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PGT-A: Yes versus No

Hesham Saleh[1,2,3*], Nehal Adel[3,4]

[1]Obstetrics and Gynecology Department, Faculty of Medicine, Alexandria University, Egypt.
[2]Agial Fertility Center, Agial Hospital, Alexandria, Egypt.
[3]Egyptian Foundation of Reproductive Medicine and Embryology (EFRE), Egypt.
[4]Madina ICSI Fertility Center, Madina women's hospital, Alexandria, Egypt.

Prof. Dr. Hisham Saleh MSc, MD, PhD is a Professor of Obstetrics and Gynecology, Alexandria University,Egypt. Founder and Chairman of the Board of Directors, Agial Hospital, Roushdy, Alexandria, Egypt.Director of Assisted Reproduction Unit, Agial Hospital, Alexandria,Egypt. Founder of Egyptian Foundation of Reproductive Medicine and Embryology (EFRE).

Dr. Nehal Adel MSc, Ph.D. is a Consultant Clinical Embryologist, Director of ICSI fertility center, Madina Women Hospital, Alexandria, Egypt, Coordinator of EFRE embryology Interest group. Managing Editor of Journal of Reproductive Medicine and Embryology (JRME), co-founder of the Egyptian Foundation of Reproductive Medicine and Embryology (EFRE).

PGT-A: Yes

The goal of IVF is a healthy full-term fetus. Still ART remains inefficient. The selection of embryos for transfer is based mainly on morphology. Morphology alone cannot select the embryo with the highest implantation potential (1). About 60% of early human embryos are aneuploid, and it increases dramatically with increasing maternal age (2,3). Embryo aneuploidy is the leading cause of implantation failure and miscarriage after IVF (4–6). Aneuploidy screening through taking a biopsy from the morphologically normal embryos & analysis of the embryo DNA allows to deselect embryos with abnormal chromosomal number. Aneuploid embryos are unable to self-correct. The proposed benefit is to improve embryo selection over morphological assessment to enhance the likelihood that a transferred embryo would result in a healthy live birth.

PGT-A: No

Preimplantation Genetic Testing for Aneuploidies (PGT-A) is a cutting-edge reproductive technology designed to identify chromosomal unbalanced translocations in pre-implantation embryos during in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (65). While PGT-A offers significant potential benefits, including the increased likelihood of successful pregnancy in cases of severe male factor and advanced maternal age patients, and reduced risk of miscarriage and implantation failure, it is essential to consider its drawbacks (66).

PGT-A main goal is to transfer a euploid embryo to shorten the time to pregnancy. This concept sheds light on financial, clinical, and procedural concerns that prospective parents and medical professionals should contemplate when considering this advanced genetic testing method. We will raise questions to be answered from what is already published in the literature.

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Debate

Despite its worldwide use, PGT-A has faced many challenges and concerns from opponents regarding its value, accuracy, validity, cost effectiveness. Several fundamental questions must be answered in this regard:

PGT-A Yes: Does PGT-A improve the cumulative live birth rate?

PGT-A, theoretically, should have the same cumulative live birth as transferring all the available blastocysts one after the other if PGT-A is 100% accurate. Although PGT-A has the same efficacy, i.e., the same number of babies per started cycle, it has a better efficiency, with less time to pregnancy, lower abortion rate, lower abnormal pregnancies, more use of single embryo transfer resulting in a lower multiple pregnancy rate and more cost effective. Moreover, without PGT-A, more embryo transfers are needed to achieve a live birth, and this is usually accompanied by a higher rate of dropouts, leading to the non-transfer of probably viable frozen embryos. Such advantages of PGT-A are even more evident in advanced maternal age (7).

PGT-A Yes: Is PGT-A useful for all IVF patients, especially patients below 35 years?

Conflicts about the value of PGT-A still exist, despite the transition from D3 to D5 biopsy & the use of new platforms (e.g., NGS) that allow the analysis of all chromosomes & the accurate diagnosis of aneuploidy. Some RCTs failed to prove any benefit on the live birth rate (8,9). In the STAR trial, a long waited RCT, when the results of multiple fertility centers are included PGT-A was found to only help patients ≥ 35 years. This means that there is clear indication of PGT-A with advanced maternal age, but it does not help young patients in all fertility centers (10). It seems that less experienced centers may be losing embryos through the process, either by excessive damage to the embryos by the biopsy and/or a high false positive. Moreover, mosaic embryos, known to have a fair implantation potential, were excluded from transfer (11).

PGT-A No: First: IS PGT- A embryo Truly Euploid or Truly Aneuplod?!

Although the error rate of PGT-A results was reported to be too low 1-3%, it cannot be neglected as it may lead to misdiagnosis of embryos even in rare cases. The main cause of error in PGT-A is the inherent biological phenomenon of Chromosomal mosaicism in human preimplantation embryos (67). Mosaicism is the presence of two or more cell populations with different genotypes in an embryo. It results from mitotic error, anaphase lagging, or chromosomal segregation errors (68). The incidence of mosaicism raises a concern that the trophoectoderm biopsy is an imperfect representative of the embryo. Thus, even when PGT-A accurately reflects the content of the TE biopsy, the result is meaningless if the TE biopsy is a poor proxy for the associated blastocyst, and provides a possibility of a misdiagnosis; then error rates expressed as false negative or positive results (69). It is also important to consider that the complexity of mosaicism increases with increasing maternal age, making decisions regarding mosaic embryos difficult, particularly for patients with a low ovarian reserve (70).

