

10/17/2021

# COMPLICATIONS AND LIMITATIONS OF EMBRYOLOGY IMPLEMENTATION IN PGT

# Preimplantation genetic testing (PGT)

- Traditionally = preimplantation genetic diagnosis
- Began its clinical application in the early 1990s.
- First used to prevent X-linked diseases, such as adrenoleukodystrophy and X-linked mental retardation (Handyside et al., 1990).
- Very soon after, live births from PGT for monogenic/single gene defects (PGT-M), such as cystic fibrosis and thalassemias, were also reported (Handyside et al., 1992).

# Preimplantation genetic testing (PGT)

(continue)

- The application of PGT was quickly broadened as an adjunct to in vitro fertilization (IVF) treatment.
- Numerous studies on spontaneous miscarriage and arrested preimplantation embryos demonstrated the correlation between maternal age and chromosomal abnormalities;
- Therefore, PGT for aneuploidies (PGT-A) was established to improve the reproductive outcome for women with advanced maternal age (AMA) and recurrent miscarriages.

# PGT-A usefulness and effectiveness

- Unlike PGT-M and PGT for chromosomal structural rearrangements (PGT-SR), PGT-A remains controversial in its usefulness and effectiveness.
- In its earliest form,
  - ✓ PGT-A was achieved by using fluorescent in situ hybridization (FISH)
  - ✓ to analyze 5 or up to 12 chromosomes only
  - ✓ and most of the work was done on single blastomeres from embryos at the cleavage stage.

# PGT-A usefulness and effectiveness

- positive data were reported (Munné et al., 1999, 2002), although not without reprimand (Mastenbroek et al., 2007; Fauser, 2008).
- the meta-analysis by Mastenbroek et al. (2011) was probably monumental in marking an end to the use of FISH in PGT-A
  - ✓ given the limitations on the number of chromosomes that could be scrutinized
  - ✓ the biopsy technique
  - ✓ as well as the embryo quality at the time of biopsy.
- Thereafter, advancement in technology has allowed PGT-A to reclaim its ground by providing comprehensive chromosome screening (CCS).

# “Second generation” PGT-A(PGS 2.0)

- ✓ Include embryo biopsy at the blastocyst stage.
- ✓ Use of array comparative genomic hybridization (aCGH)
- ✓ Single nucleotide polymorphism arrays (SNP arrays)
- ✓ quantitative polymerase chain reaction (qPCR) to detect aneuploidy
- ✓ Despite a few randomized controlled trials (RCTs) showing PGT-A could improve implantation and ongoing pregnancy rates ( much challenge remains to conduct RCTs of large enough scale to allow unbiased analyses on outcomes such as live birth rate.



# PRETESTING EXPECTATION AND CONSIDERATION

- ✓ Patients, especially those who have had a failed cycle, always want a successful transfer where the pregnancy can be carried to term, with the delivery of a healthy baby.
- ✓ The aim as a profession involved in the IVF process then is to help these patients achieve their dream by giving them a single euploid transfer, saving them time and emotional disappointment from implantation failure, pregnancy loss, or having to decide to terminate an aneuploid pregnancy.

# PRETESTING EXPECTATION AND CONSIDERATION

- ✓ In the provision of PGT-A, the service must be viewed as a continuum, with clinicians, embryologists, and geneticists as the key players.
- ✓ Although the key players have expertise in their own disciplines, they need to communicate with each other and understand the basic requirement and sometimes limitations of the other disciplines.



# To offer the service or not?

- ✓ The clinician in an IVF program has the important role of providing objective medical information and advice to patients.
- ✓ Although medical doctors generally provide evidence-based medicine, personal belief also has an influence on whether certain practice such as PGT-A will be adopted.
- ✓ It is probably too soon to assume all patients will benefit from such service; however, there is a general acceptance of offering PGT-A to those of AMA, those who experience repeated implantation failure (RIF), recurrent pregnancy loss (RPL), or previous abnormal pregnancy (PAP). There is no consensus on PGT-A for patients who have severe male factor (SMF) infertility.

# To offer the service or not?

Recently, a large multicenter RCT (the Single embryo Transfer of euploid embryo trial, or the STAR trial) on PGT-A efficacy was completed.

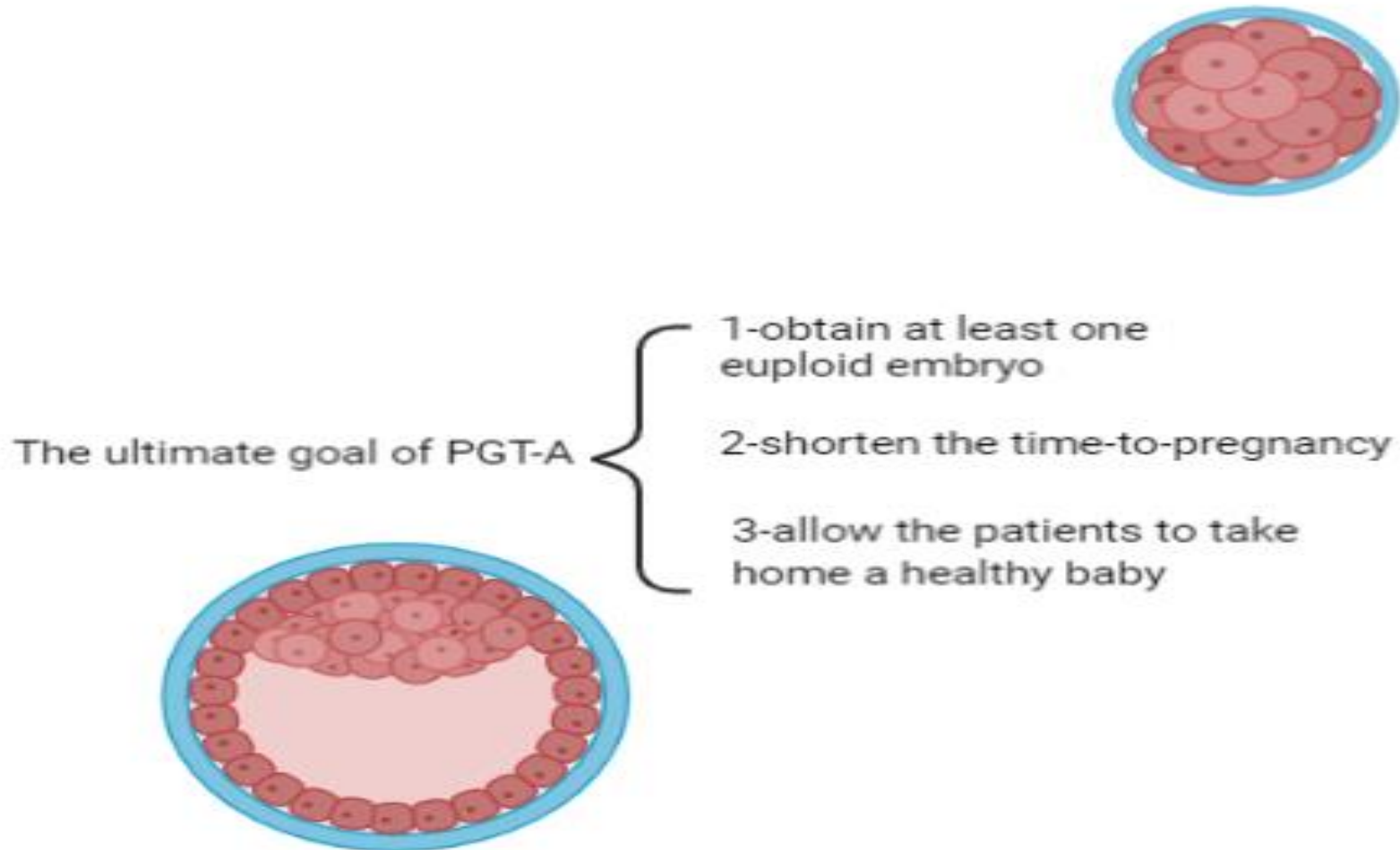
This RCT used one NGS platform for PGT-A, involving biopsies of day-5 or day-6 embryos

- from 34 clinical sites
- in four countries
- and genetic testing in nine laboratories.
- usefulness of PGT-A only in patients of  $\geq 35$  years of age.
- The majority of patient subjects were in the young group (aged 25–34 years old) and showed no significant difference in ongoing pregnancy rate with or without PGT-A.

