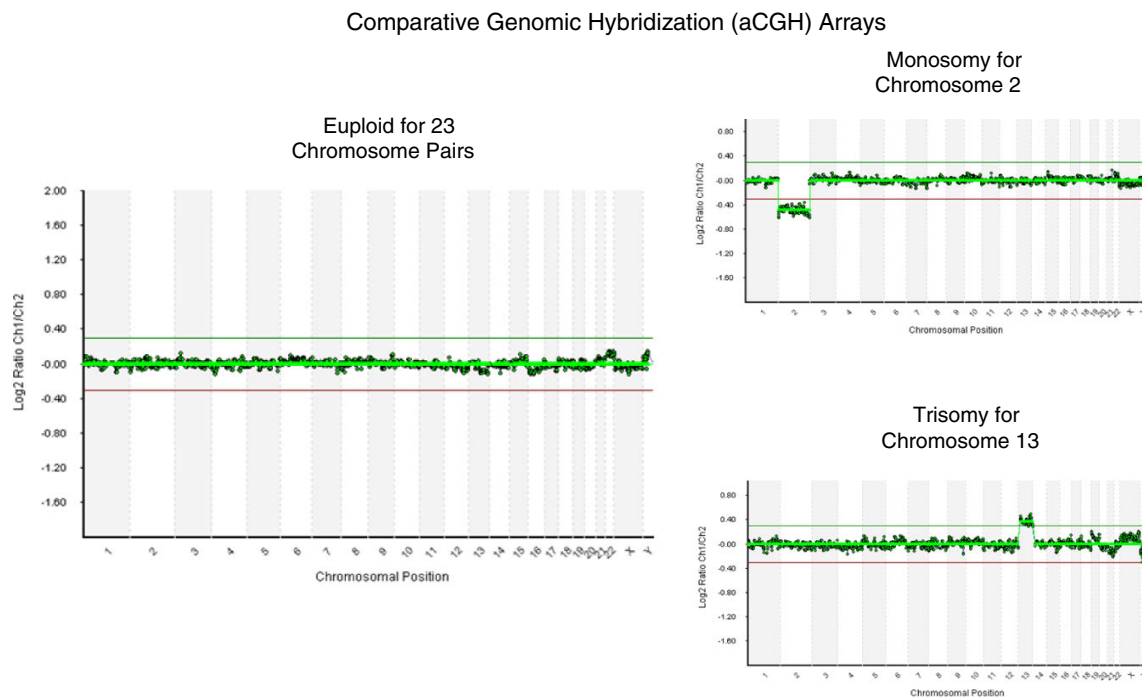

Fig. 2 Comparative genomic hybridization (CGH) arrays. Figure description: This *figure* shows examples of several different samples evaluated by CGH array for PGS. The *figure* on the *left* is diploid with a relatively equal ratio of green/red fluorescence in all 23 pairs of chromosomes. The *figure* on the *top right* shows monosomy for chromosome 2 with a clear downward deviation of the plotted line indicating a relative lack of green, as compared to red, signal intensity. Conversely, the *bottom right figure* shows trisomy for chromosome 13 with a clear upward deviation of the *plotted line* indicating a relative increase of green, as compared to red, signal intensity

chromosome analyzed [47, 48]. It does this by comparing three or four locus-specific amplicons along each chromosome to a reference gene from the same chromosome (Fig. 3). This assay can easily identify aneuploidy for all 23 pairs of chromosomes in a rapid fashion (4–12 h; depending upon the number of samples analyzed), but it is very labor intensive and automation is highly recommended [47, 48]. qPCR is unable to accurately identify structural chromosome aberrations but can identify triploidy. Since qPCR does not generate a genotype, it cannot identify uniparental disomy [45, 47]. A separate experimental design can be included to detect mitochondrion copy number.

Next generation sequencing

Next generation sequencing (NGS) is a technology that requires optimized DNA amplification to reduce the introduction of artifacts during the amplification process [49, 50]. Following the DNA amplification, artifacts can be identified and removed by bioinformatics software.