Therefore, it is advised to establish error rates through in-house evaluations and follow-up analyses for specific diagnostic tests or strategies. Additionally, prenatal diagnosis should be considered to verify PGT results in the event of a pregnancy (71).

PGT-A No: Second: Does PGT-A guarantee pregnancy?!

A systematic review and network meta-analysis published by Simopoulou et al 2021 included eleven randomized controlled trials employing PGT-A , which found that PGT-A did not improve clinical outcomes for the general population, on the other hand when performing PGT-A for women over 35-year-old, the live birth rates improved (66). They claimed this paradox by the assumption that “if all patients— being subjected to PGT-A or not—received the total number of embryos, the cumulative live-birth rate would be at least equal between the two groups.

In fact, the need for RCTs in assessing the true value of PGT-A has been questioned. It can be argued that individual clinic performances play a key role in both routine IVF and successful application of adjunct processes such as PGT-A. Non-selection studies are more appropriate tools of assessment of PGT-A (12). In highly experienced fertility centers PGT-A significantly improves ongoing pregnancy rate, reduces multiple pregnancies by elective single embryo transfer (eSET), reduces miscarriage rates, improves ongoing pregnancy rate per transfer, reduces the risk of aneuploid pregnancies, and shortens the time-to-pregnancy (TTP) (12–18).

Sanders et al.,(18) analyzed IVF live birth outcomes with and without PGT-A using UK Human Fertilization and Embryology Authority (HFEA) data collection between 2016 and 2018 using propensity score. LBR per embryo transferred and LBR per treatment cycle (including cycles with no transfer) were significantly higher for all PGT-A versus non-PGT-A and a reduced number of transfers per live birth especially for cycles with maternal age over 40 years (19).

PGT-A Yes: Does PGT-A have a high false positive predictive value?

False positive diagnosis in PGT-A stems from several factors. First, there was a previous wrong belief that mosaic embryos are abnormal and were consequently not transferred. Secondly, there was an over-diagnosis of mosaicism due to technical issues and this still exists in less experienced centers (technical mosaicism)(20). The third factor was a high false positive diagnosis of some euploid and mosaic embryos as aneuploid by older platforms as aCGH.

Until recently, mosaic embryos were considered abnormal and treated as “aneuploid” (false positive) and either remained unused (are cryopreserved) or are disposed of (21). However, mosaic embryos may result in live births (22–24). Discarding mosaic embryos reduces pregnancy rate (PR) and LBR (22,25).

The higher the technical error rate, the more likely is that euploid embryos are incorrectly diagnosed Consequently, clinical and ongoing pregnancy and live birth rates were not improved when analyzed per patient and only improved when analyzed per ET (66).

Moreover, The Society for Assisted Reproductive Technology (SART) analysed data from 31,900 patients aged ≤ 37years, and they compared cumulative live birth rates (CLBRs) in cycles with and without preimplantation genetic testing for aneuploidy (PGT-A) among patients aged <35 and 35–37years. they found that in women aged <35, the CLBR was lower with PGT-A than with the transfer of untested embryos. In women aged 35–37 years, PGT-A did not improve CLBRs. The main aim of this mega-analysis was to understand the CLBR in this younger patient population that has a lower risk of aneuploidy and determine the value of doing PGT-A (72).

We can acknowledge the benefits of PGT-A for advanced maternal age (73). However, it is important to recognize that PGT-A is effective only when good-quality euploid embryos are available for transfer. PGT-A will be beneficial primarily when the embryos analyzed come from older mothers, as oocyte aneuploidy is expected to be higher in this group (7).

PGT-A No: Third: Does it Guarantee the birth of a Healthy Baby?!

Although NGS-based CCS is widely recognized as more efficient and precise for detecting segmental mutations at a 5-10 Mb resolution, challenges remain in identifying de novo segmental mutations, which require higher resolution NGS PGT-A. This highlights the need to develop more sensitive PGT platforms for clinical applications (74).

Similarly, evaluating mitochondria is a complex challenge, making advancements in mtDNA examination through PGT technologies a critical focus.In the future, beyond just assessing mtDNA copy numbers, the ability to detect mtDNA mutations and identify candidate genes linked to implantation failure will be pivotal from a clinical perspective (75).

as mosaic & probably discarded (false positive). Euploid embryo may be misdiagnosed by older platforms (aCGH) as mosaic. Potentially, mosaic embryo may not be transferred preventing a possible pregnancy. However, the error rates for recent PGT-A techniques are low (0-2%), and the positive and negative predictive values are around 4% (26–31).

The predictive value of an abnormal result may only be resolved by performing a non-selection study. In such studies, blastocysts are biopsied and transferred prior to performing any analysis. Selection of blastocysts for transfer is based merely on morphology. Once the outcome from the cycle is known, the sample is analyzed, and it is determined if the analysis correctly predicted the clinical outcomes. In the non-selection study of Tiegs et al., 2021 a total of 402 patients underwent 484 single, frozen blastocyst transfers(32). All embryos were biopsied, and the biopsy results were blinded till the outcome was known. A significant difference in outcomes by PGT-A diagnosis was observed: embryos diagnosed as euploid had a chemical pregnancy rate, clinical pregnancy rate, and sustained implantation or delivery rate of 82.1%, 73.3%, and 64.7% respectively, while embryos diagnosed as aneuploid had a chemical pregnancy rate, clinical pregnancy rate and sustained implantation or delivery rate of 40.2%, 23.5% and 0% respectively.

