# Preimplantation Genetic Testing and Related Services (for Nebraska Only)

**Policy Number**: CS160NE.A
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Instructions for Use

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**Related Policy**

- Chromosome Microarray Testing (Non-Oncology Conditions) (for Nebraska Only)

## Application

This Medical Policy only applies to the State of Nebraska.

## Coverage Rationale

**Preimplantation Genetic Testing (PGT) for monogenic/single gene defects (PGT-M) or inherited structural chromosome rearrangements (PGT-SR) is proven and medically necessary using polymerase chain reaction (PCR), next generation sequencing (e.g., chromosomal rearrangements), or chromosomal microarray for the following**:

- The embryo is at increased risk of a recognized inherited disorder with both of the following:
  - The increased risk of a recognized inherited disorder is due to one of the following:
    - The parents are carriers of an autosomal recessive disease
    - At least one parent is a carrier of an autosomal dominant, sex-linked, or mitochondrial condition
    - At least one parent is a carrier of a balanced structural chromosome rearrangement
  - The medical condition being prevented must result in Significant Health Problems or Severe Disability and be caused by a single gene (PGT-M) or structural changes of a parents' chromosome (PGT-SR)
- Human leukocyte antigen (HLA) typing on an embryo in order for the future child to provide bone marrow or blood to treat an affected sibling

**PGT is unproven and not medically necessary for all other populations and conditions due to insufficient evidence of efficacy.** This includes but is not limited to PGT using chromosome microarray, PCR, or next generation sequencing for the following:

- Aneuploidy screening (PGT-A)
- Determining gender when the embryo is not at risk for a sex-inked disorder
- Predicting risk of polygenic disorders (PGT-P) and/or embryo selection based on polygenic scores (ESPS)

**Note**: PGT must be ordered after genetic counseling.

# Definitions

**Preimplantation Genetic Testing (PGT)**: A test performed to analyze the DNA from oocytes or embryos for human leukocyte antigen (HLA)-typing or for determining genetic abnormalities. These include:

- PGT-A: For aneuploidy screening (formerly PGS)
- PGT-M: For monogenic/single gene defects (formerly single-gene PGD)
- PGT-SR: For chromosomal structural rearrangements (formerly chromosomal PGD) (Zegers-Hochschild et al., 2017)

**Significant Health Problems or Severe Disability**: A disability or impairment that is physical or mental and substantially limits one or more major life activities. The impairment is expected to last at least 12 months or result in death (Department of Labor; Office of Disability Employment Policy; Federal Government Definition for Social Security Disability Benefits).

# Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by federal, state, or contractual requirements and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

**Coding Clarification**: For preimplantation genetic testing, please refer to the codes identified below with an asterisk (*).

| CPT Code | Description |
|---|---|
| 0254U | Reproductive medicine (preimplantation genetic assessment), analysis of 24 chromosomes using embryonic DNA genomic sequence analysis for aneuploidy, and a mitochondrial DNA score in euploid embryos, results reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplications, mosaicism, and segmental aneuploidy, per embryo tested |
| 0396U | Obstetrics (pre-implantation genetic testing), evaluation of 300000 DNA single-nucleotide polymorphisms (SNPs) by microarray, embryonic tissue, algorithm reported as a probability for single-gene germline conditions |
| 81228 | Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis |
| 81229 | Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis |
| 81349 | Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and loss-of-heterozygosity variants, low-pass sequencing analysis |
| 81479 | Unlisted molecular pathology procedure |
| *89290 | Biopsy, oocyte polar body or embryo blastomere, microtechnique (for pre-implantation genetic diagnosis); less than or equal to 5 embryos |
| *89291 | Biopsy, oocyte polar body or embryo blastomere, microtechnique (for pre-implantation genetic diagnosis); greater than 5 embryos |
| **Related Services** | |
| *58970 | Follicle puncture for oocyte retrieval, any method |
| *58974 | Embryo transfer, intrauterine |
| *76948 | Ultrasonic guidance for aspiration of ova, imaging supervision and interpretation |
| *89250 | Culture of oocyte(s)/embryo(s), less than 4 days |
| *89251 | Culture of oocyte(s)/embryo(s), less than 4 days; with co-culture of oocyte(s)/embryos |

| CPT Code | Description |
|---|---|
| **Related Services** | |
| *89253 | Assisted embryo hatching, microtechniques (any method) |
| *89254 | Oocyte identification from follicular fluid |
| *89255 | Preparation of embryo for transfer (any method) |
| *89257 | Sperm Identification from aspiration (other than seminal fluid) |
| *89258 | Cryopreservation; embryo(s) |
| *89260 | Sperm isolation: simple prep (sperm wash and swim-up) for insemination or diagnosis |
| *89261 | Sperm isolation: complex prep (Percoll gradient, albumin gradient) for insemination or diagnosis with semen analysis |
| *89264 | Sperm identification from testis tissue, fresh or cryopreserved |
| *89268 | Insemination of oocytes |
| *89272 | Extended culture of oocyte(s)/embryo(s), 4-7 days |
| *89280 | Assisted oocyte fertilization, microtechnique; less than or equal to 10 oocytes |
| *89281 | Assisted oocyte fertilization, microtechnique; greater than 10 oocytes |
| *89342 | Storage (per year); embryo(s) |
| *89352 | Thawing of cryopreserved: embryos(s) |



| HCPCS Code | Description |
|---|---|
| *S4011 | In vitro fertilization; including but not limited to identification and incubation of mature oocytes, fertilization with sperm, incubation of embryo(s), and subsequent visualization for determination of development |
| *S4015 | Complete in vitro fertilization cycle, not otherwise specified, case rate |
| *S4016 | Frozen in vitro fertilization cycle, case rate |
| *S4022 | Assisted oocyte fertilization, case rate |
| *S4037 | Cryopreserved embryo transfer, case rate |

# Description of Services

Genetic counseling is strongly recommended prior to Preimplantation Genetic Testing (PGT) in order to inform persons being tested about the advantages and limitations of the test as applied to their unique situation.

PGT is an analysis performed on an embryo prior to transfer to screen for aneuploidy (PGT-A), deletions and duplications of genomic material, generally referred to as copy number variations (CNVs) or structural rearrangements (PGT-SR) and analysis of single gene or other inherited disorders in an embryo (PGT-M). Use of this technology is hypothesized to increase the success of infertility treatment, especially in women who have worse outcomes due to advanced maternal age, history of recurrent miscarriage, failed in vitro fertilization (IVF) (CDC, 2017) or a balanced chromosome translocation. In addition, it has been explored as a way to enable single embryo transfer (SET) rather than using multiple embryos to increase the odds of having a successful pregnancy without the risk of a multiple gestation.

# Clinical Evidence

## Preimplantation Genetic Testing

Li et al. (2021) reported on their study comparing perinatal outcomes of singleton pregnancies conceived via frozen embryo transfer of a single, autologous blastocyst either with or without preimplantation genetic testing. Since genetic testing of the blastocyst removes cells from the trophectoderm (which subsequently forms the placenta), the possibility exists that preimplantation genetic testing could be associated with an increase in risk of adverse outcomes related to development of the

placenta. The study was a retrospective analysis of embryo transfer cycles that resulted in singleton live births, including cycles initiated in 2014 and 2015. Data was obtained from the Society for Assisted Reproductive Technology Clinical Outcomes Reporting System. Of 16,246 transfers that resulted in a single birth, 6,244 had undergone preimplantation genetic testing and 10,002 had not. Of note, the average maternal age and prevalence of prior spontaneous abortion were higher among women from the preimplantation genetic testing group (35.8 vs. 33.7 and 37.3% vs. 27.7%, respectively). Multivariate regression analysis, which adjusted for year of transfer, maternal body mass index, maternal age, obstetric history, smoking status, infertility diagnosis and sex of infant resulted in a significantly increased chance of preterm birth in the blastocysts that underwent preimplantation genetic testing (adjusted odds ratio, 1.20; 95% confidence interval,1.09-1.33; $p < .001$) . Other characteristics such as birthweight, odds of small or large for gestational-age, and macrosomia did not differ between the two groups. The authors concluded that this comparison revealed that frozen embryo transfer cycles which included preimplantation genetic testing were associated with a small increase in potential for preterm birth, and even though the risk was relatively small, further investigation in future studies is warranted.