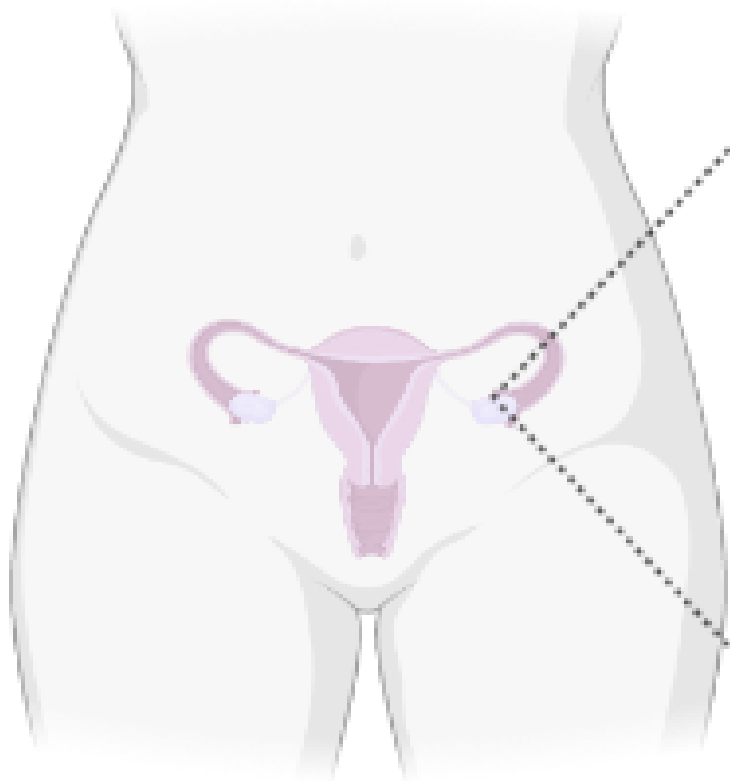
# To offer the service or not?

- The failure to replicate the outcome improvements as in previous single-centered RCTs implied that patients <35 years of age might not benefit from PGT-A; however, PGT-A did appear to help those of AMA with a good number of embryos.

# The choice of stimulation protocols



## ***The most difficult scenario: is patients who have poor ovarian response***



1-produce very few embryos

2- number of which will further be reduced after blastocyst culture and PGT-A

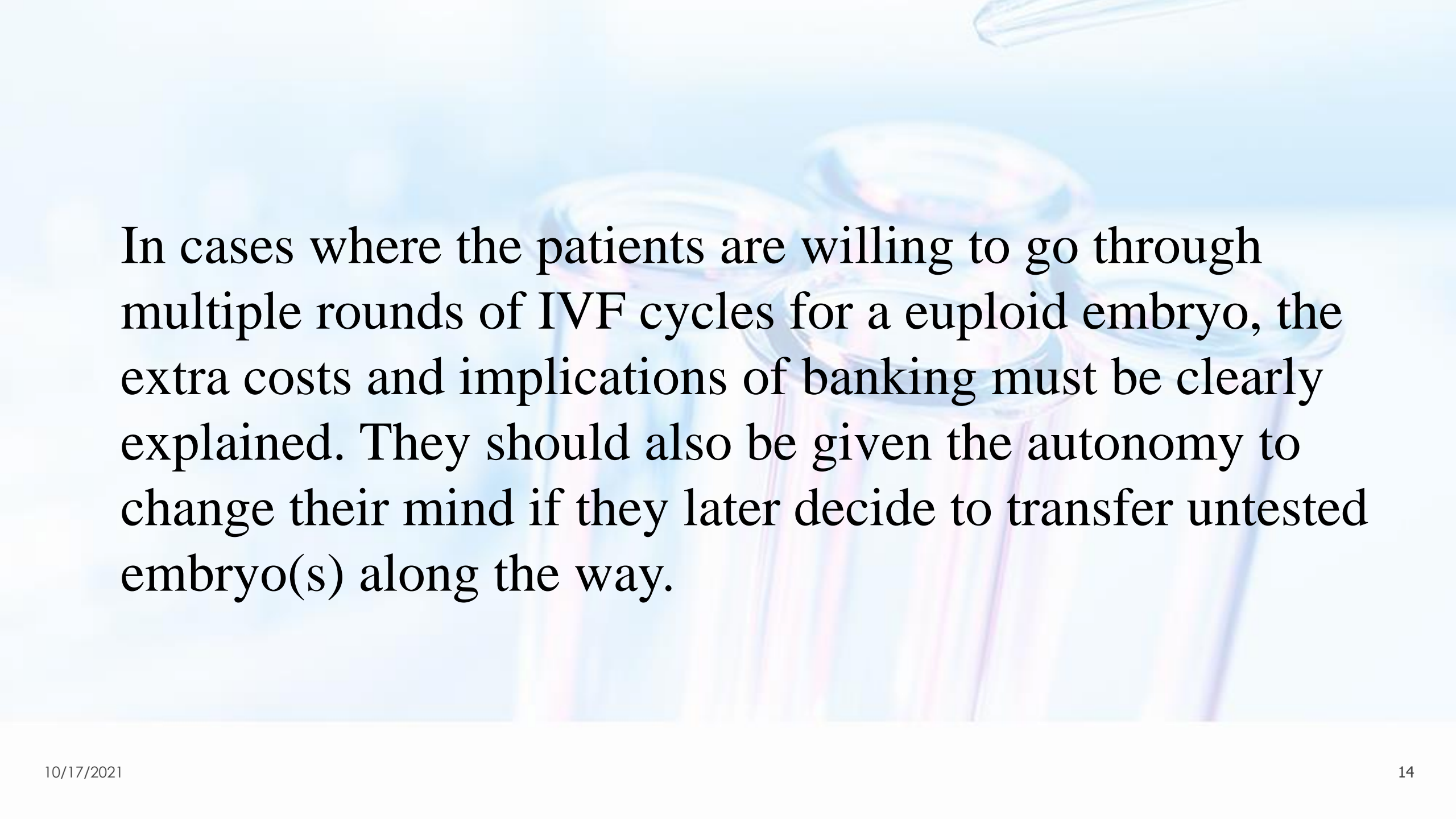
3- As the chance of having a euploid embryo from one IVF cycle is very low

4- some programs may recommend banking of embryos from more than one IVF cycles.

5- there may be minimum charges for the embryo biopsy procedure and/or genetic testing in some laboratories.

6- sometimes it may be the best for these patients to simply stay with the routine standard-of-care, that is, transfer embryo(s) that are morphologically acceptable but genetically untested (Paulson, 2016).