Although the aneuploid clinical error rate was 0% in the above study, the true error rate is unlikely to be 0%, given the numerous possibilities for introduction of error throughout the process of aneuploidy screening. Such potential sources of error include sampling error (i.e., the screening of TE cells rather than the ICM or whole embryo), de novo postzygotic mitotic errors and embryonic mosaicism, DNA amplification failure, contamination, spontaneous conception, and inadvertent mix-up of DNA samples (33–35). Therefore, although unlikely to truly be zero in a much larger sample, the aneuploid call clinical error rate for this PGT-A assay lies between 0% and 2.43%, which is exceedingly low (12,21,36).

IVF physicians and laboratory personnel need to have a thorough understanding of both current and emerging PGT platforms. While PGT-A can help avoid selecting aneuploid embryos, it does not screen for other genetic and metabolic disorders. Therefore, it is crucial to provide comprehensive counseling to patients and assist them in selecting the most appropriate PGT platform to meet their specific needs (76).

PGT-A No: Fourth: Is PGT- A safe intervention?!

The PGT-A cycle involves numerous complex steps and interventions, each of which can affect the viability of the embryos. In line with Murphy's Law, *"If it can go wrong, it will,"* we cannot ignore the fact that mistakes can happen, even with the most experienced operators. When performing PGT-A, the first step is a blastocyst trophectoderm biopsy, a highly invasive technique that can directly impact embryo viability. This is followed by the tubing of the biopsied cells, a very delicate and sensitive step. Any mistake or loss of the biopsied cells makes rebiopsy the only option. Next, Embryo cryopreservation, which further risks embryo viability and increases stress. The cycle continues with chromosomal euploidy analysis, starting with DNA extraction and amplification. If any failure or technical errors occur during this process, rebiopsy is once again the only option (77).

A recent systematic review and meta-analysis investigated the reasons why some euploid embryos fail to implant, analyzing 372 original papers and 41 reviews. One key finding was a slightly lower live birth rate (LBR) in women aged ≥ 38 undergoing PGT-A. Additionally, the study found that multiple cycles of vitrification-warming or a high number of cells biopsied might slightly reduce the LBR (78). Moreover, a retrospective study involving 18,028 blastocysts submitted for trophectoderm biopsy and PGT-A found that 400 out of 517 embryos initially categorized as inconclusive survived intact through the warming procedure, re-expanded, and were suitable for re-biopsy. Among them only 71 rebiopsied

PGT-A Yes: Does PGT-A have a high false negative rate?

Older platforms, like aCGH, had high false negative rates and may wrongly diagnose some aneuploid embryos as euploid and are therefore transferred. They, however, end in miscarriage. Chromosomal reassessment of the products of conception (POC) using NGS, a more accurate platform, revealed that such embryos were aneuploid (37).

PGT-A Yes: Mosaic embryos: What is the incidence of true mosaicism and how to transfer mosaic embryos?

ART is not a risk factor for mosaicism (38–40). We must differentiate between biological “true” mosaicism and “technical” mosaicism. The prevalence of biological mosaicism is less than 0.3% as reported in prenatal test (41). The marked drop in mosaicism between pre- and post-implantation stages has been explained by the selective elimination of aneuploid cells through competitive growth of euploid cells or apoptosis of the abnormal cells (42,43). If the incidence of mosaicism is >5% within a given clinic, consideration should be given to investigating both the embryology and PGT-A practice to identify any possible underlying explanations for that unacceptably high technical mosaicism(44). The source of technical mosaicism could be DNA amplification artifact due to incomplete cell lysis, DNA contamination, poor sample handling/transport, biopsy technique, excessive laser use, biopsy cell quality, and the choice of the algorithm used for normalizing the chromosome mapping bins (20).

The transfer of mosaic embryos is increasingly accepted as a viable option for patients who do not have euploid embryos. There have now been over 2,700 documented embryos transferred with mosaic results (45). Several retrospective studies found mosaic embryo transfer to be associated with a fair although reduced embryo implantation and sustained pregnancy, as well as increased miscarriage rate(23,24,46–51), compared with euploid embryo transfer. Such retrospective data, however, are affected by a strong selection bias. euploid blastocysts transferred, resulted in 32 clinical pregnancies (clinical pregnancy rate of 45.1%), 16 miscarriages (miscarriage rate of 41%), and 12 live births (live birth rate of 23.1%). The study found that transferring rebiopsied blastocysts resulted in a significantly lower live birth rate and higher miscarriage rate when compared to those biopsied only once (77).

PGT-A No: Nothing is more expensive than a missed opportunity.

PGT-A is criticized for discarding embryos that might still have the potential to implant, a phenomenon likened to embryocide. This is due to inconclusive results are often labeled as undiagnosed or mosaic embryos, which can mislead clinical decisions and waste crucial opportunities for patients with advanced maternal age. It is clear that many mosaic embryos possess considerable developmental competence and should not be overlooked for transfer, as highlighted in the PGDIS 2019 Position Statement (79).

Scriven (2022) explained that the presence of a few aneuploid cells in a euploid/aneuploid mosaic embryo has minimal impact on the embryo's potential for a clinical live birth. Consequently, the inclusion of a few aneuploid cells in a trophectoderm biopsy may merely indicate random over-sampling of a small clone of aneuploid cells. A test that excludes every embryo to reduce miscarriage risk will also prevent any pregnancy, which is an absurd approach (80). Additionally, it is important to note that over 2 million DNA repair processes occur during the first cell cycle, and these self-corrective mechanisms are likely responsible for converting mosaic embryos into fully euploid ones during development (81, 82).