In 2016, Chang and colleagues published a review of the outcomes of in vitro fertilization utilizing preimplantation genetic testing (PGT) from 2011-2012, from the United States Assisted Reproductive Technology Surveillance Data (Chang et al., 2016). Overall, they included 97,069 non-PGT cycles and 9,833 cycles that used PGT in their analysis. Most were for aneuploidy screening (55.6%), 29% were for “other reasons,” and 15% were for preventing genetic disease. In the “other reasons” category, only 2% of clinics provided information on the reason for PGT, and it was primarily for gender selection. In 2011, 98% of clinics reporting doing at least one PGT cycle, and in 2012, 100% of reporting clinics had performed PGT cycles. The clinical characteristics between the three groups differed. The aneuploidy screening group tended to be older (> 37 years) and had a higher rate of prior miscarriages. As a group, they had fewer miscarriages than other age matched groups in the study, and had a higher chance of a live birth compared to the age matched non-PGT group. They were more likely to have multiple births compared to the non-PGT group. This group was also more likely to have low birth weight babies. The genetic disease group was younger and did not have a history of prior miscarriages. In this group, in women ages 35-37, the adjusted odds of achieving a pregnancy and live birth were lower than the non-PGT group. In all categories, women using PGT who were < 35 years old and transferred one embryo, the odds of clinical pregnancy and live birth were lower than compared to the non-PGT group. Information was not available on the PGT techniques used by the different clinics, on biopsy type, protocol to select chromosome abnormalities, number of embryos, embryo morphology, and number of embryos discarded. The authors concluded that PGT might improve outcomes in populations at risk of a genetically affected child, including aneuploidy, on the basis of family history, but additional data collection and outcome data is necessary to better understand the overall value and effectiveness of PGT. Prospective, randomized studies are needed.

## Preimplantation Genetic Testing for Monogenic/Single Gene Defects (PGT-M)

Ben-Nagi et al. (2019) conducted an observational study to determine if live birth rate is affected by oocyte yield as well as number of blastocysts biopsied, and/or the number of acceptable blastocysts to transfer post PGT-M or PGT-SR. Participants were 175 couples referred to an IVF center from 2014 to 2017, that chose to undergo either PGT-M or PGT-SR. One hundred forty-five (83%) of couples had PGT-M, while 30 (17%) had PGT-SR. Forty-four (25%) couples had second or third cycles of IVF, for a total of 249 oocyte retrievals and 230 frozen embryo transfers (FET); 196 (79%) due to single-gene disorders and 53 (21%) for chromosomal rearrangement. One hundred twenty-two (53%) of the frozen embryo transfers resulted in live birth, 16 (7%) resulted in ongoing pregnancy, 21 (9%) resulted in miscarriage, and 69 (30%) resulted in failed implantation. The authors found that the number of oocytes collected ($p = 0.007$; OR 1.06), the number of blastocysts biopsied ($p = 0.001$; OR 1.14), and the number of suitable embryos to transfer ($p = 0.00$; OR 1.38) were all significantly positively associated achieving a live birth. The likelihood of live birth increased by 14% per additional blastocyst biopsied and by 38% per suitable embryo to transfer. Stratified analysis determined that the odds of live birth per acceptable embryo for transfer was 1.28 for single-gene disorders and 3.23 for chromosomal rearrangement.

Rechistky and Kuliev (2018) report on the use of PGT to select embryos at risk for inherited cancer syndromes. In their experience through Reproductive Genetics Innovations, cancer was the largest category of PGT-M for conditions with a genetic pre-disposition. In the PGT-M cohort, there were 5,037 cycles, resulting in 3,669 transfers of 6,038 embryos. In the sub-cohort for cancer pre-disposition, there were 24 cancer syndromes reported, which included hereditary breast and ovarian cancer (BRCA1 and BRCA2), Li Fraumeni syndrome (LFD1), and familial adenomatous polyposis (APC). There were 383 at risk couples that underwent 702 PGT cycles. PGT utilized different methodologies depending on the type of mutation and available parental genetic information. Generally, a polar body or embryo biopsy was taken. Mutation testing using direct mutational analysis or linkage with parental haplotyping was performed. Aneuploidy screening using a 24-chromosome single nucleotide polymorphism (SNP) array or next generation sequencing (NGS) was the final step for couples with advanced maternal age.

This resulted in 684 embryos in 484 transfer cycles, and 282 pregnancies. Three hundred and sixteen children were born without the cancer predisposition mutations. The authors reported that the inclusion of 24 chromosome aneuploidy screening for advanced maternal age couples increased the pregnancy rate in the PGT-M group from 50% to 70% and reduced the miscarriage rate from 14% to 9%.

Kubikova et al. (2018) reported on the development of a multiplex polymerase chain reaction (PCR) test for PGT-M of the beta-globin gene (HBB), responsible for beta-thalassemia and sickle cell anemia. The analysis utilized the amplification of overlapping small HBB segments to cover the entire gene, with analysis using next generation sequencing. In addition, 17 closely linked single nucleotide polymorphisms (SNPs) were tested simultaneously to aid in defining haplotypes in combination with HBB sequencing. A validation study on five family trios representing 14 different mutations was conducted, and results were consistent with previously obtained genetic results. Three of the families continued on using this protocol for PGT-M. One couple had a single-cell embryo biopsy at an early cleavage stage, and the other two families had about five cells extracted from the trophectoderm from blastocyst stage embryos. A total of 21 embryos were tested and had successful whole genome amplification, and NGS analysis was successful. Typical karyotyping and linkage analysis was performed simultaneously as a comparison for standard PGT methods. All but one embryo had an average read depth of 1,000x for HBB. The single embryo that failed was found to have nullisomy for chromosome 11 where the HBB gene is located. In one couple, there were low call rates and a high allele dropout rate in the standard karyotype method, likely associated with suboptimal amplification after blastocyst biopsy. Results were resolved using linkage analysis of parental SNPs to confirm mutations and haplotypes found in the embryos. The allele drop out was not found in the NGS analysis. The authors concluded that the use of a trophectoderm biopsy with next generation sequencing provided better accuracy than traditional PGT testing. Pregnancy rates, outcomes and confirmation of PGT results post-natally were not reported in this study.

Current technology allows for PGT-M testing for a multitude of single gene disorders, but the efficacy depends on the performance of gene amplification in a small sample, often a single cell. Volozonoka et al. (2018) examined the difference between multiple displacement amplification (MDA) and Omniplex whole genome amplification when used for comparative genome hybridization (CGH), Sanger sequencing, SNaPshot (single-base extension sequencing) and fragment size analysis. Nine couples at risk for single-gene disorders consented to participate in the study. Disease genes involved included ACTA2, HTT, KRT14, ALOX12B, TPP1, GLB1, MTM1, and DMD. A total of 62 embryos were tested, and 1-8 trophectodermal cells were taken from the outer layer. All embryos survived the extraction. Thirty-nine embryos underwent whole genome amplification using MDA and the remaining went through OmniPlex linear amplification. Amplification detection was determined by capillary electrophoresis. Direct mutation analysis used Sanger sequencing or SNaPshot, and chromosomes were analyzed using CGH. Whole genome amplification, regardless of method, and testing was successful and provided a conclusive result in all embryos. Five unaffected and euploid embryos were transferred, resulting in four clinical pregnancies and the live birth of two healthy children. Key differences were noted, however. The MDA approach to whole genome amplification resulted in heavier DNA strings and resulting electrograms were clearer, and the base error rate was lower compared to other PCR based approaches. MDA had significant amplification bias that caused high CGH noise. The authors concluded that methodology choice should depend on which downstream analysis is most needed, and both amplification techniques could be used if there are enough embryonic cells available.