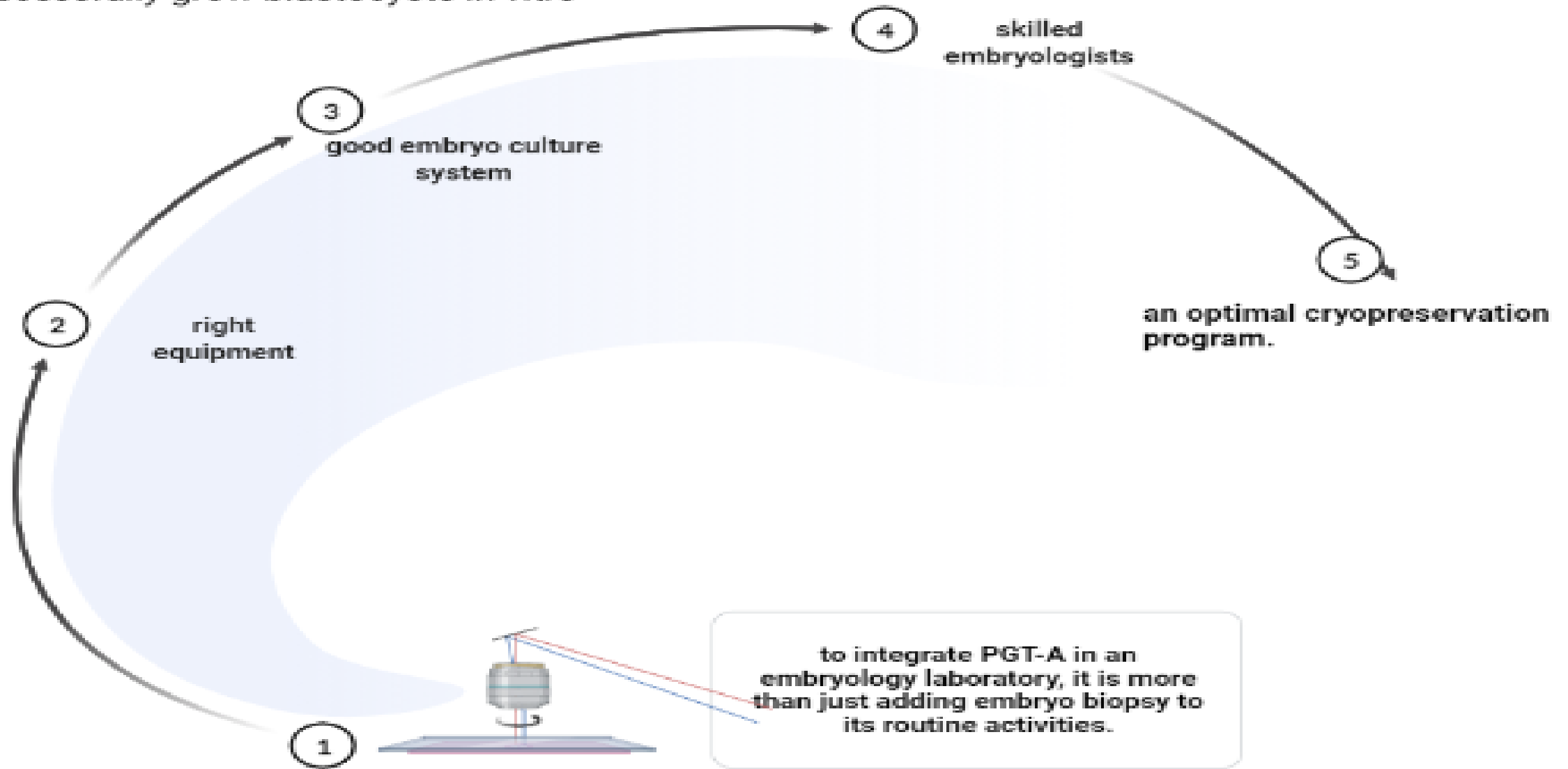


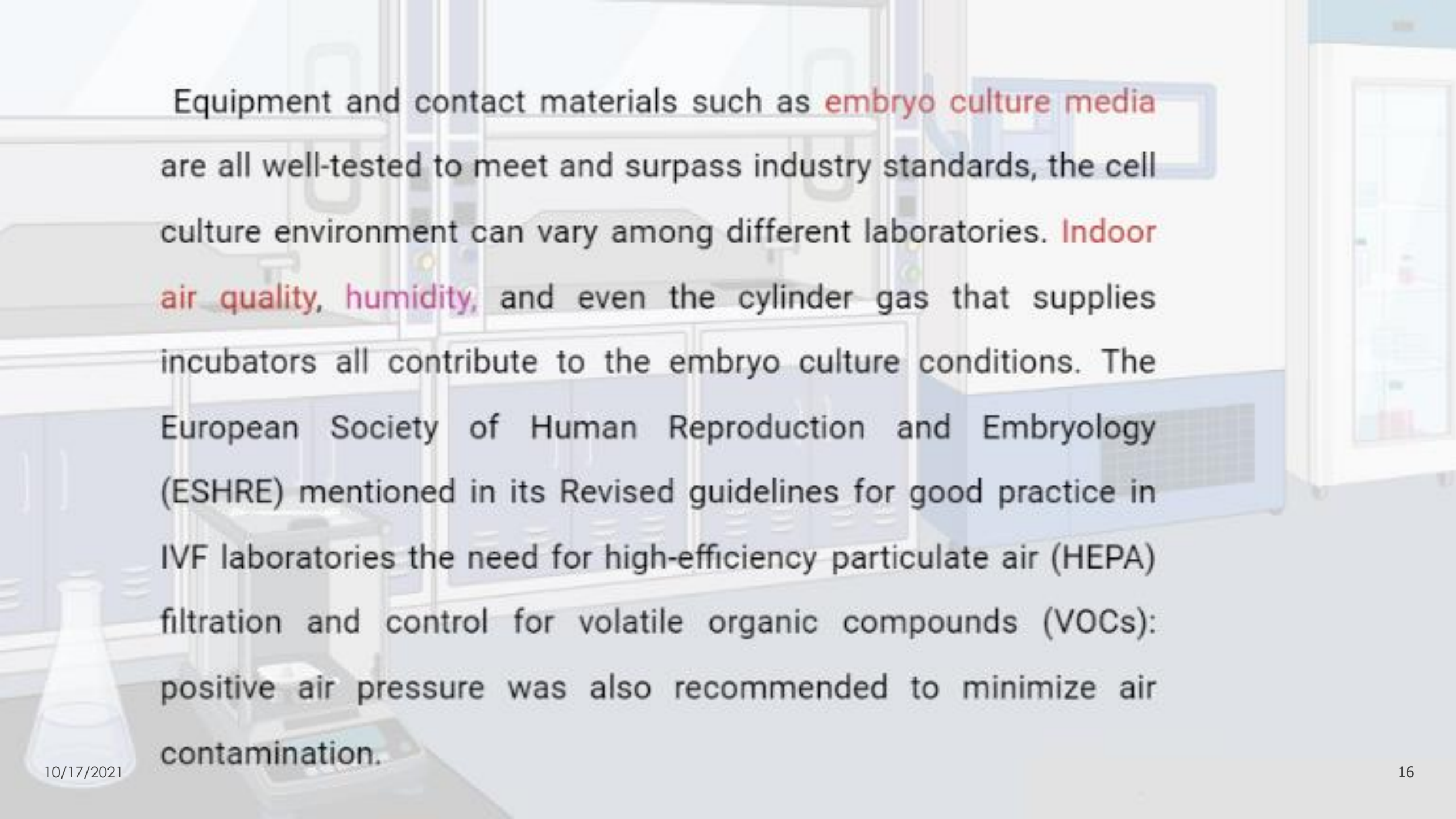


In cases where the patients are willing to go through multiple rounds of IVF cycles for a euploid embryo, the extra costs and implications of banking must be clearly explained. They should also be given the autonomy to change their mind if they later decide to transfer untested embryo(s) along the way.

# Is the embryology laboratory ready?

The most critical prerequisite for a successful PGT-A service, or an IVF laboratory in general, is its ability to successfully grow blastocysts in vitro





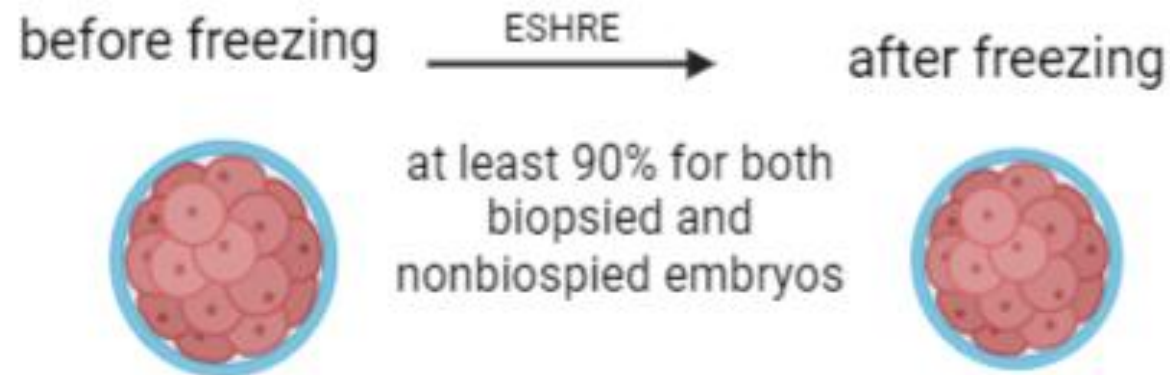
Equipment and contact materials such as **embryo culture media** are all well-tested to meet and surpass industry standards, the cell culture environment can vary among different laboratories. **Indoor air quality, humidity,** and even the cylinder gas that supplies incubators all contribute to the embryo culture conditions. The European Society of Human Reproduction and Embryology (ESHRE) mentioned in its Revised guidelines for good practice in IVF laboratories the need for high-efficiency particulate air (HEPA) filtration and control for volatile organic compounds (VOCs): positive air pressure was also recommended to minimize air contamination.