On the other hand, Barad (2023) described the pursuit of "*perfect" PGT-A embryos as the enemy of “good” ones, stating, "As we work to provide our patients with PGT-A “perfect” embryos, we risk eliminating many “good” embryos with reasonable chances of achieving their goal."* In other words, By selecting only top-quality blastocysts for biopsy with PGT-A, we may

In particular, retrospective analyses do not take into account the fact that mosaic embryos are transferred as a last option and, consequently, that their reproductive performance is often measured on a highly selected subpopulation of women who had previous failed implantations with euploid embryos. Mosaic embryos are also transferred in those individuals producing only aneuploid embryos, introducing again a strong selection bias toward a poor-prognosis population.

On the other hand, a prospective non-selection study concluded that mosaicism levels under 50% do not impact early embryonic development, with ongoing pregnancy rates and miscarriage rates similar to euploid embryos (52). It is assuring that <1% of mosaic embryos transferred resulted in an ongoing aneuploid pregnancy related to the original PGT-A result (45). The abnormal cells in the mosaic embryo probably die out or don't grow as fast as the normal ones. Moreover, mosaic diagnosis can be technical (not true) and self-correction is unlikely as UPD is extremely rare (53,54).

PGT-A Yes: Concordance between trophectoderm biopsy (TEB) and inner cell mass (ICM).

TEB consists of sample of 3 to 10 blastomeres from the trophectoderm which represents the future placenta. However, the embryo is derived from the ICM. There have been great doubts that sampling few TE blastomeres can represent the ICM. Concerns were raised about the concordance between TEB and the ICM (55–59). It is to be noted that several old concordance studies used outdated platforms for analysis and suffered methodological problems that exaggerated the discordance rates between the TEB and ICM(58). Capalbo et al.,2020 conducted a well-designed concordance study on 73 unselected human blastocysts donated for research (52). The ICM was isolated and the trophectoderm was divided into 4 biopsies. All 5 samples (4 trophectoderm and one ICM) underwent blinded NGS analysis. When the index TEB was euploid, low mosaic, or medium mosaic, the ICM was euploid in 99.6%, 99.3%, and 95.5% respectively. When the index TEB was aneuploid the ICM was 98% aneuploid. The results show a very high concordance rate between the TEB and reduce,or at best not improve, the overall chance of transferring a "good" embryo (83).

This approach could reduce the overall chances of achieving a healthy pregnancy, especially for patients with advanced maternal age or poor ovarian reserve. This hypothesis is supported by an observational study involving over 10,000 women published by Zou et al. (2023), which found that transferring low-grade CC blastocysts was not linked to an increased risk of adverse birth outcomes. Although the live birth rate (LBR) was lower in women aged 38 years or older, it still exceeded 15% with low-grade CC blastocysts (84).

PGT-A No: Finally, Is PGT-A cost-effective?!

PGT-A entails significant costs for IVF, embryo biopsy, embryo cryopreservation, and genetic analysis, as well as the expense of pooling embryos in cases of low ovarian reserve (85). Despite these investments, if the final results show no viable or euploid embryos, all of this effort and expenses may be in vain.

PGT-A No: Considerations:

1. PGT-A is solely focused on selecting embryos based on chromosomal euploidy.
2. PGT-A cannot detect all mutations within an embryo, such as de novo segmental mutations, or identify metabolic disorders.
3. PGT-A cannot guarantee the birth of a healthy child if pregnancy occurs.
4. PGT-A is a multi-step procedure involving highly invasive techniques.
5. PGT-A may jeopardize embryo viability and compromise valuable opportunities, especially in patients of advanced maternal age or poor ovarian reserve.
6. The potential for false-positive and false-negative results, though rare, cannot be ignored.

the ICM, mosaic diagnosis was confined to the trophectoderm and was not reflected in the ICM which was mostly euploid if the TEB is euploid, low or medium mosaic, and aneuploid if the TEB was aneuploid. However, when the TEB showed high mosaicism the ICM was 65% aneuploid.

PGT-A Yes: The biopsy: Does it affect the implantation potential of the embryo?

Scott et al., 2013 reported a dramatic relative reduction of 39% in implantation rate when cleavage stage biopsy was conducted with respect to control (60). This can be explained by the fact that embryos at this stage of preimplantation development are relatively fragile since embryonic genome activation has not taken place yet. Thus, downstream developmental processes can be compromised by removing a cell from the embryo. Moreover, the biopsied blastomere represents 12.5 to 16.6% of the total blastomeres. Such an impact in fact reflects also in a lower blastocyst rate after cleavage stage biopsy with respect to undisturbed embryos, as reported in several papers(61–63). Blastocyst biopsy is gradually replacing cleavage stage biopsy. The power of TEB resides in its higher technical and biological robustness. This approach in fact entails both lower influence of technical errors and lower impact of mosaicism on the molecular analysis. However, proper techniques are required for blastocyst culture and vitrification, which is an important prerequisite for the widespread implementation of this strategy. Blastocyst biopsy, in experienced hands, is a procedure that does not affect embryo viability and implantation potential as proved by well-designed non-selection studies (32,36). A possible explanation for this is that only 5 to 6 trophectoderm cells are removed from a 200-blastomere blastocyst. Moreover, cells are removed from a nonembryonic portion of the blastocyst and at a stage of preimplantation development perhaps more tolerant to manipulation.