Sallevelt et al. (2017) reported on the use of PGT-M using a single blastomere for mitochondrial disorders. Mitochondrial diseases are transmitted only from the mother, and the expression of disease is dependent on the mutation load, meaning the number of mitochondria carrying the mutation compared to the number of wildtype mitochondria present. Prenatal diagnosis has a potential problem in that the mutational load across all tissues may not be able to be identified completely, and therefore the future phenotype of the fetus cannot be predicted easily. PGT-M is the preferred choice for female carriers, as only mutation free embryos can be transferred. If no mutation free embryos are available, embryos with a low mutation load can be transferred, which reduces the risk of an affected child, but cannot eliminate it. To date, two blastomeres have been used in PGT-M for mitochondrial disease to better predict the mutation load. This has a negative impact on the live birth rate. The authors studied the value of using only one blastomere in a cohort of nine women carrying a m.3243A > G mutation that causes mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS). These women produced 73 embryos that had two or more blastomeres removed, from which 294 single blastomeres were analyzed. Only one blastomere was concluded to have a false negative result. This was based on this cell having a mutation load of about 5%, within the range where an embryo transfer might have been considered, but surrounding blastomeres from the same embryo had a higher mutational load of 22-30%. The authors concluded that as the false negative rate was 0.34%, a single blastomere would be sufficient for PGT-M. Pregnancy rates and outcomes were not highlighted by the authors because their goal was to determine first if a single cell would provide the correct diagnosis. Single cells were analyzed but excluded from the data reporting, as well as multi-cells, and

used for embryo transfer which they feel would confound the data. PGT-M was first reported in 1990, for sex selection for an X-linked disorder. The field has evolved since then to encompass many genetic diseases, including early and adult-onset disorders. Testing for tissue typing also occurs, with the hopes that a baby will be born that can provide a blood or bone marrow transplant for an older affected sibling. Testing for adult-onset disorders and tissue typing to rescue an older sibling raises a number of ethical issues. Analysis can be completed with chromosome microarray, polymerase chain reaction (PCR), or next generation sequencing. Technical challenges exist for all methods, such as timing of the biopsy and which cells are biopsied. Collecting too many cells at an early cleavage stage may impact implantation and pregnancy rates, and early-stage biopsies may result in poor DNA amplification. Single cell PCR may also result in allele drop out, which occurs in 10-20% of cases. Alleles drop out can result in misdiagnosis between carrier and affected embryos. Because PGT-M is using only 1-2 cells, there is additional risk of contamination of the sample with DNA from the technician or even sperm sticking on the zona pellucida. Errors can occur from transfer of the wrong embryo, from mislabeling, and from couples having unprotected sex during the cycle that results in pregnancy, versus the transferred embryo. In 2005, the European Society of Human Reproduction and Embryology (ESHRE) reported on the reanalysis of 940 un-transferred embryos and noted that 93.7% of embryos were correctly classified, with a sensitivity of 99.2% and a specificity of 80.9%. Recent ESHRE data suggested that the success rate of pregnancy after PGT-M was similar to other assisted reproduction, with a live birth rate per oocyte retrieved of 24%. Genetic counseling is strongly recommended prior to PGT-M so that couples have a clear understanding of the pros and cons of this approach (Lee et al., 2017).

Trachoo et al. (2016) reported on the case of a healthy infant born after intracytoplasmic sperm injection-in vitro fertilization (ICSI-IVF) with a preimplantation genetic diagnosis (PGD) to parents who are carriers of the recessive disease pantothenate kinase-associated neurodegeneration (PKAN). Their first child was diagnosed with PKAN due to a PANK2 gene mutation. To avoid this disorder in subsequent offspring, the couple underwent assisted reproduction in order to take advantage of the availability of preimplantation genetic diagnosis. After a single cycle using ICSI-IVF, seven embryos were tested for the familial PANK2 mutation and for aneuploidy. Two were likely affected, three were likely carriers, one was likely unaffected, and one failed genome amplification and was not genotyped. The unaffected embryo was transferred after a freeze-thaw cycle and resulted in a viable pregnancy. Amniocentesis confirmed the PGT results. The pregnancy resulted in a healthy male live birth at 38 weeks. Post-natal testing also confirmed prior testing, and at age 24 months the baby continues with normal growth and development.

## Preimplantation Genetic Testing for Aneuploidy Screening (PGT-A)

There is insufficient evidence to support the use of PGT for aneuploidy screening. Evidence at this time is limited to small study populations. Further studies with a larger number of patients and longer follow-up are needed.

To investigate whether cumulative live-birth rate is improved when preimplantation genetic testing for aneuploidy (PGT-A) is performed compared to conventional in vitro fertilization (IVF), Yan et al. (2021) conducted a multicenter randomized controlled trial including 1,212 sub-fertile women between 20 and 37 years of age. Three blastocysts either underwent next-generation sequencing in the PGT-A test group or were selected based on morphologic criteria in the conventional IVF group. Primary outcome to be reported was the cumulative live birth rate after as many as three embryo transfers within one year after randomization. 606 individuals were assigned to each of the two trial groups. Live births occurred in 77.2% of women in the PGT-A group and in 81.8% in the conventional IVF group. Cumulative frequency of pregnancy loss was 8.7% and 12.6%, respectively and incidence of complications or other adverse events were similar between the two groups. Although the frequency of loss of pregnancy appeared to be lower in the PGT-A group, this result did not translate into either a higher cumulative live birth rate or a shorter mean time until live birth. In addition, birth weight and incidence of complications and congenital anomalies were similar in the two groups. The authors concluded that among individuals with three or more high-quality blastocysts, conventional IVF resulted in a cumulative live birth rate that was non-inferior to the rate with PGT-A.

In a 2021 publication, Tiegs et al. reported the outcome of their prospective, multicenter, blinded, non-selection study to evaluate the value of a diagnosis of aneuploidy [made via targeted next-generation sequencing preimplantation genetic testing (PGT-A)] in predicting failure of a successful delivery. A secondary outcome measured was the impact of trophectoderm biopsy on lasting implantation. A total of 402 individuals with infertility received 484 single, frozen blastocyst transfers. Unblinded PGT-A results performed using NextSeq 500/550 NGS-based PGT-A were compared to clinical outcomes of embryo transfers and a calculation of predictive values was made. Significant difference in outcome by PGT-A diagnosis was found: 64.7% (202/312) of euploid embryos progressed to either sustained implantation or delivery while none of the 102 embryos diagnosed as whole chromosome aneuploid progressed to either sustained implantation or delivery. Thus, the clinical error rate in aneuploid diagnoses was 0%. There was no difference in sustained implantation between the control group, which was aged matched

and had not undergone biopsy, and the PGT-A testing group. The authors assert that the PGT-A assay evaluated was found to be prognostic of failure to deliver when such testing revealed an aneuploid result and did not result in the discard of embryos that have significant reproductive potential. They do, however, note limitations, including the inability to analyze predictive values associated with segmental PGT-A or whole chromosome mosaic diagnoses due to the low incidence of those results. Additionally, the retrospective identification of a control group to evaluate impact of cell biopsy on sustained implantation limits the study's strength. Lastly, about half of the study subjects were less than 35 years of age; however, the false positive rates of aneuploidy are typically higher in this group compared with older subjects, so this may have further challenged the accuracy of the assay used in this study. The researchers recommend non-selection studies be performed for every new PGT-A assay as additional technologies emerge.

Konstantinidis et al. (2020) studied the incidence and patterns of trisomies and recombination separately and in conjunction with each other at the blastocyst stage by single nucleotide polymorphism (SNP) testing with array comparative genomic hybridization (aCGH). Interesting findings regarding recombination and aneuploidy origin were revealed. SNP microarrays were performed on 1,442 blastocyst embryos from 268 couples who underwent PGT for known single gene disorders; 24-chromosome aneuploidy screening by aCGH was done concurrently. One hundred percent of meiotic trisomies were maternal in origin and incidence increased significantly with maternal age ($p < 0.0001$). Meiosis I trisomies and meiosis II trisomies were 55.8% and 44.2%, respectively. Recombination studies were performed for 11,476 chromosomes and 17,763 recombination events were reported. The average number of recombination sites was 24.0 ±0.3 for male meiosis and 41.2 ±0.6 for autosomal female meiosis. One hundred ninety euploid embryos and 69 embryos with maternal meiotic trisomies were compared which revealed similar recombination rates ($p = 0.425$) and non-recombinant chromatid rates ($p = 0.435$). Although the study provided unique data regarding recombination and aneuploidies in embryos, further research and data is needed to establish clinical validity and clinical utility.