## Laboratory infrastructure

- The laboratory should be well ventilated to minimise the effect of any noxious fumes. This is particularly important if cells are fixed using methanol and acetic acid. In this case the use of a fume cabinet for the fixation steps is recommended.
- FISH outcomes, including cell spreading and fixation, are dependent on humidity. The humidity in the FISH laboratory should be controlled and stable. FISH protocols should be optimised in these conditions.
- FISH signals may be bleached or weakened in bright light. It is recommended that the FISH laboratory be fitted with variable intensity incandescent lighting. Fluorescent lighting is acceptable. The slides should be stored cool and in light-tight storage boxes or folders.

## A highly efficient cryopreservation program

- Slow-freezing
- Vitrification
- A recent review and meta-analysis comparing slow-freezing and vitrification showed that cryosurvival rate for embryos and blastocysts was significantly improved after vitrification (Rienzi et al., 2017).





# Training

- Depending on regulatory or accreditation requirement, **embryos from mice** are often used for training; alternatively, supernumerary **embryos may be obtained through donation by patients.**
- ESHRE PGD Consortium guidelines for PGT recommended training on at least 50 blastomeres for fixation and 100 cells for chromosomal analyses.
- Training emphasis should also be made on sample tubing because the successful placement of the biopsied sample into a PCR tube is critical.



# Training

- Although it is possible for the DNA in a sample to be fragmented or degraded in some circumstances.
- the most probable cause for amplification failure in PGT is that there has been no sample in the PCR tube.
- The competency level of detectable DNA in an embryo biopsy should be maintained at  $\geq 90\%$  (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017)

# Witnessing

- Different personnel from the biopsy embryologist should be available to check and match the identifiers of all embryos and their corresponding TE samples on dishes, PCR tubes, vitrification devices, and the paperwork.
- Particular attention may be required for numbers such as “1” and “7”, “6” and “9” when labeling is handwritten instead of printed.
- It is estimated that an embryology laboratory already providing blastocyst culture needs to devote additional resources and approximately 7 hours of personnel time for every biopsy case.
- Electronic witnessing can be an option to ease manpower, but its implementation will add considerable asset and maintenance costs especially to smaller IVF practices.

# Manpower consideration

- It requires at least one embryologist who is skilled to perform the biopsy procedure and another to help with witnessing the correct designation of embryos.
- Depending on the number of biopsy cases in a year, it is crucial for an IVF laboratory to devote at least a manpower equivalence of one full day of one embryologist on the day when there is an embryo biopsy.
- ESHRE and the American Society for Reproductive Medicine (ASRM) specify a minimum of two qualified persons to perform up to 150 oocyte retrieval and/or cryopreservation cycles per year and one additional embryologist per additional 200 cycles.

# The choice of genetic laboratory

- Some IVF programs choose to set up and have their own diagnostic facility of PGT-A, while many others send their biopsied samples to reference laboratories for testing.
- In providing PGT-A, a genetic laboratory must be proficient in at least one of the PGT-A methods (aCGH, SNP arrays, qPCR, or NGS) to give consistent and reliable diagnostic results.



# Change of circumstances

- ✓ There may be only one or two good quality blastocysts available for biopsy.
- ✓ In these scenarios, a clinician may wish to counsel the patients based on their fertility background regarding the decision for PGT-A.
- ✓ For example:
  - if a patient suffering from recurrent pregnancy loss has only one blastocyst suitable for biopsy, it may be a better option for her to proceed testing as the original intention was to avoid another possible miscarriage, instead of transferring this embryo without testing.
  - if a patient is undergoing her first-time IVF with PGT-A due to AMA and has only one blastocyst developed from a good number of cleavage embryos, she may consider withdrawing her PGT-A decision because she may want to proceed with embryo transfer after all, on the premise that the extended culture has already helped to select out the best one among the crop.

# Change of circumstances

- ❖ Before reaching the final decision, a joint meeting with the responsible clinician, embryologist and the couple should be considered to rediscuss the pros and cons and ultimate objective of testing.

# When and what to biopsy

- ❖ Gamete handling and embryo culture in the laboratory are basically the same for PGT or non-PGT cases, except that ICSI is recommended for fertilization to avoid extraneous sperm from contaminating the DNA amplification of the biopsied samples and compromising test results.
- ❖ There are different stages at which a biopsy can be performed. When there are religious or regulatory restrictions, PGT-A can only be done on the first and/or second polar bodies removed from an oocyte and/or a zygote to avoid moral or legal concerns with meddling with an embryo.

# When and what to biopsy

- ❖ In order to interrogate both the maternal and paternal attributes in an embryo, the removal of one or two blastomeres at cleavage-stage has once been the “norm” due to its relative ease and also flexibility to accommodate fresh embryo transfer on day-5.
- ❖ It is now known that 40%–70% of embryos can be mosaic, in which more than one cell line is present within the same embryo. The analysis of one single cell from a mosaic embryo can thus lead to a false positive/negative result.
- ❖ The removal and testing of around 5–10 cells obtained from the trophectoderm (TE) provides diagnosis that is more representative of the embryo while avoiding damage to the inner cell mass (ICM).

# When and what to biopsy

- ❖ As demonstrated in a paired randomized clinical trial (Scott et al.,2013), the implantation potential of biopsied embryos was impaired when biopsy was done at cleavage stage but not so at blastocyst stage.
- ❖ This RCT, however, only biopsied high-quality blastocysts in patients with good prognosis; it did not address the impact of day-5 biopsy on high-quality versus low-quality blastocysts.
- ❖ Based on common practice, most laboratories culture embryos up to day-6 for blastocyst assessment. For those that also perform embryo biopsy, they need to set up a robust policy on when and what to biopsy to tailor the relevant activities into their workflow.



# When and what to biopsy

✓ For example:

- many busy units assess embryos only in the mornings of day 5 and day 6, followed by biopsy of those that have full or expanded blastocoel with both ICM and TE graded B or above on the system of Gardner.
- Some labs, on the other hand, will reassess embryos a second time in the afternoon and perform biopsy when needed. Whether or not to biopsy blastocysts of poorer morphology also depends on the laboratory policy.
- In most circumstances, one PGT-A cycle will involve TE biopsies on both day 5 and day 6. It has been shown that euploids of both days give comparable clinical outcomes

# When and what to biopsy

- ❖ Interestingly, there were increasing reports on the viability of embryos that had slow development but reached blastocysts on day 7 in culture. Although the aneuploidy rate was higher compared to those of day 5 and day 6, a small proportion of day 7 blastocysts could still produce healthy live births.
- ❖ Based on etiology and cycle characteristics of the women, embryologists may consider the possibility of allowing at least some biopsy cases to undergo further culture to day 7, or perform biopsy on embryos at morula stage on day 6.

# Potential harm

- ❖ PGT-A is an invasive procedure that may lead to the loss of healthy embryos because the prerequisites for prolonged culture, biopsy, and freeze–thaw process may impair the developmental potential of the embryo. Secondly, while PGT-A is generally applied to select euploids out of the aneuploids, it is not a test with absolute accuracy.
- ❖ Much like other diagnostic tests, it may still give false positive results. One important source of a false positive result is the biological phenomenon of mosaicism in which a biopsy sample does not represent the true ploidy status of the majority of the TE nor the ICM

# Potential harm

- ❖ Laboratory errors may be another source of false positive results but it ought to be rare.
- ❖ Occasionally, the result may be inconclusive due to amplification failure or failed quality control. In such a situation, the embryo may be rebiopsied, revitrified, and rewarmed, which may further impair its potential to successfully implant.
- ❖ For all these reasons, PGT-A is not without potential harm and may well lead to wastage of embryos capable of producing live birth at the expense of an increase in implantation rate and a reduction in miscarriage rate.