It should be emphasized that blastocyst biopsy can still have an impact on the implantation potential of the embryo in less experienced hands. This explains why centers differ significantly in the results when implementing PGT-A (12,64). If the risk of damage is high and embryos are being lost,

PGT-A only improves embryo selection above a threshold of aneuploidy rates. At lower technical embryo loss (<10%) it may improve embryo selection even in young patients with only 20% aneuploidy rates. At high embryo loss rates (30% or more) the injury is only compensated by selecting against 60% or more aneuploidy rates, i.e., among women >35 years old.

PGT-A Yes: Conclusions

PGT-A has a similar CLBR as transferring untested embryos one after the other, but with the advantage of less time to pregnancy, lower abortion rate, lower abnormal pregnancies, more use of single embryo transfer resulting in a lower multiple pregnancy rate and more cost effective. If dropouts are considered, PGT-A may practically have a higher CLBR. PGT-A is useful for all patients especially those above 35 years of age. In experienced centers all ages benefit from PGT-A. PGT-A has a low false positive and low false negative predictive values. The incidence of mosaicism in any IVF clinic should be less than 5%. Low and medium mosaic embryos should be deprioritized for transfer after euploid embryos as they have a good reproductive outcome. They should never be discarded. The concordance between TEB and ICM is very high, and this implies that TEB is predictive of the ICM. Blastocyst biopsy has no effect on the implantation potential of the embryo especially when performed in highly experienced IVF clinics.

References

1. ANAGNOSTOPOULOU C, MALDONADO ROSAS I, SINGH N, GUGNANI N, CHOCKALINGHAM A, SINGH K, et al. Oocyte quality and embryo selection strategies: a review for the embryologists, by the embryologists. Panminerva Med. 2022 Jul;64(2).
2. Griffin DK. The incidence, origin, and etiology of aneuploidy. Vol. 167, International Review of Cytology. 1996.
3. Gruhn JR, Zielinska AP, Shukla V, Blanshard R, Capalbo A, Cimadomo D, et al. Chromosome errors in human eggs shape natural fertility over reproductive life span. Science (1979). 2019 Sep 27;365(6460):1466–9.
4. Schaeffer E, Porchia L, López-Luna A, Hernández-Melchor D, López-Bayghen E. Aneuploidy Rates Inversely Correlate with Implantation during In Vitro Fertilization Procedures: In Favor of PGT. In: Modern Medical Genetics and Genomics. IntechOpen; 2019.
5. Hodes-Wertz B, Grifo J, Ghadir S, Kaplan B, Laskin CA, Glassner M, et al. Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. Fertil Steril. 2012 Sep;98(3):675–80.
6. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two

centers involving 956 screened blastocysts. Human Reproduction. 2014 Jun;29(6):1173–81.
7. Sacchi L, Albani E, Cesana A, Smeraldi A, Parini V, Fabiani M, et al. Preimplantation Genetic Testing for Aneuploidy Improves Clinical, Gestational, and Neonatal Outcomes in Advanced Maternal Age Patients Without Compromising Cumulative Live-Birth Rate. J Assist Reprod Genet. 2019 Dec 12;36(12):2493–504.
8. Kucherov A, Fazzari M, Lieman H, Ball GD, Doody K, Jindal S. PGT-A is associated with reduced cumulative live birth rate in first reported IVF stimulation cycles age ≤ 40: an analysis of 133,494 autologous cycles reported to SART CORS. J Assist Reprod Genet. 2023 Jan 1;40(1):137–49.
9. Verpoest W, Staessen C, Bossuyt PM, Goossens V, Altarescu G, Bonduelle M, et al. Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. Human Reproduction. 2018 Sep 1;33(9):1767–76.
10. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril. 2019 Dec;112(6):1071-1079.e7.
11. Pagliardini L, Viganò P, Alteri A, Corti L, Somigliana E, Papaleo E. Shooting STAR: reinterpreting the data from the 'Single Embryo TrAnsfeR of Euploid Embryo' randomized clinical trial. Reprod Biomed Online. 2020 Apr;40(4):475–8.
12. Wang L, Wang X, Li M, Liu Y, Ou X, Chen L, et al. PGT-A: The biology and hidden failures of randomized control trials. Prenat Diagn. 2022 Aug 25;42(9):1211–21.
13. Neal SA, Morin SJ, Franasiak JM, Goodman LR, Juneau CR, Forman EJ, et al. Preimplantation genetic testing for aneuploidy is cost-effective, shortens treatment time, and reduces the risk of failed embryo transfer and clinical miscarriage. Fertil Steril. 2018 Oct;110(5):896–904.
14. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012 Dec 2;5(1):24.
15. Scott RT, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013 Sep;100(3):697–703.
16. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril. 2013 Jul;100(1):100-107.e1.
17. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017 May;107(5):1122–9.
18. Sanders KD, Silvestri G, Gordon T, Griffin DK. Analysis of IVF live birth outcomes with and without preimplantation genetic testing for aneuploidy (PGT-A): UK Human Fertilisation and Embryology Authority data collection 2016–2018. J Assist Reprod Genet. 2021 Dec 12;38(12):3277–85.
19. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Fertilization and early embryology: Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Human Reproduction. 1993 Dec;8(12):2185–91.
20. Capalbo A, Rienzi L. Mosaicism between trophectoderm and inner cell mass. Fertil Steril. 2017 May;107(5):1098–106.
21. Barad DH, Albertini DF, Molinari E, Gleicher N. IVF outcomes of embryos with abnormal PGT-A biopsy previously refused transfer: a prospective cohort study. Human Reproduction. 2022 May 30;37(6):1194–206.
22. Greco E, Minasi MG, Fiorentino F. Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. New England Journal of Medicine. 2015 Nov 19;373(21):2089–90.
23. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril. 2021 May;115(5):1212–24.
24. Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. Fertil Steril. 2019 Feb;111(2):280–93.
25. Clinical management of mosaic results from preimplantation genetic testing for aneuploidy of blastocysts: a committee opinion. Fertil Steril. 2023 Nov;120(5):973–82.
26. Wells D, Kaur K, Grifo J, Glassner M, Taylor JC, Fragouli E, et al. Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human