The effectiveness and safety of PGT-A was evaluated by Cornelisse et al. (2020) who performed a systematic review of six databases and two trial registries in September 2019. Thirteen randomized controlled trials involving 2,794 women reporting data on clinical outcomes in patients who underwent IVF with PGT-A versus IVF without PGT-A were included. The quality of evidence ranged from low to moderate. Cumulative live birth (cLBR) was the primary outcome; live birth rate (LBR) after first embryo transfer, miscarriage rate, ongoing pregnancy rate, clinical pregnancy rate, multiple pregnancy rate, proportion of women obtaining an embryo transfer and mean number of embryo transfers represented the secondary outcomes. The authors reported results were as follows: One trial used polar body biopsy with array comparative genomic hybridization (aCGH). It is uncertain whether the addition of PGT-A by polar body biopsy increases the cLBR compared to IVF without PGT-A [odds ratio (OR) 1.05, 95% confidence interval (CI) 0.66 to 1.66, 1 RCT, n = 396, low-quality evidence]. The evidence suggests that for the observed cLBR of 24% in the control group, the chance of live birth following the results of one IVF cycle with PGT-A is between 17% and 34%. It is uncertain whether the LBR after the first embryo transfer improves with PGT-A by polar body biopsy (OR 1.10, 95% CI 0.68 to 1.79, 1 RCT, n = 396, low-quality evidence). PGT-A with polar body biopsy may reduce miscarriage rate (OR 0.45, 95% CI 0.23 to 0.88, 1 RCT, n = 396, low-quality evidence). No data on ongoing pregnancy rate were available. The effect of PGT-A by polar body biopsy on improving clinical pregnancy rate is uncertain (OR 0.77, 95% CI 0.50 to 1.16, 1 RCT, n = 396, low-quality evidence). Another trial used blastocyst stage biopsy with next-generation sequencing. It is uncertain whether IVF with the addition of PGT-A by blastocyst stage biopsy increases cLBR compared to IVF without PGT-A, since no data were available. It is uncertain if LBR after the first embryo transfer improves with PGT-A with blastocyst stage biopsy (OR 0.93, 95% CI 0.69 to 1.27, 1 RCT, n = 661, low-quality evidence). It is uncertain whether PGT-A with blastocyst stage biopsy reduces miscarriage rate (OR 0.89, 95% CI 0.52 to 1.54, 1 RCT, n = 661, low-quality evidence). No data on ongoing pregnancy rate or clinical pregnancy rate were available. IVF with PGT-A versus IVF without PGT-A with the use of FISH for the genetic analysis; eleven trials were included in this comparison. It is uncertain whether IVF with addition of PGT-A increases cLBR (OR 0.59, 95% CI 0.35 to 1.01, 1 RCT, n = 408, low-quality evidence). The evidence suggests that for the observed average cLBR of 29% in the control group, the chance of live birth following the results of one IVF cycle with PGT-A is between 12% and 29%. PGT-A performed with FISH probably reduces live births after the first transfer compared to the control group (OR 0.62, 95% CI 0.43 to 0.91, 10 RCTs, n = 1,680, $I^2 = 54\%$, moderate-quality evidence). The evidence suggests that for the observed average LBR per first transfer of 31% in the control group, the chance of live birth after the first embryo transfer with PGT-A is between 16% and 29%. There is probably little or no difference in miscarriage rate between PGT-A and the control group (OR 1.03, 95%, CI 0.75 to 1.41; 10 RCTs, n = 1,680, $I^2 = 16\%$; moderate-quality evidence). The addition of PGT-A may reduce ongoing pregnancy rate (OR 0.68, 95% CI 0.51 to 0.90, 5 RCTs, n = 1,121, $I^2 = 60\%$, low-quality evidence) and probably reduces clinical pregnancies (OR 0.60, 95% CI 0.45 to 0.81, 5 RCTs, n = 1,131; $I^2 = 0\%$, moderate-quality evidence). The authors concluded that due to the poor quality of evidence regarding cumulative live birth rate, live birth rate after transfer or miscarriage rate between IVF with and IVF without PGT-A, routine clinical practice of PGT-A is not supported.

Trophectoderm (TE) biopsy, a technique to assess aneuploidy for PGT, can result in false positive and false negative test results because the chromosome number in TE cells is not always concordant with the chromosome number of the inner cell mass, which develops into the fetus. Huang et al. (2019b) conducted an investigational study to determine the effectiveness of noninvasive preimplantation genetic testing for aneuploidy (niPGT-A) as compared to the standard TE biopsy method. Fifty-two frozen donated blastocysts were analyzed by next-generation sequencing to serve as a gold standard. TE biopsy PGT-A and niPGT-A results were generated for all samples and compared with sequencing results from corresponding embryos. The false negative rate for niPGT-A was zero. The positive predictive value and specificity were higher for niPGT-A than for TE biopsy PGT-A. In addition, the authors stated that the concordance rates for embryo ploidy and chromosome copy number were also higher for niPGT-A than seen in TE biopsy PGT-A. Based on this study, the authors concluded that niPGT-A by DNA sequencing of DNA released in culture media from both trophectoderm and ICM provides a non-invasive method which is less prone to errors linked to embryo mosaicism, though future studies with larger sample sizes are necessary.

Simon et al. (2018) conducted a retrospective study examining IVF outcomes using single nucleotide polymorphism (SNP) based PGT-A. Outcome data was collected on procedures performed at two U.S. fertility centers from 2010-2013. Women 18-55 years of age who underwent IFV treatment were eligible for inclusion; those who did not elect 24 chromosome SNP-based PGT-A were excluded from analysis. During the study timeframe, 974 women (20-46 years of age) underwent 1,884 IVF cycles (1,621 non-donor, 262 donor) and elected to use SNP-based PGT-A. An implantation rate of 69.9%, clinical pregnancy rate per transfer of 70.6%, and live birth rate per transfer of 64.5% were observed in the non-donor cycles. Data were stratified by maternal age for analysis, with no significant difference observed in outcome rates per transfer, even for women > 40 years of age. No difference in pregnancy outcome was seen in single embryo transfers (SET) compared with double embryo transfers which supported the authors' recommendation for the utilization of SET when SNP-based PGT-A is used. Larger, prospective studies are recommended to further assess the impact of SNP-based PGT-A on pregnancy outcomes.

A randomized clinical trial was conducted by Verpoest et al. (2018) to determine if women age 36-40 who used PGT-A of the first and second polar body in intracellular sperm injection (ICSI) cycle to select embryos for transfer had a better live birth rate compared to women who did not have PGT-A. Women were excluded from the study if they had three or more failed IVF cycles, three or more clinical miscarriages, poor response or low ovarian reserve. Three hundred and ninety-six women were enrolled between June 2012 and December 2016, in the multicenter study. Two hundred and five were allocated to the PGT-A group using CGH and 191 were in the non-PGT-A control group. Overall, the live birth rate between the two groups was the same, at 24%. Fewer women in the PGT-A group experienced a miscarriage, and fewer transfers were needed to achieve the same pregnancy rate. The authors noted that the sample size was smaller than targeted which reduced the power of the study. More studies are needed to evaluate whether the potential benefits outweigh drawbacks such as the impact of prolonged culture times in order to complete the PGT-A testing before transfer.

Zore et al. (2018) compared the outcomes of frozen single embryo transfer between euploid embryos and those with segmental mosaicism. Three hundred and twenty-seven women had 377 frozen embryo transfers. All embryos underwent biopsy at the blastocyst stage where two or more cells were taken from the trophectoderm. CGH was used to determine if embryos were euploid or had segmental mosaicism. Three hundred and fifty-seven were euploid, and 20 had segmental mosaicism. The spontaneous miscarriage rate was 18.2% in euploid embryos, compared to 40% in segmental mosaic embryos. Furthermore, the live birth rate for euploid embryos was 53.8%, whereas for segmental mosaics the live birth rate was 30%. The authors concluded that reporting segmental mosaicism was important to help with selection of embryos for transfer, and noted that although reduced, segmental mosaics still had the potential to result in a live birth.

Munné (2018) reported on the outcomes of the 2018 Preimplantation Genetic Diagnosis International Society (PGDIS) conference regarding PGT-A. Studies and data were reviewed at the conference that demonstrated improved pregnancy rates per transfer in experience centers and in women over the age of 35 who utilize PGT-A, but not in younger women. Studies using cell-free embryo DNA in spent media were promising, showing 80-90% concordance with biopsy. Mosaicism in the trophectoderm was a topic of debate, the outcome of which was PGDIS agreeing to update their guidelines. However, the guidelines will still recommend transferring euploid embryos favorably over mosaic embryos.