# POSTTESTING EXPECTATION AND CONSIDERATION

This is the stage when laboratory testing is complete and the final results are released to the clinical team and/or embryology laboratory. In most IVF practice, the clinicians carry the responsibilities to disclose PGT-A results to patients; and therefore, a PGT-A report must contain clear interpretation on the diagnosis so that the clinician can make appropriate recommendation.

# To rebiopsy or not

- When there is no result due to failed DNA amplification or failed QC parameters because of the quality of amplified DNA, some clarification maybe required before deciding to rebiopsy or discard an embryo.
- Having to rebiopsy an embryo implies that it needs to be thawed or warmed, to undergo a second biopsy, followed by refreezing or revitrification, and possibly a second thawing or warming if it were a euploid.
- As the embryology laboratory may indeed biopsy morphologically poor embryo(s) for PGT-A when a cohort of blastocysts was of generally suboptimal quality, the decision to rebiopsy any embryo should balance the likelihood of it surviving the extra micromanipulation, the additional costs incurred for the process, and the possibility of compromised implantation potential after two rounds of biopsy.



# To rebiopsy or not

Preliminary data available on rebiopsy showed that blastocysts with “no diagnosis” results still had a good chance to be euploids (Brower et al., 2014; Neal et al., 2017a). However, when compared to euploids that were only biopsied once, euploids that were twice biopsied had lower clinical pregnancy rate and live birth rate (Bradley et al., 2017).

It is important to note that these rebiopsy data were from experienced groups with large volumes of PGT-A; and at a “no diagnosis” rate of <4%, the sample size for a retrospective analysis on rebiopsy cases was still very small.

Also, the quality of blastocysts and the size of the cell mass sampled at the time of their first and second biopsies were not addressed. Additional well planned studies with a sufficiently large sample size are required to verify if repeat biopsy of the blastocyst does not indeed impair the developmental potential of the embryo.

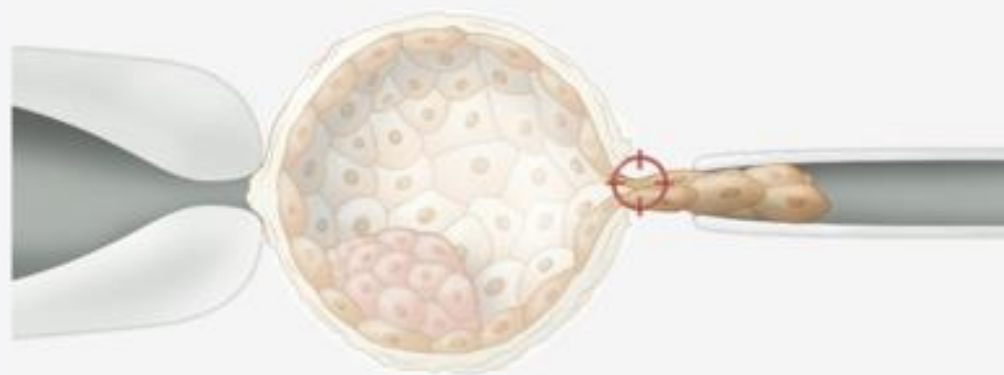
# Understanding mosaicism

- ❖ With the use of advanced technology such as NGS is its capability of detecting mosaicism.
- ❖ Mosaicism is the coexistence of two or more cell lines within the same entity.
- ❖ A difficult situation arises when a euploid cell line coexists with one or more aneuploid cell lines in the same embryo, and this may further be complicated by their relative level or proportion and perhaps also by which particular chromosome(s) involved.

# Understanding mosaicism

- ❖ Although such mosaic result is based on sampling only a small localized area of TE cells and may not be fully representative of the ICM, there could still be placental implications for the established pregnancy, which in turn might affect the fetus and/or live birth.
- ❖ According to the Preimplantation Genetic Diagnosis International Society(PGDIS) Position Statement on chromosome mosaicism (PGDIS,2016), embryos with a euploid result should always be prioritized for transfer over those with a mosaic result.
- ❖ When a patient has no euploid but at least one mosaic embryo, clinicians should always discuss the option of a further IVF cycle.
- ❖ If the patient does not consider other alternative but transferring a mosaic blastocyst, appropriate genetic counseling should be provided before such transfer.

A

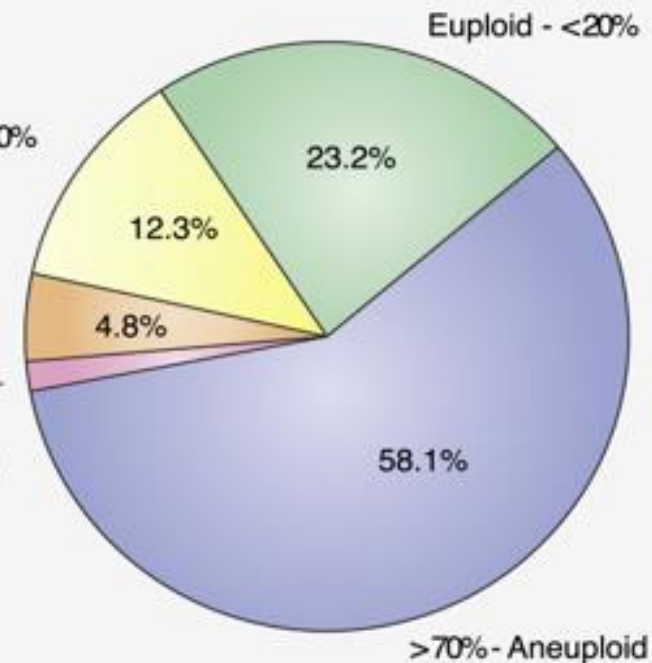


Clinical trophoctoderm biopsies N = 6,766  
Mean age =  $38.06 \pm 3.65$  years old