embryos prior to implantation. J Med Genet. 2014 Aug 16;51(8):553–62.
27. Fiorentino F, Biricik A, Bono S, Spizzichino L, Cotroneo E, Cottone G, et al. Development and validation of a next-generation sequencing–based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril. 2014 May;101(5):1375-1382.e2.
28. Treff NR, Tao X, Ferry KM, Su J, Taylor D, Scott RT. Development and validation of an accurate quantitative real-time polymerase chain reaction–based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril. 2012 Apr;97(4):819-824.e2.
29. Scott RT, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril. 2012 Apr;97(4):870–5.
30. Gutiérrez-Mateo C, Colls P, Sánchez-García J, Escudero T, Prates R, Ketterson K, et al. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. Fertil Steril. 2011 Mar;95(3):953–8.
31. Werner MD, Leondires MP, Schoolcraft WB, Miller BT, Copperman AB, Robins ED, et al. Clinically recognizable error rate after the transfer of comprehensive chromosomal screened euploid embryos is low. Fertil Steril. 2014 Dec;102(6):1613–8.
32. Tiegs AW, Tao X, Zhan Y, Whitehead C, Kim J, Hanson B, et al. A multicenter, prospective, blinded, nonselection study evaluating the predictive value of an aneuploid diagnosis using a targeted next-generation sequencing–based preimplantation genetic testing for aneuploidy assay and impact of biopsy. Fertil Steril. 2021 Mar;115(3):627–37.
33. Frumkin T, Malcov M, Yaron Y, Ben-Yosef D. Elucidating the origin of chromosomal aberrations in IVF embryos by preimplantation genetic analysis. Mol Cell Endocrinol. 2008 Jan;282(1–2):112–9.
34. Wilton L, Thornhill A, Traeger-Synodinos J, Sermon KD, Harper JC. The causes of misdiagnosis and adverse outcomes in PGD. Human Reproduction. 2009 Feb 27;24(5):1221–8.
35. Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, SenGupta SB, et al. The ESHRE PGD Consortium: 10 years of data collection. Hum Reprod Update. 2012 May 1;18(3):234–47.
36. Scott RT, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril. 2012 Apr;97(4):870–5.
37. Maxwell SM, Colls P, Hodes-Wertz B, McCulloh DH, McCaffrey C, Wells D, et al. Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. Fertil Steril. 2016 Nov;106(6):1414-1419.e5.
38. Kalousek D. K, Dill FJ. Chromosomal Mosaicism Confined to the Placenta in Human Conceptions. Science (1979). 1983 Aug 12;221(4611):665–7.
39. Huang A, Adusumalli J, Patel S, Liem J, Williams J, Pisarska MD. Prevalence of chromosomal mosaicism in pregnancies from couples with infertility. Fertil Steril. 2009 Jun;91(6):2355–60.
40. Zamani Esteki M, Viltrop T, Tšuiko O, Tiirats A, Koel M, Nõukas M, et al. In vitro fertilization does not increase the incidence of de novo copy number alterations in fetal and placental lineages. Nat Med. 2019 Nov 4;25(11):1699–705.
41. HSU LYF, YU MT, RICHKIND KE, VAN DYKE DL, CRANDALL BF, SAXE DF, et al. INCIDENCE AND SIGNIFICANCE OF CHROMOSOME MOSAICISM INVOLVING AN AUTOSOMAL STRUCTURAL ABNORMALITY DIAGNOSED PRENATALLY THROUGH AMNIOCENTESIS: A COLLABORATIVE STUDY. Prenat Diagn. 1996 Jan;16(1):1–28.
42. Bolton H, Graham SJL, Van der Aa N, Kumar P, Theunis K, Fernandez Gallardo E, et al. Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. Nat Commun. 2016 Mar 29;7(1):11165.
43. Singla S, Iwamoto-Stohl LK, Zhu M, Zernicka-Goetz M. Autophagy-mediated apoptosis eliminates aneuploid cells in a mouse model of chromosome mosaicism. Nat Commun. 2020 Jun 11;11(1):2958.
44. Huang A, Adusumalli J, Patel S, Liem J, Williams J, Pisarska MD. Prevalence of chromosomal mosaicism in pregnancies from couples with infertility. Fertil Steril. 2009 Jun;91(6):2355–60.
45. Treff NR, Marin D. The “mosaic” embryo: misconceptions and misinterpretations in preimplantation genetic testing for aneuploidy. Fertil Steril. 2021 Nov;116(5):1205–11.
46. Lledó B, Morales R, Ortiz JA, Blanca H, Ten J, Llácer J, et al. Implantation potential of mosaic embryos. Syst Biol Reprod Med. 2017 May 4;63(3):206–8.

47. Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, et al. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid–aneuploid blastocysts. Hum Genet. 2017 Jul 9;136(7):805–19.
48. Maxwell SM, Colls P, Hodes-Wertz B, McCulloh DH, McCaffrey C, Wells D, et al. Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. Fertil Steril. 2016 Nov;106(6):1414-1419.e5.
49. Spinella F, Fiorentino F, Biricik A, Bono S, Ruberti A, Cotroneo E, et al. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. Fertil Steril. 2018 Jan;109(1):77–83.
50. Zhang L, Wei D, Zhu Y, Gao Y, Yan J, Chen ZJ. Rates of live birth after mosaic embryo transfer compared with euploid embryo transfer. J Assist Reprod Genet. 2019 Jan 24;36(1):165–72.
51. Munné S, Blazek J, Large M, Martinez-Ortiz PA, Nisson H, Liu E, et al. Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing. Fertil Steril. 2017 Jul;108(1):62-71.e8.
52. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. The American Journal of Human Genetics. 2021 Dec;108(12):2238–47.
53. Gueye NA, Devkota B, Taylor D, Pfundt R, Scott RT, Treff NR. Uniparental disomy in the human blastocyst is exceedingly rare. Fertil Steril. 2014 Jan;101(1):232–6.
54. Mounts EL, Besser AG. Lack of evidence to support recommendation for prenatal uniparental disomy (UPD) analysis following mosaic embryo transfer. Genetics in Medicine. 2021 Jan;23(1):230–1.
55. Orvieto R. The reproducibility of trophectoderm biopsies – The chaos behind preimplantation genetic testing for aneuploidy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2020 Nov;254:57–8.
56. Victor AR, Griffin DK, Brake AJ, Tyndall JC, Murphy AE, Lepkowsky LT, et al. Assessment of aneuploidy concordance between clinical trophectoderm biopsy and blastocyst. Human Reproduction. 2019 Jan 1;34(1):181–92.
57. Gleicher N, Vidali A, Braverman J, Kushnir VA, Barad DH, Hudson C, et al. Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos. Reproductive Biology and Endocrinology. 2016 Dec 5;14(1):54.
58. Tortoriello D V., Dayal M, Beyhan Z, Yakut T, Keskintepe L. Reanalysis of human blastocysts with different molecular genetic screening platforms reveals significant discordance in ploidy status. J Assist Reprod Genet. 2016 Nov 16;33(11):1467–71.
59. Orvieto R. Preimplantation genetic screening-the required RCT that has not yet been carried out. Reproductive Biology and Endocrinology. 2016 Dec 24;14(1):35.
60. Scott RT, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013 Sep;100(3):624–30.
61. Hardarson T, Hanson C, Lundin K, Hillensjo T, Nilsson L, Stevic J, et al. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. Human Reproduction. 2008 Sep 1;23(12):2806–12.
62. Debrock S, Melotte C, Spiessens C, Peeraer K, Vanneste E, Meeuwis L, et al. Preimplantation genetic screening for aneuploidy of embryos after in vitro fertilization in women aged at least 35 years: a prospective randomized trial. Fertil Steril. 2010 Jan;93(2):364–73.
63. Staessen C, Verpoest W, Donoso P, Haentjens P, Van der Elst J, Liebaers I, et al. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. Human Reproduction. 2008 Sep 1;23(12):2818–25.
64. Xiong S, Liu W, Wang J, Liu J, Gao Y, Wu L, et al. Trophectoderm biopsy protocols may impact the rate of mosaic blastocysts in cycles with pre-implantation genetic testing for aneuploidy. J Assist Reprod Genet. 2021 May 4;38(5):1153–62.
65. Penzias A, Bendikson K, Butts S, Coutifaris C, Falcone T, Fossum G, et al. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. Fertil Steril. 2018;109:429–36.
66. Simopoulou, M., Sfakianoudis, K., Maziotis, E. et al. PGT-A: who and when? A systematic review and network meta-analysis of RCTs. J Assist Reprod Genet 38, 1939–1957 (2021). https://doi.org/10.1007/s10815-021-02227-9
67. Campos G, Sciorio R, Fleming S. Healthy Live Births after the Transfer of Mosaic Embryos: Self-Correction or PGT-A Overestimation? Genes. 2024; 15(1):18. https://doi.org/10.3390/genes15010018