Friedenthal et al. (2018) evaluated the difference in pregnancy outcomes using NGS compared to CGH in single frozen thawed transferred embryos (STEET) in a retrospective review. A total of 916 STEET cycles from 2014 to 2016, were reviewed, and included 548 NGS cases, and 368 cases using CGH. The outcomes analyzed included implantation rate, live birth rate, and miscarriage rate. The NGS group had a higher implantation rate (72% vs. 65%) than CGH, and a higher live birth rate compared to CGH (62% vs. 54%). The miscarriage rate was similar between the two groups. The authors concluded that NGS was better

at detecting reduced viability embryos caused by mosaicism, and using NGS may results in better pregnancy outcomes when compared to using CGH.

Gleicher and Orvieto (2017) conducted a comprehensive literature review through January 2017, on research related to current PGS methodologies and outcomes using comparative chromosome screening on 5-6-day TE biopsies, which they call PGS 2.0. This includes aCGH and SNP-based array technologies. Overall, they noted that the literature has a skewed view of clinical utility, and uses embryo transfer as the starting point for measuring success, whereas generally IVF literature uses the initiated IVF cycles as the starting point. When using initiated cycles as a starting point, non-PGS cycles result in a higher live birth rate over PGS cycles. In addition, they report from their analysis that TE mosaicism may be present in at least half of all embryos, and mathematical models suggest that that the likelihood of false negative and positive results is too high to safely determine which embryos should be transferred or not. Their overall conclusion is that PGS 2.0 does not have clinical utility and may in fact reduce live birth rates.

Barad et al. (2017) conducted a retrospective analysis of the impact of PGT-A on pregnancy outcomes in donor oocyte-recipient cycles. The authors utilized the data obtained between 2005 and 2013, from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System. This database relies on voluntary reporting, and 90% of the U.S. IVF centers participate. In this cohort, first embryo transfers with day 5/6 embryos were reviewed, for a total of 20,616 control cycles and 392 PGT-A cycles. The data showed that the pregnancy and live birth rates were lower in the PGT-A group by 35% when compared to the control group. The authors concluded that PGT-A was not associated with improved odds of pregnancy, live birth, or miscarriage rate.

Gleicher et al. (2017) addressed the issue of trophectoderm mosaicism, in a collaboration between The Center for Human Reproduction in New York City and the Center for Studies in Physics and Biology and the Brivanlou Laboratory of Stem Cell Biology and Molecular Embryology, using mathematical modeling. As molecular methodologies improve, it has become more apparent that the trophectoderm has more mosaicism than previously appreciated. Recent studies have shown that over a third of embryos considered to be aneuploid were actually mostly euploid-normal on follow up studies. This has raised concerns about the impact on PGT-A results and whether or not mosaic embryos can be transferred. The authors developed two models to assess the likelihood of false positive and false negative results on an average six-cell biopsy from a 300-cell trophectoderm, with the understanding that trophectoderm biopsies often include only one cell. The models assumed that mosaicism was distributed evenly throughout the trophectoderm, even though in reality it is often clonal. In their first model that examined the probability of a false negative with results from one or more euploid cells, they determined that there is a high probability of selecting a euploid cell, even when the ratio of euploid cells is low. In the second model, the probability of a false positive from an aneuploid result was examined. The authors found that even with 1-2 cells being aneuploidy, the embryo could theoretically still be mostly euploid. When three cells were found to be aneuploid, it is mathematically more likely consistent with embryo aneuploidy. The author's goal was to examine, through mathematical modeling, the likely reliability of being able to choose or discard an embryo based on ploidy results of a single-cell trophectoderm biopsy. They concluded that mathematically, one cannot use the results of a single cell to determine the ploidy of an embryo, and therefore cannot reliably predict which embryos should be used or discarded.

To determine the impact of PGT-A on pregnancy rates, Kang et al. (2016) did a retrospective review of outcomes between women utilizing embryo transfer after 24-chromosome PGT-A with women who had fresh, non-biopsied transfers. The cases occurred between 2010 and 2014, and included 274 women who had PGT-A and 863 controls. In women who were < 37 years old, there were no differences in live birth rates between the control and PGT-A group for single or double embryo transfers, and miscarriage rates were similar. In women > 37 years old, there was an increase in live birth rates with an odds ratio of 8.2 for women who had a single or double embryo transfer and PGT-A, though the miscarriage rate was the same. When the data was analyzed on a per-retrieval basis, the differences between the control and PGT-A group were no longer significant. The authors concluded that women < 37 gained no advantage from using PGT-A, and while the data suggests that it may have some benefit for women > 37, the advantage disappears when considered on a per retrieval basis.

The intra-laboratory and intra-embryonic concordance of embryo biopsy aneuploidy was studied by Gleicher et al. (2016). Eleven couples donated an embryo determined to be aneuploid for further research. The embryos were disassociated into 37 anonymized samples and sent to a different national laboratory than originally tested the sample for repeat analysis. Only two of the eleven embryos were found to have identical results between the two labs and were confirmed to be aneuploidy. Four embryos were chromosomally normal, two were mosaic, and five were aneuploidy, but had different aneuploidies reported between the two labs. In intra-embryo analysis, 50% of the embryos differed between biopsy sites. The authors also note that an

additional eight couples chose to transfer aneuploidy embryos resulting in five chromosomally normal pregnancies. Four were ongoing pregnancies at the time of publication, and one had a healthy live birth. The authors concluded that trophectoderm mosaicism may be higher than previously anticipated, and this plus the difference in diagnostic platforms could explain the difference between the two labs. The study size was too small, however, to derive definitive conclusions and more data is needed to assess if a single cell trophectoderm can be used to select embryos for transfer.

Tortoriello et al. (2016) reported on the comparison of NGS and CGH, as well as intra-laboratory and intra-embryo comparison, on 37 abnormal blastocyst stage embryos. Eight patients donated their embryos for further research after being identified as aneuploid. The embryos were disassociated and sent to two different laboratories for comparison between the labs, CGH and NGS. Only 33% of embryos initially reported to be aneuploidy were found to be aneuploidy on repeat analysis by CGH. When 27 confirmed aneuploidy embryos were re-tested using NGS, 11 (41%) were found to be euploid. Three gender discrepancies between CGH and NGS were found. The authors concluded that such inconsistencies from trophectoderm biopsies could be due to different sensitivities between platforms and questions the current clinical validity of PGT-A.

Capalbo et al. (2015) compared SNP based microarray screening, aCGH, and qPCR techniques for screening embryos. The authors conducted a prospective double-blind observational study from Oct. 2012-Dec. 2013. TE biopsies were done on day 5-6. Forty-five patients were included who had indications of advanced maternal age, recurrent miscarriage, or parental carrier of a balanced translocation. A total of 124 blastocysts underwent aCGH. Of these, 122 survived warming and re-expansion and underwent TE biopsy and qPCR analysis. Two samples failed qPCR and were excluded. Eighty-two percent of embryos showed the same diagnosis between aCGH and qPCR and 18% were discordant for at least one chromosome. Discordant blastocysts were warmed and TE was biopsied again on 21 embryos that survived another rewarming and underwent a blinded SNP array analysis. A conclusive result was obtained in 18 of the 21. In four of these, the qPCR, aCGH, and SNP array did not match and were considered mosaic aneuploid. Overall, when the data is viewed per chromosome, the aCGH and qPCR results were consistent in 99.9% of cases where both methods were performed on TE biopsy from the same embryo. The SNP based reanalysis, however, showed a higher discordant rate between aCGH and qPCR. The authors concluded that TE biopsies can be a highly reliable and effective approach for PGS, and that until aCGH is studied for their clinical negative predictive value, this comparative study can only demonstrate that aCGH results in a higher aneuploidy rate than other contemporary and better validated methods of chromosome screening.