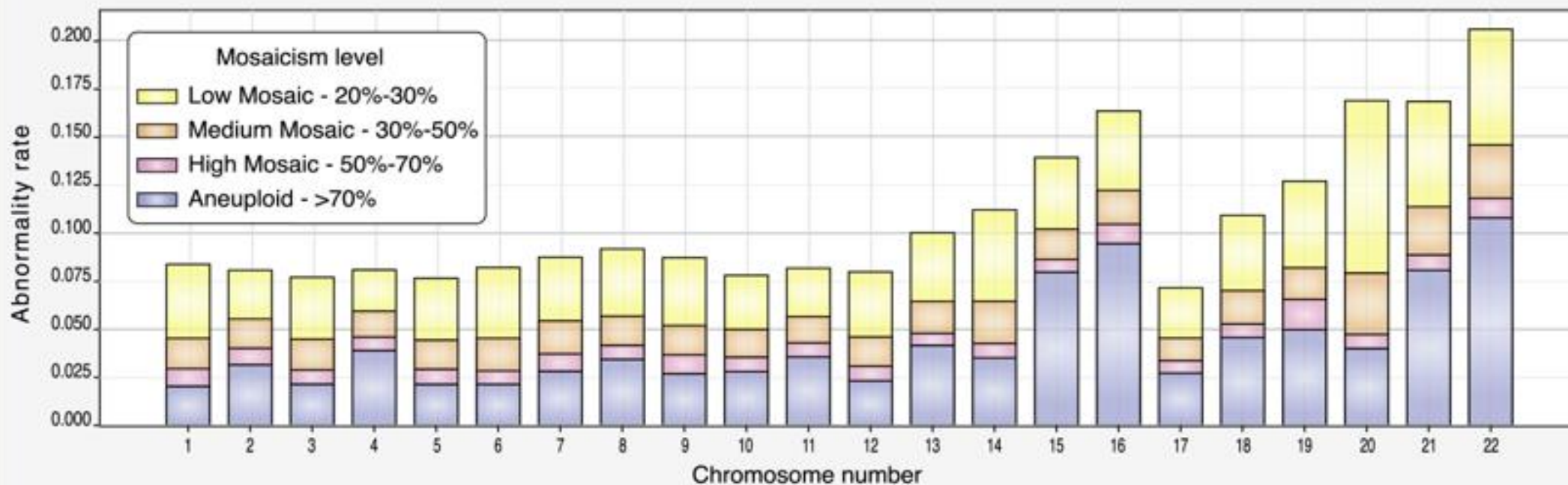
Low Mosaic - 20%-30%

Medium Mosaic 30%-50%

High Mosaic - 50%-70%  
1.6%



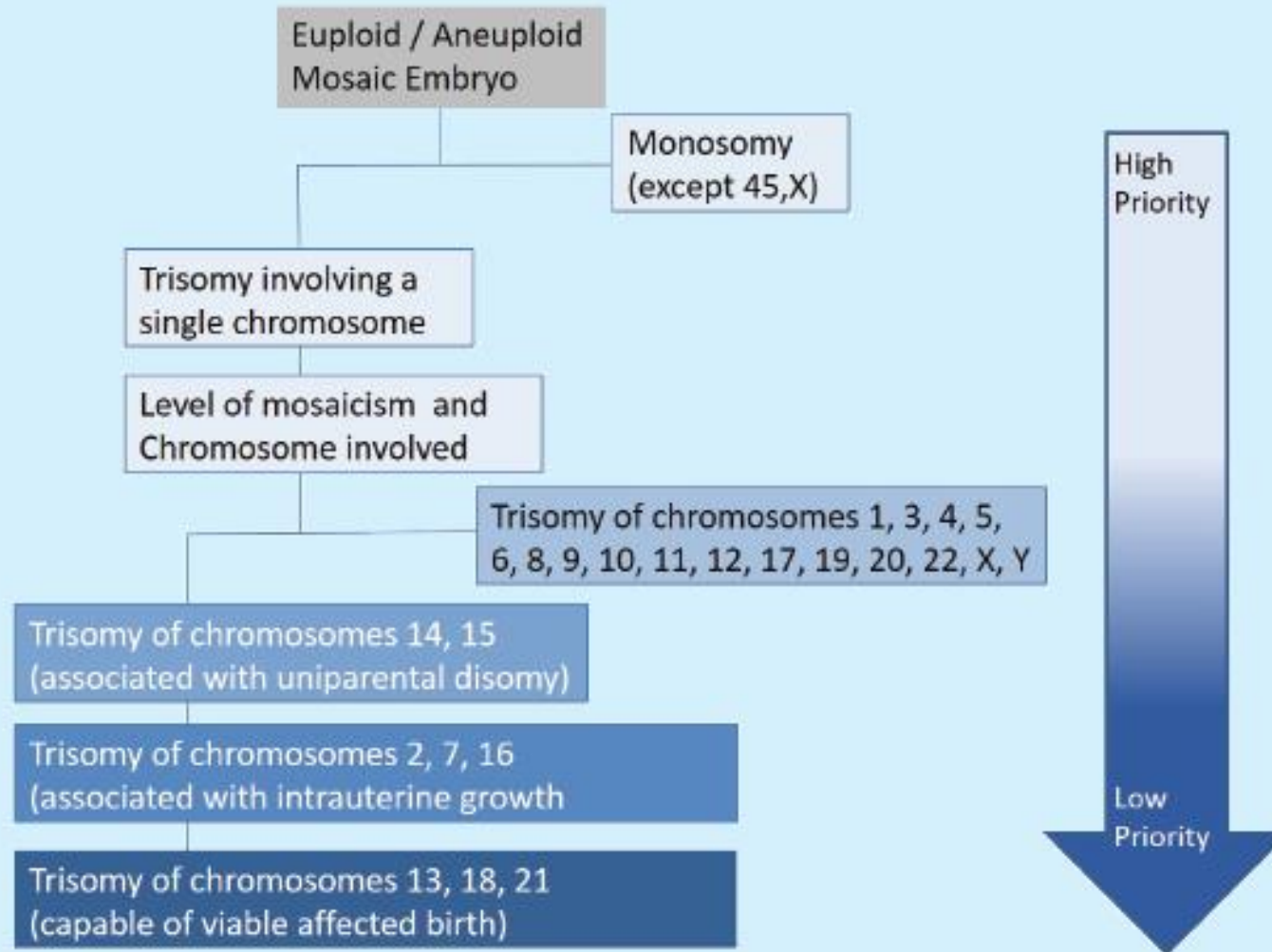
>70% - Aneuploid



# recommendations by the PGDIS(2016)

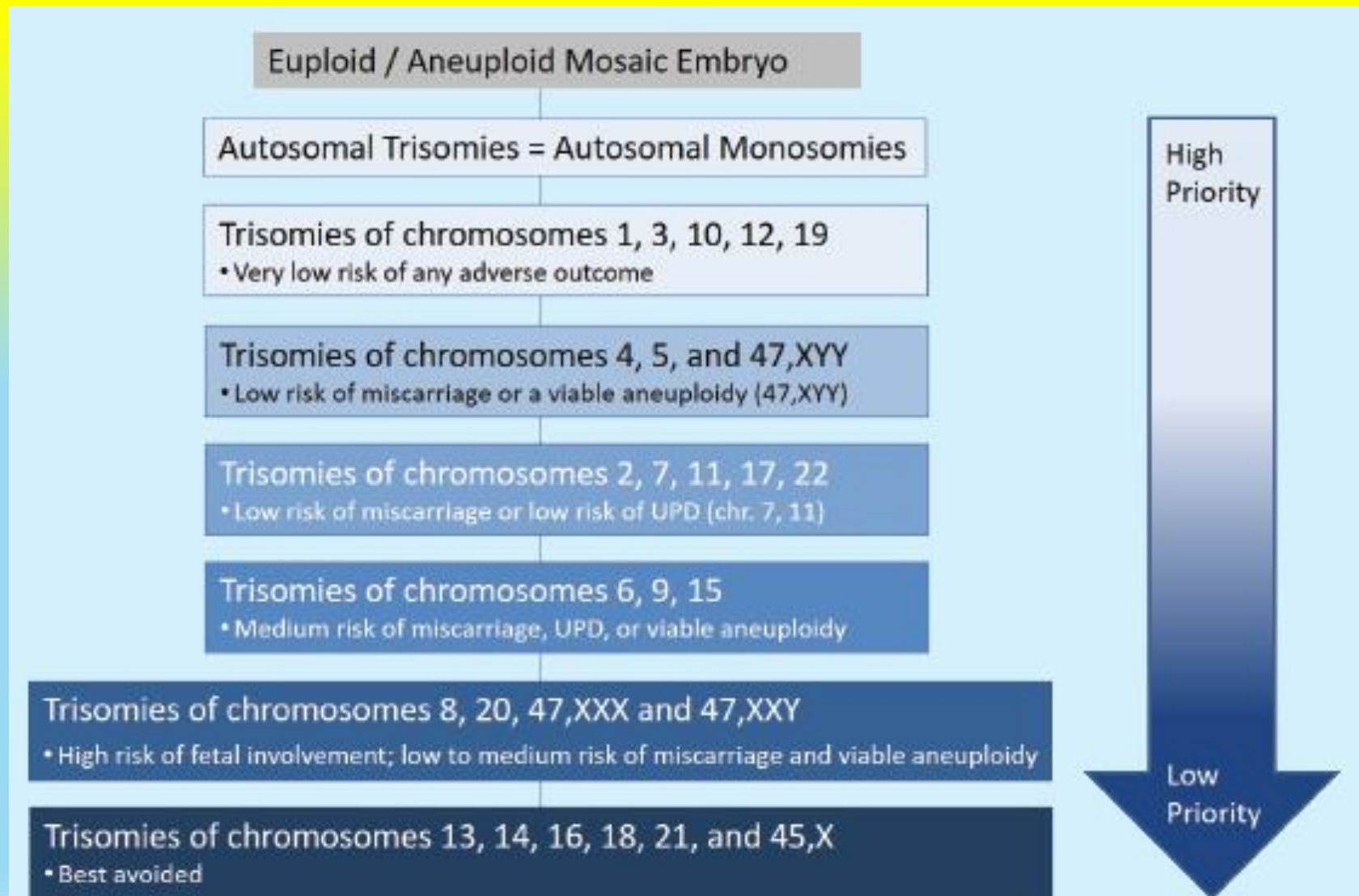
- ❖ Based on reproductive outcomes reported in the literature, some mosaic embryos would still produce viable pregnancies, except that their implantation rate would be lower and miscarriage rate would be higher than euploid embryos.
- ❖ Mosaic monosomies, with the exception of 45,XO, have a higher priority than mosaic trisomies. Among mosaic trisomic embryos, those that do not involve chromosomes implicated in uniparental disomy nor intrauterine growth retardation would be considered first; and those that are capable of producing live births would be last.