There are two main platforms currently being used for PGT. These are the MiSeq from Illumina and the Personal Genome Machine (PGM) from Thermo-Fisher Scientific [37, 38, 51–53]. Following DNA amplification, approximately 50 ng of each DNA sample is enzymatically digested into millions of fragments and pooled for library preparation. Library preparation is where all of the DNA fragments are fused with an adapter and a barcode. A robust library produces a representative, non-biased representation of nucleic acids and is critical for accurate molecular analysis.

Following library preparation, either an emulsion PCR step is accomplished (PGM) or a bridge PCR step is done (MiSeq). Upon completion of these steps, significant differences exist in the actual sequencing of the DNA fragments.

Quantitative PCR (qPCR)
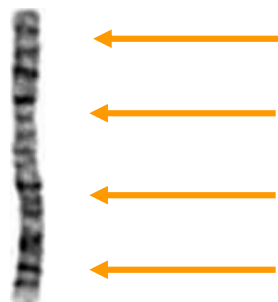**or**
Real-Time PCR (RT-PCR)

Fig. 3 Quantitative PCR (qPCR) or real-time PCR (RT-PCR). Figure description: This *figure* shows a representation of the regions of a given chromosome evaluated by qPCR or RT-PCR technology. Note the very low number of regions evaluated by this technology

For the MiSeq, an optics-based sequencing by synthesis occurs (Fig. 4). The PGM uses an ion-sensitive field effect which can detect ions as they are released by DNA polymerase during sequencing by DNA synthesis. This is based upon the release of a hydrogen ion which occurs each time a nucleotide triphosphate is added. The proton release causes a slight pH shift which is detected by a sensitive sensor (Fig. 5). Both the MiSeq and the PGM sequence the entire genome at a depth of approximately 1X and compare the sequenced data to a human hap map reference genome. Both platforms permit the simultaneous analyses of 22 (MiSeq) to 60 (PGM) DNA samples at a time. Both platforms can complete the analyses (from DNA amplification to the generation of a final report) in 13 to 16 h.

Following the sequencing analysis, significant differences exist between the MiSeq and the PGM data output and analysis. The MiSeq DNA undergoes a first round of quality assurance metrics which is followed by a detailed analysis using BlueFuse software. The PGM DNA output undergoes a first round of quality assurance metrics using a Torrent Browser followed by a detailed analysis by the Ion Reporter software.

The MiSeq can identify whole chromosome aneuploidy but is only designed for the identification of whole chromosome aneuploidy. As Illumina states in their technical bulletin specifications for PGS using their VeriSeq genome analysis on the MiSeq, "VeriSeq is intended only to detect whole genome aneuploidy and mosaics down to a level of 50 %" [38, 46]. It should not be used for detecting any structural chromosome abnormalities. The MiSeq can also identify mitochondrial copy number.

In contrast, the PGM analysis can identify whole chromosome aneuploidy, large dels or dups, and clinically significant dels or dups down to a resolution of approximately 800 kb to 1 Mb [37, 46]. The PGM can also identify mosaicism down to approximately the 20 % level and mitochondrial copy number.

In fairness, some commercial laboratories using the MiSeq do report out large structural chromosome abnormalities and mosaicism at a level below 50 %. Additionally, some laboratories using the PGM only report out copy number for aneuploidy and no structural chromosome aberrations or mosaicism.

Both the PGM and the MiSeq can also test for single gene mutations.