Kurahashi et al. (2015) conducted a comprehensive review of the literature regarding the analytical validity of CMA for PGS. The authors reported that while oligonucleotide arrays (CMA) are the standard for clinical analysis of individuals with developmental delay and congenital anomalies, the need to use a single cell and then perform WGA when using CMA for PGS may introduce amplification bias. Uneven amplification can occur of various regions of the DNA sampled from the embryo and lead to inaccuracy in the test results. Newer technologies including bacterial artificial chromosome (BAC) and a multiple displacement method are being explored as ways to mitigate amplification bias. Mosaicism in the embryo is also reported by the authors as a factor to overcome in using CMA for PGS. It has been demonstrated in the oocyte and blastomere that the spindle assembly process that regulates chromosome segregation is transiently deficient, which leads to a high rate of mosaicism during this stage and raises the question of whether or not a single cell biopsied during this stage is representative of the whole embryo. In addition, self-correction of the mosaicism to a euploid embryo has been demonstrated, so low-level mosaicism may not be a concern. Studies have shown that CMA can identify mosaicism in only 25% of embryos and so may miss low levels of mosaicism. This review further describes issues of cell cycle replication as a confounding factor for CMA. DNA replication begins at more than 10,000 sites in a genome, and during S phase, some parts of the genome have finished replicating and have two copies while other regions have not completed replicating and have a single copy of DNA. This variation in copy number could be incorrectly interpreted as abnormal or as high background noise. The risk of cell cycle issues may be mitigated by performing cell sampling just after cell division, or by trophectoderm biopsy in the blastocyst state. Finally, CMA is not optimal for identifying polyploidy which is a significant limitation because triploidy is one of the most common chromosome abnormalities found in miscarriages. Microarrays that are SNP based can be used for detection of polyploidy, but at the time of publication, SNP arrays have not been optimized for WGA. Overall, the authors conclude that CMA for PGS is slowly becoming a clinical standard, but states that the procedure needs to be optimized on an individual basis and tailored protocols are required.

Novik et al. (2014) published a comparison of fluorescence in situ hybridization (FISH) methods used to evaluate chromosomal mosaicism in IVF embryos with CMA to determine the limits of mosaicism detection, accuracy, and mosaicism prevalence. Chromosomal mosaicism is higher in IVF created embryos than in other prenatal specimens and may be found in 71-73% of human embryos. Low levels of mosaicism in prenatal specimens suggest selective pressure against mosaic embryos for

ongoing pregnancy. Mosaicism has been reported in embryos evaluated by CMA using trophectoderm (TE) biopsies, but the effect of TE mosaicism on development, implantation and pregnancy outcome is unknown. To determine the limits of mosaicism detection, the authors mixed different ratios of amplified DNA from aneuploid and euploid cells, as well as tested clinical samples. Overall, they were able to identify the limit of mosaicism detection with CMA at 25-37% for gains of DNA, and 37-50% for losses. They used the CMA technique developed to CMA was used to determine if an embryo was euploid, non-mosaic aneuploidy, or mosaic aneuploid. The diagnostic accuracy of the CMA test was assessed by FISH analysis on non-transferred embryos. In 47 embryos, 26 were considered to be non-mosaic aneuploid by CMA, and 100% were confirmed by FISH. In the mosaic category, 95% were confirmed by FISH. The single embryo not confirmed by FISH did have a discordant result with 7% of nuclei with an aneuploid FISH signal that was below the threshold to call the embryo abnormal. Embryos predicted to the euploid by CMA were not tested by FISH. The authors concluded that CMA testing can identify mosaicism in day 5/6 blastocysts and that FISH confirms that the mosaicism is real and not likely a technical artifact.

Liang et al. (2013) explored the clinical utility of using an oligo microarray for embryo PGS. The team analyzed 383 blastocysts from 72 patients who were advanced maternal-age or experience recurrent miscarriage. Biopsied cells underwent Rubicon WGA and screened with an oligo microarray. Some aneuploidy blastocysts were analyzed further by FISH to evaluate the accuracy of results. Overall, 58% of embryos were abnormal. Transfer of normal embryos resulted in an implantation rate of 54%. The FISH and microarray analysis matched in all aneuploid embryos analyzed. The authors concluded that the oligo array platform was able to identify aneuploidy and other small gains and losses, and improved embryo implantation rates.

## Preimplantation Genetic Testing for Chromosomal Structural Rearrangements (PGT-SR)

Huang et al. (2019a) performed a retrospective cohort study of 194 reciprocal translocation carrier couples who had experienced two or more adverse pregnancy histories. Two hundred sixty-five PGT-SR cycles were examined to assess the impact of PGT-SR on normal live birth, birth defect, and miscarriage rates in reciprocal translocation carrier couples. Prior to PGT-SR, the reproductive history of the couples consisted of 592 pregnancies – 83.6% resulted in miscarriages, 6.1% live birth with defects, 4.9% were terminated due to unwanted pregnancy, and 2.9% resulted in normal live births. Post PGT-SR, 118 clinical pregnancies resulted in 85.6% normal live births, 11% miscarriage, 3.4% with birth defects. The authors concluded that reciprocal translocation carriers in this study had a low risk of miscarriage and birth defects and a higher frequency of normal live births following post PGT-SR.

Next generation sequencing is emerging as an important technique for genetic analysis. Zhou et al. (2018a) examined the validity of using massive parallel sequencing (MPS) on trophectoderm samples for PGT-A for chromosome translocation carriers. Twelve couples with chromosome translocations participated in a study. Nine had balanced translocations, and three were carriers of a numerical chromosome abnormality. A total of 105 embryos were biopsied on day three and had one cell removed. The cells underwent whole genome amplification and were then tested for genomic imbalances using MPS and CGH and confirmed using routine karyotyping. Results were obtained for MPS and CGH for 101 embryos, and there was concordance between MPS and CGH for 19 euploid and 82 unbalanced or aneuploidy embryos. There were four discrepancies, however. In one blastomere, MPS found a deletion of a X chromosome not found by CGH. This might be caused by a low density of SNPs on the CGH platform in that region. In another case, MPS identified a 186 Mbp duplication on chromosome 1, and a 15.6 Mbp duplication on chromosome 5, whereas CGH identified the duplications but of a different size. This could be related to amplification bias impacting CGH that would have been corrected in the MPS bioinformatics process. In the third embryo, karyotyping and MPS identified an unbalanced translocation between chromosome 3 and 6, and CGH only identified the imbalance in chromosome 3. In the final discrepant embryo, karyotype and MPS identified an unbalanced translocation between chromosomes 13 and 22, and CGH only identified the imbalance in chromosome 13. Twelve of the nineteen embryos that were found to be free of genomic imbalances were used for frozen-thaw embryo transfer, resulting in 1 live birth and 5 ongoing pregnancies.

Brunet, et al. (2018) examined the use of next generation sequencing to identify complex chromosome rearrangements in the embryos of chromosomal translocation carriers. Six couples with complex rearrangements underwent PGT-SR. Biopsies were done on day 5 or 6 blastocysts. A total of 84 oocytes were retrieved, resulting in 25 embryos that had trophectoderm biopsy and NGS analysis. Vitrified warm single embryo transfers were done with six euploid embryos resulting in four healthy live births for four couples. One couple chose to confirm the PGT-SR results with prenatal diagnosis, and the other three did not. Two couples did not have any transferable embryos after two cycles.

Segmental mosaicism is a concern for both PGT-A and PGT-SR. Zhou et al. (2018b) examined the frequency of de novo segmental aneuploidy identified by next generation sequencing (NGS). The study took place over a three-year time period and

involved 5,735 blastocysts from 1,854 couples who underwent PGT-A (n = 770) and PGT-SR (n = 1,084) on trophectoderm biopsies. Biopsied cells underwent whole genome amplification using GenomePlex amplification, and low coverage massively parallel sequencing (MPS) on the Proton platform. Overall, 581 blastocysts were found to have 782 de novo segmental aneuploidies. Most carried only one, but 115 had two, and 38 had three or more. There was no association with advanced maternal age or a specific chromosome. In 1,377 cycles, 1,686 blastocysts were transferred resulting in clinical pregnancies in 49% of the PGT-SR group and 47% of the PGT-A group. The miscarriage rate was about 9% in both groups. At the time of publication, there were 84 prenatal diagnosis tests and 645 delivered babies that were considered normal and healthy. Forty blastocysts with de novo segmental aneuploidy were donated for further research, and they were additionally analyzed by FISH as a comparison analysis. Of the donated blastocysts, 39 were successfully analyzed and FISH confirmed the segmental aneuploidy identified by NGS. Because de novo segmental aneuploidy can be caused by either meiosis during gamete formation or during mitosis during embryo development, the trophectoderm and inner cell mass were evaluated for 26 blastocysts. Five showed pure segmental mosaicism in both the trophectoderm and inner cell mass, but fourteen showed different levels of mosaicism between the two tissue types. The authors concluded that this analysis showed that segmental de novo aneuploidy is a real issue and is not an artifact of whole genome amplification. Further studies are needed to understand de novo segmental mosaicism and its impact on embryo development.