Fig. 1. Recommendations on the order of prioritization of mosaic embryos by Preimplantation Genetic Diagnosis International Society (PGDIS, 2016).





# system devised by Grati et al. (2018)

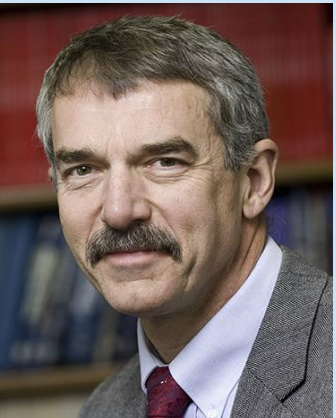






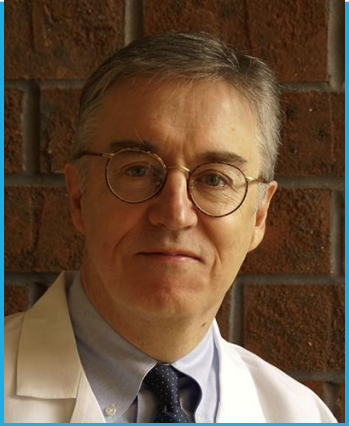
### **Pro 1. Richard T. Scott, Jr**

- PGT-A is clinically beneficial and cost effective
- There are other important benefits to PGT-A. Fewer clinical losses, less time to achieve an ongoing pregnancy, and reduced transfer order resulting in the near elimination of multiple gestation



### **Con 1. Richard J. Paulson**

- CON: PGT-A risks outweigh clinical benefits
- Blastomeres divide at very high rates, particularly in the TE, increasing the risk for mitotic errors and mosaic aneuploidy, even if the inner cell mass remains euploid. This limits the accuracy of PGT-A.
- an incorrect diagnosis of aneuploidy, resulting in the potential discarding of embryos that would otherwise lead to normal births.
- Clinical outcome data strongly suggest that far more potential live births are lost due to PGT-A, most likely due to the additional manipulation that biopsied embryos undergo.



## **Pro 2. Michael C. Summers**

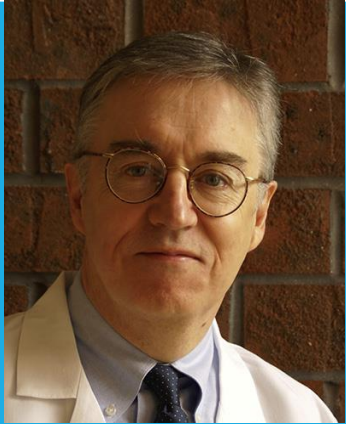
PGT-A is clinically beneficial and cost effective

- For women aged 40-45 years, approximately 87,000 embryo transfers (mostly single-embryo transfer [SET]) were performed resulting in about 8,700 live births and 6,900 early pregnancy losses.
- This represents a staggering level of embryo loss in this patient group, when 90% of embryo transfers do not result in a pregnancy and, of those who conceive, 44% suffer a miscarriage.
- It is now generally accepted that embryo aneuploidy is the most common reason for failed IVF treatment



## **Con 2. Norbert Gleicher**

- The preimplantation genetic screening (PGS) hypothesis has not only remained unconfirmed but has been mostly refuted.
- Even many of its proponents no longer claim that it improves pregnancy and live birth rates.
- 
- Paulson recently calculated an approximately 40% false positive rate in PGS-PGT-A, which in practical terms means that approximately 40% of embryos recommended for disposal are really chromosomally normal. In other words, PGS/PGT-A not only does not improve IVF outcomes but, actually, adversely affects some IVF cycles by leading to the disposal of large numbers of perfectly normal embryos.



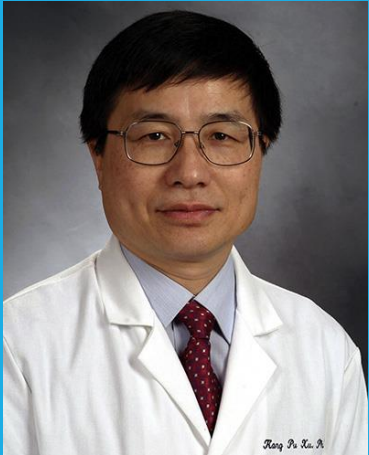
## **Pro 2. Michael C. Summers(CONTINUE)**

- PGT-A using NGS-based analysis is quite robust for detecting euploid and full copy aneuploid blastocysts.
- We do not discard any embryos based on the findings of PGTA, but simply shift the rank order of embryos for transfer after taking note of the pattern of early embryo cleavage and blastocyst morphology.
- In our clinic using only SET implantation rates of 80% to 82% and ongoing pregnancy/delivery rates of 66% to 70% depending on the patient group have been maintained for the last two years.
- The challenge is identifying the most appropriate patient group(s) where PGT-A shows an unequivocal clinical gain.



## **Con 2. Norbert Gleicher (CONTINUE)**

- A new “threshold concept,” based on alleged aneuploid DNA load in a single TE biopsy, now designated embryos as “normaleuploid,” “mosaic,” or “aneuploid-abnormal.” The chosen thresholds of 20% and 80% to demark “normal” from “mosaic” and “mosaic” from “aneuploid,” however, have absolutely no scientific basis, and neither do new claims by proponents of PGS/PGT-A that 40% to 50% aneuploid DNA load among mosaic embryos differentiates between better0 and poorer pregnancy chances.



- For patients who have many morphologically high-grade embryos available for transfer, selecting embryos based on genetics is an effective option.
- Data published by us and others clearly show that transferring euploid embryos into young, as well as older, women results in similar implantation rates.
- qPCR and digital PCR are platforms used to obtain rapid results (within hours), allowing a same-day biopsy and transfer for patients . However, effective cryopreservation renders this fast turnaround time unnecessary for most patients.
- As advancements are made in NGS based PGT, it may soon become the primary platform globally.



## F&S Reviews

Volume 2, Issue 1, January 2021, Pages 43-56



# Preimplantation genetic testing: a review of current modalities

Benjamin S. Harris M.D., M.P.H.<sup>a</sup>  , Katherine C. Bishop M.D.<sup>b</sup>, Jeffrey A. Kuller M.D.<sup>a</sup>, Sarah Alkilany B.S.<sup>a</sup>, Thomas M. Price M.D.<sup>a</sup>

# Aneuploidy is the most common cause of miscarriages

- responsible for 50%–70% .
- The risk of oocyte aneuploidy increases with maternal age, rising rapidly after age 35 years .
- At age 30 years, the rate of aneuploidy in TE biopsies is 23.2%,
- increasing to 34.5% at age 35
- 47.9% at age 38
- 75.1% at age 42
- 84.3% at age 45
- Most commonly, oocyte aneuploidy results from meiotic errors, specifically premature separation of sister chromatids and whole chromosome nondisjunction during meiosis I and II, respectively . The estimated prevalence of errors in meiosis I and II are 42% and 37%, respectively
- Aneuploidy is most frequently observed for chromosomes 13, 15, 16, 18, 19, 21, and 22, which is important given that some of these conditions may result in clinically detectable abnormal pregnancies and not simply implantation failure .