Another variation using the MiSeq NGS platform, termed low density NGS or LD-NGS, offered by one reference laboratory applies NGS technology with less resolution (fewer tested data points) than is reported by the other NGS technologies described in this paper [54] (Fig. 6). Their DNA amplification protocol differs from whole genome amplification in that they amplify approximately 10,000 genomic loci throughout the genome. Once complete, they do next generation sequencing on these amplified products. This NGS
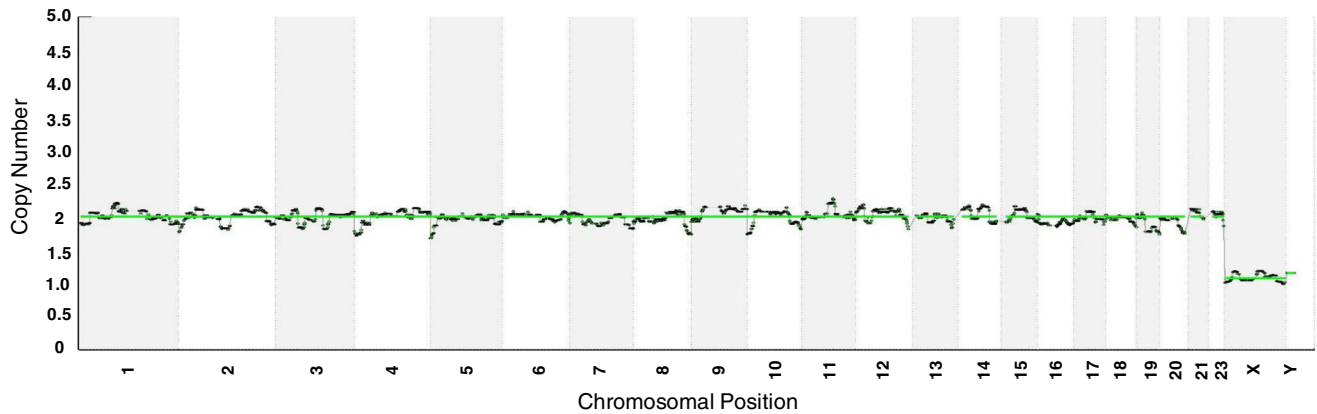
46, XY As Evaluated By NGS: MiSeq

Fig. 4 46, XY as evaluated by NGS: MiSeq. Figure description: In this *figure*, a 46, XY diagnosis can be seen on NGS MiSeq data report

methodology provides sequencing information at each locus but not between each locus (in contrast, the PGM technology sequences tiles across the entire length of each chromosome). This reference laboratory technology is only validated for whole chromosome aneuploidy and not segmental aneuploidy (>50 Mb) or clinically significant dels or dups. This methodology also cannot detect mosaicism. If approximately 50 % of the sample is a mosaic, they classify this embryo as abnormal, not mosaic abnormal.

It is paramount that healthcare providers recommending these diagnostic platforms must understand the differences between the available NGS technologies and never "assume" that they are all equal.

What technology should you use for your patients?

PGS is a powerful new tool in the optimization of assisted reproductive technologies. The evolution of how PGS is performed has dramatically matured and improved over the past decade. Current medical literature supports the contention that PGS improves ART results in many patient populations. Current recommendations support the use of technologies that evaluate the ploidy status of all 23 chromosome pairs at the blastocyst stage with trophectoderm biopsy when performing PGS. Future studies evaluating the data in a directly comparative manner are needed to more completely

46, XX, del(13q) and 46, XX, dup(9p)
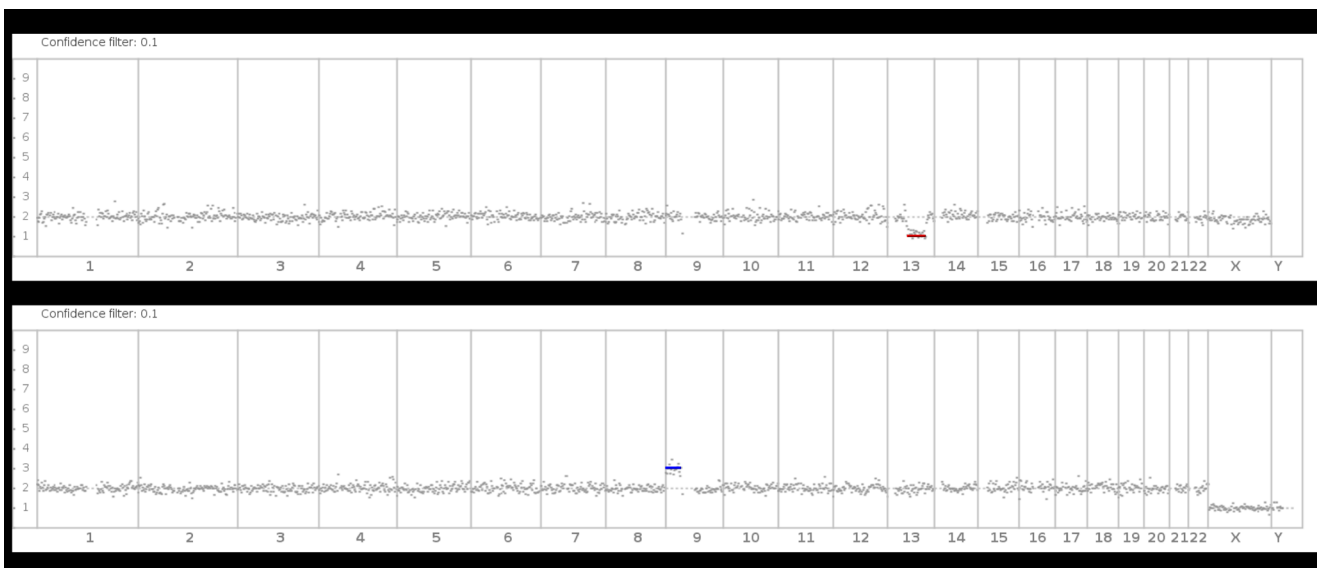## As Evaluated By NGS: PGM