Maithripala et al. (2018) reviewed the reproductive choices of 36 couples who experienced recurrent miscarriage as a result of one member of the couple carrying a balanced chromosome translocation. The couples were identified through a retrospective chart review of 2,321 couples seen in a highly specialized reproductive assistance clinic between 2005 and 2013. The pre-diagnosis obstetrical history was obtained, and it was similar for all couples. The date of parental diagnosis was identified for each couple and used in determining the time from diagnosis to live birth as a point of comparison between couples that chose natural conception and those that picked PGD as their reproductive choice. Twenty-three couples chose to pursue natural conception, and thirteen chose PDT-SR. In the natural conception group, there were 24 live births with a live birth incidence of 1 birth per 4.09 years, and 74% of women had at least one live birth in the follow up period. In the PGT-SR group, six live births were recorded, reflecting a live birth incidence of 1 birth per 5.63 years, and 38% of women had at least one live birth in the follow up period. There was no significant difference between the groups in post-parental diagnosis miscarriage or live birth rates. It should be noted that in the PGT-SR group, the miscarriage rate did not take into consideration PGT-SR specific variables. There were 8 failed PGT-SR cycles, which included four euploid embryo transfers that did not result in pregnancy. While failed PGT-SR and miscarriage cannot be equated, the authors felt it was meaningful to report as cycle failure represents a significant effort resulting in a failure to achieve a live birth.

Iews et al. (2018) conducted a systematic review of the literature to examine the evidence support the use of PGT-SR in couples who have experienced recurrent miscarriage due to an inherited structural chromosome rearrangement. Meta-analysis was not possible because of significant differences between the studies. The authors identified 20 studies after a comprehensive review of the literature. Live birth was the primary outcome that was analyzed, and secondary outcomes reviewed included miscarriage rate and time to successful pregnancy. A pooled total of 847 couples that conceived naturally had a live birth rate of 25-71%. A pooled total of 562 couples had PGT-SR and had a similar live birth rate of 26-87%. There were no large comparative or randomized studies found. The studies also had different inclusion criteria and some evaluated patients for additional causes of miscarriage, such as auto-immune disease, whereas others did not. Some studies found a lower miscarriage rate in the PGT-SR group, and others did not. Two studies were identified as the best comparative analysis for examining the miscarriage rate and time to live birth post-parental diagnosis, and the studies had conflicting results. One found a lower miscarriage rate in the PGD group, and the other did not. Both found a similar time to live birth rate for PGT-SR and natural conception.

The ability of NGS to detect complex chromosome rearrangements as compared with CGH was the focus of a study by Chow et al. (2018). The authors used archived whole genome amplified DNA from 342 embryos at risk of genomic imbalance because of translocation or inversion carrier parents. All embryos had been previously analyzed by CGH. There were 287 blastomere biopsies and 55 trophectoderm biopsies. Overall, the concordance rate on abnormal results was 100% between NGS and CGH, regardless of the biopsy type. The concordance in normal embryos was 98% in the blastomere biopsy group, and 79% on trophectoderm biopsies. NGS detected a de novo segmental aneuploidy and low-level mosaicisms that were not identified by CGH. The authors concluded that NGS was an acceptable technology to use in PGT-SR.

Zhang et al. (2017) examined the utility of using SNP-microarray in families with balanced translocations to accurately identify euploid embryos for transfer. In 68 blastocysts from 11 translocation families, SNP-microarray identified 42 unbalanced or aneuploidy embryos, and 26 balanced or normal chromosomes. Ten families became pregnant on the first cycle; one family was successful on cycle three. Amniocentesis on the resulting pregnancies matched the embryo microarray analysis, resulting

in a 100% sensitivity and sensitivity in this cohort, but the authors caution that a larger sample size is needed to further validate sensitivity and sensitivity.

Tobler et al. (2014) conducted a retrospective analysis comparing SNP-array and aCGH in 543 embryos from 63 couples, of which one parent carried a reciprocal translocation. Couples were from 16 different fertility centers with samples being analyzed at one lab. SNP-array was used for molecular karyotyping from 2007 to 2011, and from 2011 to 2014, aCGH was used. No embryo was analyzed by both methods. A cell was obtained from the embryo at day 5 or the blastocyst stage and placed in a stabilizing buffer and frozen for transport. Whole genome amplification (WGA) was accomplished for the SNP-array using a phi 29 polymerase protocol, and aCGH WGA was done using a Klenow fragment and a modified random priming protocol. Molecular karyotypes were obtained on 92% (498) of the biopsied embryos. In the 8% (45) samples that failed, WGA failed and was strongly correlated with poor embryo quality. Overall, 45% of embryos were chromosomally normal, and the remaining had translocation errors or aneuploidy. The pregnancy rates were equivalent for SNP (60%) and aCGH (65%). The pregnancy rate was slightly higher if the biopsy was done on blastocysts (65%) vs. cleavage stage embryos (59%). Overall, the authors concluded that SNP or aCGH microarray technologies demonstrate equivalent clinical findings that maximize the pregnancy potential in patients with known parental reciprocal chromosome translocations.

## Clinical Practice Guidelines

### *American College of Obstetricians and Gynecologists (ACOG)*

Committee Opinion Number 799 (ACOG, 2020) indicates that the clinical utility of PGT-M and PGT-SR is firmly established, but the utility of PGT-A has not yet been fully determined. ACOG further recommends:

- Confirmation of PGT-M results by chorionic villus sampling (CVS) or amniocentesis should be offered to all patients
- Confirmation of PGT-SR results by CVS or amniocentesis should be offered to all patients
- Traditional diagnostic testing or screening for aneuploidy should be offered to all patients who have had PGT-A, in accordance with recommendations for all pregnant patients

### *American College of Medical Genetics and Genomics (ACMG)*

In a 2021 position statement, the ACMG addressed direct-to-consumer prenatal testing for multigenic or polygenic disorders indicating that issues surrounding testing for such disorders are very complex. These disorders have been shown to be controlled, at least in part, by multiple genetic loci and the potential influence of unknown environmental factors. The ACMG ultimately recommends that prenatal testing for diseases or disorders that that exhibit polygenic or multigenic heritability is not appropriate for clinical use at this time and should not be offered direct-to-consumer.

### *American Society for Reproductive Medicine (ASRM)*

The ASRM Practice Committee released an opinion report on clinical management of mosaic results from PGT-A of blastocysts in 2020. The report states that there is not yet data to suggest that PGT-A is appropriate for all cases of IVF. Data on outcomes related to pregnancies and children resulting from mosaic embryos is only beginning to emerge. The authors emphasized the importance of comprehensive genetic counseling, based on the most recent empirical studies, for patients undergoing or considering IVF; this counseling is critically important in the case of a mosaic embryo. The Practice Committee recommends that clinicians inform patients that there is no evidence-based method which can determine which mosaic embryos have the best change of resulting in successful pregnancy and concludes that while PGT-A is a technique which may have a role among technologies that can be offered to patients looking to ensure they have healthy children, there is not enough data to endorse its universal application.

The ASRM Ethics Committee published an opinion statement regarding the disclosure of fetal sex when incidentally revealed as part of preimplantation genetic testing. The committee summarized that clinics should have policies in place regarding the determination and disclosure of fetal sex when performing PGT. Patients should give consent as to whether they wish to know available information on sex of embryo(s). Nondiscrimination policies should be developed by clinics performing PGT and patients should be made aware of such policies. In addition, clinics should have policies for using randomized selection of embryos in cases where more embryos are available than can be transferred. Finally, clinics should also develop policies that prohibit consideration of sex of embryo as a factor for transfer and prioritize embryo quality for selection instead (Ethics Committee of the American Society for Reproductive Medicine, 2018).

The Ethics Committee of ASRM published a comprehensive review of the use of PGT-M for adult-onset conditions in 2018. The committee concluded that PGT-M for monogenic adult-onset conditions is ethical when the condition is serious and no safe,

effective interventions are available. Genetic counselors experienced with PGT-M should provide comprehensive counseling to couples considering PGT-M for adult-onset diseases (Ethics Committee of the American Society for Reproductive Medicine, 2018).