# aneuploidy in sperm

- Less attention has traditionally been paid to aneuploidy in sperm, which occurs in all men and is more frequent for certain chromosomes (21, 22, X, and Y) and in men with infertility.
- In men with normal semen parameters, it is estimated that 3%–5% of sperm are aneuploid. Most published studies have been performed on men with azoospermia undergoing testicular biopsy, and in those patients the aneuploid rates are higher by two- to three fold.
- In embryos, aneuploidy is paternally derived in up to 5%–10% of autosome aneuploidies and 5%–100% of sex chromosome aneuploidies.



# Advantages, and Limitations of PGT-A

- ❖ The limitations of FISH led to the development of more accurate techniques that could analyze all chromosomes. In a validation study of aCGH, Gutierrez-Mateo et al. found that aCGH detected 42% more abnormalities and 13% more abnormal embryos than FISH and had an error rate of only 1.9% .
- ❖ A large study comparing aCGH and qPCR showed a sensitivity of 98.2% and 98.8%, between the two methods, respectively, and a specificity of 99.9% and 99.6%, respectively.
- ❖ While SNP array, aCGH, and qPCR all have accuracy rates >98%
- ❖ NGS has been shown to be the most accurate method with sensitivity and specificity of 100%

# The error rate of the diagnostic test is important

- ❖ The false-positive rate of aCGH is estimated to be 9% , and the false-positive rate associated with SNP array is similar at ~ 0% . The false-positive and false-negative rates of NGS are both 0%
- ❖ SNP arrays have limited ability to identify triploidy, but can identify uniparental disomy (UPD).
- ❖ aCGH cannot identify triploidy or UPD
- ❖ NGS has been shown to be the most accurate method with sensitivity and specificity of 100%

# Mosaism identification

- SNP array can identify mosaicism if enough TE cells are analyzed.
- aCGH is more limited in its ability to identify mosaicism.
- aCGH can be completed faster than SNP array (12–15 hours compared with 30–40 hours).
- qPCR can identify aneuploidy rapidly, in 4-12 hours, and has the ability to identify triploidy, but not UPD or structural chromosome abnormalities.
- NGS can be performed in 13–16 hours.
- the two main platforms, MiSeq or PGM. MiSeq was designed to detect whole-genome aneuploidy, mosaics down to 50%, and mitochondrial copy number, but not structural chromosome abnormalities. NGS using PGM is able to identify deletions or duplications as small as 800 kb to 1 Mb, mosaicism down to 20%, and mitochondrial copy number.

# PGT-M

- PGT-M was initially developed to identify embryos carrying genes for debilitating childhood-onset diseases (e.g., cystic fibrosis, spinal muscular atrophy, sickle cell disease, Duchenne muscular dystrophy).
- Over time, PGT-M has been used for identification of adult-onset single-gene diseases (e.g., Huntington disease, early-onset Alzheimer diseases), cancer predisposition syndromes (e.g., BRCA mutations, Lynch syndrome), and some nonfatal but serious conditions identified at birth.
- Since 2008, the ASRM has recommended PGT-M with IVF as a significant advance over post conception invasive prenatal diagnosis.

# Advantages, Risks, and Limitations of PGT-M

- ❖ Currently, PGT-M is commonly performed with the use of SNP array after WGA .
- ❖ Polar body (PB) biopsy for PGT-M is another option for couples with ethical concerns surrounding genetic testing and in countries where genetic testing is prohibited.
- ❖ PB testing may be used to identify maternal mutations.
- ❖ Limitations include the small amount of DNA obtained, lack of information involving paternal origin, embryo sex, or downstream mitotic errors.
- ❖ PB testing can be highly accurate with sequential biopsy of both polar bodies and use of multiplex PCR (simultaneous amplification of two or more DNA sequences) for detection of ADO and contamination , however sequential biopsy requires multiple procedures and can be costly.

# Cleavage-stage biopsy specimens for PGT-M

- have the limitation of only one cell being available for testing.
- Removal of two blastomeres can jeopardize pregnancy and implantation rates .
- Single-blastomere biopsy for PGTM has not been shown to adversely affect embryo development to blastocyst stage.
- Generally, testing results are available within 2 days, allowing biopsied cleavage-stage embryos to be cultured to blastocyst (day 5 or 6) and providing an opportunity for transfer of normal embryos during the same IVF cycle.
- Although still performed, this technique has given way to blastocyst biopsy.

# Blastocyst -stage biopsy specimens for PGT-M

- Trophectoderm biopsy (three to eight TE cells) allows for improved amplification efficiency compared with single-cell biopsy and has been shown to have superior implantation rates compared with cleavage-stage transfer in a paired randomized clinical trial.
- In a study by Scott et al., biopsied cleavage-stage embryos had significantly reduced implantation rates compared with nonbiopsied control embryos (31% vs. 54%).
- Conversely, implantation and delivery rates after TE biopsy were similar to those of nonbiopsied control embryos (52% vs. 54%, respectively)
- Increased genetic material obtained from TE biopsy is advantageous compared with cleavage-stage biopsy and translates to reduced risk of amplification failure and ADO .
- The limitation of blastocyst biopsy is the time required to obtain results, which typically precludes a fresh transfer and requires that biopsied blastocysts be cryopreserved and transferred in a subsequent cycle.



# (Advantages, Risks, and Limitations of PGT-SR)

- PGT-SR is differentiated from PGT-A in that it is used to detect cases of unbalanced hereditary chromosomal abnormalities, which are a result of one or both parents having a balanced chromosomal rearrangement (e.g., reciprocal translocations, Robertsonian translocations, and inversions).
- Balanced translocations are the most common chromosomal abnormality in the population, seen in 0.2% of newborns.
- Although individuals with balanced translocation have a complete chromosome composition, the rearrangement causes gene abnormalities to occur at the breakpoints of the translocation.
- Many genes affected by these breakpoints have been identified with the associated disorders including intellectual disability, hearing loss, dysmorphic features, and cardiac arrhythmia syndromes .
- Therefore, patients with balanced translocations should undergo genetic counseling even with normal phenotype

# (Advantages, Risks, and Limitations of PGT-SR)

- There is also evidence that aneuploidy rates may be higher in gametes from men and women with balanced translocations.
  - An interchromosomal effect on meiosis is proposed during which the chromosome break from the translocation affects the meiotic disjunction in the egg or sperm, leading to aneuploidy (Support for this idea includes the increased rate of balanced translocation found in parents of children with Down syndrome.
  - Owing to a possible increase in aneuploidy rate, concurrent PGT-A should be performed, which is typical with CCS (comprehensive chromosome screening)
- . As with PGT-A, the most commonly used techniques for PGT-SR currently include aCGH, SNP array, and NGS . aCGH is unable to discern normal embryos from balanced carriers. This can be accomplished with the use of SNP microarray, a combination of SNP and NGS, and a newer technique of Nanopore sequencing .

# ETHICAL CONCERNS OF PGT

➤ Prominent areas of ethical debate include PGT for:

- Adult-onset conditions
- Sex selection outside of medical indications
- Transferring of mosaic embryos and embryos affected by known pathogenic variants based on patient wishes.

➤ Ethical arguments in favor of PGT include:

- The right to reproductive choice
- The medical successes of preventing inherited genetic disease
- Avoiding abortion after postconception genetic diagnosis
- and reducing the societal burden of disease .

# ETHICAL CONCERNS OF PGT

➤ Ethical arguments against use of PGT include:

- Cost
- Misdiagnosis
- Unknown procedural risk
- Unknown benefit in the context of unpredictable medical advances over time
- Possible negative effects on individuals living with or have predisposition to a given genetic disease.

# According to the ASRM

- Testing for adult onset disorders with PGT-M is “ethically justifiable when the conditions are serious and when there are no known interventions for the conditions, or the available interventions are either inadequately affected or are perceived to be significantly burdensome”
- Similarly, for conditions that are mild or have lower penetrance, PGTM is deemed to be ethically acceptable as a reproductive liberty.

Thank you





*Thank You.*