Fig. 5 46, XX, del(13q) and 46, XX, dup(9p) as evaluated by NGS: PGM. Figure description: In this *figure*, a 46, XX, del(13q) and 46, XX, dup(9p) as evaluated by NGS: PGM diagnosis can be seen on NGS PGM data report. The *figure* shows del(13q) with a clear downward deviation of the *plotted line*. Conversely, dup(9p) is demonstrated with a clear upward deviation of the *plotted line*

Fig. 6 Comparison of PGS technologies. Figure description: This *figure* summarizes the relative strengths and weaknesses of diagnostic platforms currently utilized in PGS. The technologies are listed along the *x-axis* with diagnostic capabilities listed along the *y-axis*

	SNP	aCGH	RT-PCR	MiSeq NGS	Low Density NGS	PGM NGS
Whole chromosome aneuploidy	X	X	X	X	X	X
Mosaicism	X	+/-		X		X
Triploidy	Depends on DNA quality +/-		X	X		X
Large deletions (del) or duplications (dup) (> 50 Mb)	X			Not designed to detect +/-		X
Clinically significant dels or dups (~ 800 kb – 1 Mb)	Depends on the size and chromosome location					X
Uniparental disomy	X			X		X
Mitochondrion copy number			X	X		X

define the relative merits of the different diagnostic technologies currently utilized in PGS.

PGS is emerging as one of the most valuable tools to enhance pregnancy success with assisted reproductive technologies. The emerging complexity of results, especially with the utilization of more sophisticated tools such as NGS, may lead to a different way of resulting PGS results in coming years. As rates of mosaicism are quantified in NGS results, mosaics of non-viable chromosomes could be considered for transfer when there are no euploid embryos are available. One could imagine a time in the near future when PGS results are given with all rates of mosaicism identified and, in addition to a clear recommendation to or not to transfer, also have a "middle category" in which some embryos with aneuploid/euploid mosaics could be considered for transfer when there are not euploid embryos available. In such a scenario, patients would be counseled extensively on the implications of such a transfer. Also, in such scenarios, the type of ploidy would be quite important, with possible non-lethal aneuploid-euploid mosaics, such as embryos harboring some level 46, XX/ 46, XXY or 46, XY/47, XYY being considered, not considering for uterine transfer any embryos with viable genetic syndrome mosaics such as 46, XY/47, XY, +13 or 46, XX,/47, XX, +18. One could also consider the transfer of all lethal monsomies (except for chromosomes 13, 18,

21, X, or Y) if no euploid embryos are available for transfer. Our laboratory has employed SNP and CGH microarrays, qPCR analysis, and both the MiSeq and PGM NGS platforms. Ultimately, the choice of which diagnostic platform is utilized should be individualized to the needs of both the clinic and the patient. Such a decision must incorporate the risk tolerance of both the patient and provider, fiscal considerations, and other factors such as the ability to counsel patients on their testing results and how these may or may not impact clinical outcomes.

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54. Wherever testing can show the way forward, Good Start Genetics offers an option. 2016. at https://www.goodstartgenetics.com/clinicians/genetic-testing-services-portfolio/.)