### *American Society for Reproductive Medicine (ASRM)/Society for Assisted Reproductive Technology (SART)*

In this joint Practice Committee Opinion from 2018, ASRM and SART state that while some studies have demonstrated higher birth rates after the use of PGT-A and single-embryo transfer, the studies have important limitations. They conclude that the value of PGT-A as a screening test for in vitro fertilization patients has yet to be determined. Large, prospective studies evaluating a variety of approaches to embryo selection are needed to determine the safety and risks of various technologies (Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2018).

### *European Society of Human Genetics (ESHG)*

In a December 2021 publication, Forzano et al. (on behalf of the Executive Committee and the Public and Professional Policy Committee of the ESHG) states the utility for embryo selection using polygenic risk score (PRS) analysis is “severely limited” with no clinical research assessing its diagnostic effectiveness in embryos performed to date. The ESHG recommends education regarding the use of PRS and its limitations and indicates that societal debate focused on what could be considered acceptable regarding individual trait selection must take place before any further implementation of this technology.

### *European Society of Human Genetics and the European Society for Human Reproduction and Embryology*

In a 2017 consensus opinion, the European Society of Human Genetics and the European Society for Human Reproduction and Embryology (Harper et al., 2017) reviewed the pros and cons of PGT for PGT-M and PGT-A. The authors noted that randomized controlled trials for PGT-A are lacking, and that what constitutes success in the literature has been defined differently by different authors, creating a situation where it is not possible to conduct a meta-analysis of available literature. The data to date suggests that PGT-A may improve the clinical outcome for patients with normal ovarian reserve, but more data is needed to determine the validity of PGT-A in other patient populations and at which stage of embryo biopsy.

### *The European Society for Human Reproduction and Embryology (ESHRE)*

In 2020, ESHRE published a series of four papers promoting best practices in PGT; however, the authors note that the papers should not be interpreted as standard of care or inclusive/exclusive of other methods of care. ESHRE recommends that PGT should only be applied when the reliability of the diagnosis is high and potential contraindications (such as age, ability to retrieve gametes, signs/symptoms of autosomal dominant or x-linked disorder which could cause medical complications during the IVF/pregnancy process) have been considered. Physical and psychological problems should be addressed as well. PGT testing is inappropriate in case of uncertain genetic diagnosis (for example genetic/molecular heterogeneity), or in case of uncertain mode of inheritance. For identifying chromosome structural rearrangements, PGT-SR is a routine procedure in most IVF/PGT centers for patients unable to achieve a pregnancy or at high risk of pregnancy loss and of abnormal live born births resulting from inheritance of unbalanced products of the rearrangement, but PGT-SR is only recommended if the technique applied can detect all expected unbalanced forms of the chromosomal rearrangement. PGT-M testing is carried out to confirm pathogenic germline genetic variant(s), that may have serious health effects potentially manifesting at birth, in childhood or in adulthood. Exclusion or non-disclosure testing may be appropriate for late-onset disease, such as Huntington's disease, to avoid pre-symptomatic testing of the individual with a family history of the disease. Exclusion testing is preferred over PGT with non-disclosure of test results to the couple. Cited indications for PGT-A have included advanced maternal age, recurrent implantation failure, severe male factor (SMF) and recurrent miscarriage in couples with normal karyotypes, however, the value of PGT-A for all or a subset of IVF patients remains heavily debated and is the subject of ongoing discussions and research.

### *Preimplantation Genetic Diagnosis International Society (PGDIS)*

The PGDIS recently updated their position statement regarding the transfer of mosaic embryos to include new evidence. The position statement indicates that embryos with higher-level mosaicism appear to be associated with less favorable outcomes when compared to lower-level mosaicism, and relative percentage of mosaicism seems to better predict outcome than the involvement of specific chromosomes. As such, relative percentage of mosaicism should be included in patient discussions and in reporting. The PGDIS further states that decision to transfer a mosaic embryo can be prioritized based either on the level

or type of mosaicism, and if there is a choice between similar levels of mosaicism, preference may be considered based on morphology of embryo or the nature of the variation. Comprehensive patient education and support regarding potential mosaic embryos and prioritization of euploid blastocysts continue to be part of the recommendations for clinicians (PGDIS Position Statement On The Transfer Of Mosaic Embryos 2021).

The PGDIS issued an updated position statement on the transfer of mosaic embryos stating that transfer of an euploid embryo is preferred, but if that is not feasible, priority for transfer of a mosaic embryo should be based on the level of mosaicism over the specific chromosome involved, with preference given to embryos with a mosaicism of less than forty percent. In the event where there must be a choice between the transfer of two unequivocal mosaic embryos, mosaicism involving uniparental disomy, intra-uterine growth retardation, or live-born syndromes should be given lower priority. Patients should be educated on the risks associated with the transfer of mosaic embryos, and it is recommended that an additional cycle of PGT-A be considered to increase the likelihood of obtaining an euploid embryo for transfer (Cram et al., 2019).

The PGDIS position statement on chromosome mosaicism and preimplantation aneuploidy at the blastocyst state states that only a validated next generation sequencing (NGS) platform that can quantitatively measure copy number should be used, and can accurately measure 20% of mosaicism in a known sample (PGDIS Position Statement on Chromosome Mosaicism and Preimplantation Aneuploidy Testing at the Blastocyst Stage, 2016).

# U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

A search of the FDA website identified an approval (K042279) for the Affymetrix Genechip Microarray Instrumentation System on December 23, 2004. Refer to the following website for more information: http://www.accessdata.fda.gov/cdrh_docs/pdf4/K042279.pdf. (Accessed January 31, 2022)

## Additional Products

180K Oligo Array and SNP+CGH Array (Ambry Genetics Corp.); Cytogenomic SNP Microarray (2003414), Cytogenomic SNP Microarray, Prenatal (2002366), and Cytogenomic SNP Microarray, Products of Conception (2005633) (ARUP Laboratories); Chromosomal Microarray Analysis – HR (Test #8655), Chromosomal Microarray Analysis HR+SNP Screen (Test #8665), Chromosomal Microarray Analysis – CytoScan HD SNP Array – Non-Tumor (Test #8650), Targeted Chromosomal Microarray Analysis – Prenatal [Test #8656 (Amniocentesis) or #8657 (CVS)], and Expanded Chromosomal Microarray Analysis – Prenatal [Test #8670 (Amniocentesis) or #8671 (CVS)] (Baylor College of Medicine Medical Genetics Laboratories); Whole-Genome Chromosomal Microarray (GenomeDx), Whole-Genome Chromosomal Microarray, Prenatal, and Whole-Genome Chromosomal Microarray, Products of Conception (GeneDx Inc.); Reveal SNP Microarray – Pediatric; Reveal SNP Microarray – Prenatal, and Reveal SNP Microarray – POC (Integrated Genetics); Chromosomal Microarray, Postnatal, Clarisure Oligo-SNP (Test 16478), Chromosomal Microarray, Prenatal, Clarisure Oligo-SNP (Test 90927), and Chromosomal Microarray, POC, Clarisure Oligo-SNP (Test 90929) (Quest Diagnostics Inc.); Signature ChipOS, Signature ChipOS+SNP, Signature PrenatalChipOS, Signature PrenatalChipOS+SNP, Signature PrenatalChipTE, and Signature PrenatalChipTE+SNP (Signature Genomic Laboratories LLC), HumanKaryomap-12 DNA Analysis Kit (Illumina), IdentifySGD (Progenity, Inc.), Spectrum PGS (Natera, Inc.), Spectrum-PGD+PGS (Natera, Inc.), NexCCS (Foundation for Embryonic Competence).

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## Policy History/Revision Information

| Date | Summary of Changes |
|---|---|
| 10/01/2023 | • New Medical Policy |

## Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the federal, state or contractual requirements for benefit plan coverage must be referenced as the terms of the federal, state or contractual requirements for benefit plan coverage may differ from the standard benefit plan. In the event of a conflict, the federal, state or contractual requirements for benefit plan coverage govern. Before using this policy, please check the federal, state or contractual requirements for benefit plan coverage. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

UnitedHealthcare may also use tools developed by third parties, such as the InterQual® criteria, to assist us in administering health benefits. The UnitedHealthcare Medical Policies are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.