68. Currie, C.E., Ford, E., Benham Whyte, L. et al. The first mitotic division of human embryos is highly error prone. Nat Commun 13, 6755 (2022). https://doi.org/10.1038/s41467-022-34294-6
69. Popovic M, Dhaenens L, Boel A, Menten B, Heindryckx B. Chromosomal mosaicism in human blastocysts: the ultimate diagnostic dilemma. Hum Reprod Update. 2020 Apr 15;26(3):313-334. doi: 10.1093/humupd/dmz050. Erratum in: Hum Reprod Update. 2020 Apr 15;26(3):450-451. doi: 10.1093/humupd/dmaa015. PMID: 32141501.
70. Armstrong A, Kroener L, Miller J, Nguyen A, Kwan L, Quinn M. The nature of embryonic mosaicism across female age spectrum: an analysis of 21,345 preimplantation genetic testing for aneuploidy cycles. F S Rep. 2023 Apr 8;4(3):256-261. doi: 10.1016/j.xfre.2023.03.008. PMID: 37719098; PMCID: PMC10504531.
71. ESHRE PGT Consortium Steering Committee; Carvalho F, Coonen E, Goossens V, Kokkali G, Rubio C, Meijer-Hoogeveen M, Moutou C, Vermeulen N, De Rycke M. ESHRE PGT Consortium good practice recommendations for the organisation of PGT. Hum Reprod Open. 2020 May 29;2020(3):hoaa021. doi: 10.1093/hropen/hoaa021. PMID: 32524036; PMCID: PMC7257038.
72. Mejia RB, Capper EA, Summers KM, Mancuso AC, Sparks AE, Van Voorhis BJ. Cumulative live birth rate in women aged ≤37 years after in vitro fertilization with or without preimplantation genetic testing for aneuploidy: a Society for Assisted Reproductive Technology Clinic Outcome Reporting System retrospective analysis. F S Rep. 2022 May 11;3(3):184-191. doi: 10.1016/j.xfre.2022.05.004. PMID: 36212571; PMCID: PMC9532873.
73. Shi WH, Jiang ZR, Zhou ZY, Ye MJ, Qin NX, Huang HF, Chen SC, Xu CM. Different Strategies of Preimplantation Genetic Testing for Aneuploidies in Women of Advanced Maternal Age: A Systematic Review and Meta-Analysis. J Clin Med. 2021 Aug 30;10(17):3895. doi: 10.3390/jcm10173895. PMID: 34501345; PMCID: PMC8432243.
74. Hsin-Fu Chen, Ming Chen, Hong-Nerng Ho, An overview of the current and emerging platforms for preimplantation genetic testing for aneuploidies (PGT-A) in in vitro fertilization programs, Taiwanese Journal of Obstetrics and Gynecology, Volume 59, Issue 4, 2020, Pages 489-495, ISSN 1028-4559, https://doi.org/10.1016/j.tjog.2020.05.004.
75. Akifumi I, Hiroe U, Tomonari H, Shunsuke M, Ai M, Haru H, Sumiko S, Shiori T, Marina S, Haruka H, Teppei T, Yasushi Y, Etsuko M, Hiroki K, Hideya S, Mariko M. Mitochondrial DNA mutations can influence the post-implantation development of human mosaic embryos. Frontiers in Cell and Developmental Biology, VOLUME 11, 2023. https://www.frontiersin.org/journals/cell-and-developmental
76. Giuliano R, Maione A, Vallefuoco A, Sorrentino U, Zuccarello D. Preimplantation Genetic Testing for Genetic Diseases: Limits and Review of Current Literature. Genes (Basel). 2023 Nov 17;14(11):2095. doi: 10.3390/genes14112095. PMID: 38003038; PMCID: PMC10671162.
77. Nohales, M., Coello, A., Martin, A. et al. Should embryo rebiopsy be considered a regular strategy to increase the number of embryos available for transfer?. J Assist Reprod Genet 40, 1905–1913 (2023). https://doi.org/10.1007/s10815-023-02875-z
78. Cimadomo D, Rienzi L, Conforti A, Forman E, Canosa S, Innocenti F, Poli M, Hynes J, Gemmell L, Vaiarelli A, Alviggi C, Ubaldi FM, Capalbo A. Opening the black box: why do euploid blastocysts fail to implant? A systematic review and meta-analysis. Hum Reprod Update. 2023 Sep 5;29(5):570-633. doi: 10.1093/humupd/dmad010. PMID: 37192834.
79. Leigh D., Cram D.S., Rechitsky S., Handyside A., Wells D., Munne S., Kahraman S., Grifo J., Katz-Jaffe M., Rubio C., Viotti M., Forman E., Xu K., Gordon T., Madjunkova S., Qiao J., Chen Z.-J., Harton G., Gianaroli L., Simon C., Scott R., Simpson J.L., Kuliev A. PGDIS position statement on the transfer of mosaic embryos 2021. RBMO, VOLUME 45, ISSUE 1 2022.
80. Scriven PN. Elucidating the PGT-A paradox: marginalising the detriment relegates the benefit. J Assist Reprod Genet. 2022;39:2475–81.
81. Gleicher N, Patrizio P, Brivanlou A, Preimplantation Genetic Testing for Aneuploidy – a Castle Built on Sand, Trends in Molecular Medicine, Volume 27, Issue 8, 2021, Pages 731-742, ISSN 1471-4914, https://doi.org/10.1016/j.molmed.2020.11.009.
82. Lin J, Vitek WS, Scott EL. Order from chaos: case report of a healthy live birth from a genetically “chaotic” embryo. F&S Reports 2022;3: 301–4.
83. Barad, D.H. PGT-A “perfect” is the enemy of good. J Assist Reprod Genet 40, 151–152 (2023). https://doi.org/10.1007/s10815-022-02706-7
84. Zou H, Kemper JM, Hammond ER, Xu F, Liu, Xue GL, Bai X, Liao H, Xue S, Zhao S, Xia L, Scott J, Chapple V, Afnan M, Morbeck DE, Mol BWJ, Liu Y, Wang R, Blastocyst quality and reproductive and perinatal outcomes: a

multinational multicentre observational study, Human Reproduction, Volume 38, Issue 12, December 2023, Pages 2391–2399, https://doi.org/10.1093/humrep/dead212

85. Facadio Antero M, Singh B, Pradhan A, Gornet M, Kearns WG, Baker V, Christianson MS. Cost-effectiveness of preimplantation genetic testing for aneuploidy for fresh donor oocyte cycles. F S Rep. 2020 Dec 9;2(1):36-42. doi: 10.1016/j.xfre.2020.11.005. PMID: 34223271; PMCID: PMC8244284.