

## Fact Sheet

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



#### Key points:

- Error/s in the early development of the sperm, egg or embryo can lead to an abnormal number of chromosomes in the developing embryos (i.e. missing or extra chromosomes)
- An abnormal chromosome number can cause implantation failure, miscarriage, or the birth of a child with a chromosome abnormality (e.g. Down syndrome).
- Some individuals have an increased risk of producing embryos with an abnormal chromosome number.
- Preimplantation Genetic Testing for Aneuploidy (PGT-A) can be used to screen embryos for abnormalities in chromosome number.
- PGT-A is NOT 100% accurate. Confirmatory prenatal diagnosis is recommended if a pregnancy is achieved following PGT-A.

### Using Preimplantation Genetic Testing as an embryo selection tool

Whenever more than one embryo is obtained from an IVF cycle, a selection process takes place to decide which embryos are the most suitable for transfer or freezing. Current selection criteria include the appearance and the rate of development of the embryos. However, as even beautiful looking (morphology) embryos can be genetically abnormal, it is important to consider direct testing of the genetic status of the embryo.

Preimplantation genetic testing for aneuploidy (PGT-A) offers the opportunity to add a further selection criterion, the genetic complement of the embryo, to enhance this selection process and improve the chance of selecting an embryo that is capable of a healthy live birth. This screening may assist in speeding up conception, while also reducing miscarriage rates.

### What is aneuploidy?

An individual's genetic information is packaged into strings of DNA called chromosomes. Normal embryos contain 46 chromosomes, or 23 chromosome pairs. These chromosome pairs are labelled 1 to 22 (the autosomes) and X and Y (the sex chromosomes).

Some embryos can have an abnormal number of chromosomes (ie: missing or extra chromosome/s) due to errors in cell division in the developing egg, sperm or embryo. This is known as chromosomal aneuploidy. While most aneuploid embryos will not implant, some aneuploid embryos can implant and result in pregnancy. In most cases, these aneuploid pregnancies will end in miscarriage. However, in a small percentage of cases, a baby can be born with a chromosome abnormality. The most common chromosome aneuploidy detected at birth is trisomy 21, commonly known as Down syndrome. The incidence of aneuploidy in embryos, and therefore in live births, increases as a woman ages (Figure 1).

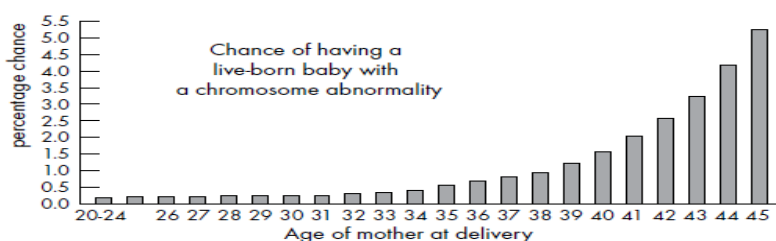


Figure 1: Chance of having a live-born baby with any chromosomal abnormality according to the mother's age at delivery. From The Australian Genetics Resource Book, 8<sup>th</sup> Edition, Centre for Genetics Education, 2007.

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#### What is mosaicism?

Some embryos may have a mosaic chromosomal pattern. That means they are made up of both normal and abnormal cells. This happens during early embryo cell divisions where one cell makes an error and this error is passed on to all the daughter cells that arise from that original abnormal cell. Some of these embryos may have the potential to give rise to healthy babies. It is believed some of these embryos can “self-correct” and push those abnormal cells out into the placenta, leaving just normal cells in the inner cell mass (baby).

Monash IVF may allow some of these mosaic embryos to be transferred after genetic counselling has been provided.

#### What is Preimplantation Genetic Testing for Aneuploidy?

Preimplantation Genetic Testing for Aneuploidy (PGT-A) can be used to screen for aneuploidy involving any chromosome. This testing may be appropriate for:

- Individuals with advanced maternal age (generally older than 37 years)
- Individuals who have experienced repeated miscarriage
- Individuals who have experienced repeated IVF failure
- Individuals who have previously had a pregnancy with a chromosomal abnormality
- Individuals where one partner has an altered sex chromosome complement (e.g. XXY)
- If embryos of only a specific gender are suitable for transfer, to reduce the risk of a specific genetic condition in a child.

Your fertility specialist will be able to advise whether PGT-A would benefit your treatment.

Social sex selection (transferring embryos of a specific gender because of parental preference) is not considered ethical under NHMRC\* guidelines in Australia and is therefore not allowed.

#### What is involved?

Individuals requesting PGT-A must undertake an IVF cycle to stimulate the woman’s ovaries to produce a number of eggs. These eggs are collected and fertilised using the male partner’s sperm. Most embryos created with the intention of undergoing PGT-A are created using a fertilisation method called Intracytoplasmic Sperm Injection (ICSI). ICSI involves the injection of a single sperm into the egg, and is specifically used to minimise any risks associated with the presence of additional sperm around the developing embryo.

The resulting embryos are grown in the laboratory until Day 5 or 6 of development. By this time, the embryo should have developed to the expanded blastocyst stage, and should consist of an inner cell mass (which will go on to form the fetus), fluid filled cavity (blastocoel) and an outer rim of trophectoderm cells (which will go on to form the placenta). PGT-A can only be performed on embryos that are suitable for freezing. Any embryo that has not formed a viable blastocyst by day 6 is not suitable for freezing and will not be biopsied

To proceed with PGT-A, scientists need to obtain a sample of the embryo’s DNA. There are two different methods that can be used to access embryonic DNA. Both of these methods are described in more detail below. In some cases your doctor may request a combination of both testing. Your doctor will guide you as to the best test for your circumstances. For all PGT-A testing, the embryos will be frozen while a genetic result is obtained.

#### 1. PGT using cell biopsy

In this method, a few cells are biopsied from the embryo for genetic testing. Embryos need to have a clear inner cell mass and a suitable number of healthy trophectoderm cells to be considered suitable for biopsy. This is to ensure it is possible for the scientist to selectively biopsy the placental cells (trophectoderm), without damaging the future baby (inner cell mass). A small hole is made in the outer shell of the embryo on Day 3 of development and the embryo is returned to the culture dish. By Day 5/6, some of the trophectoderm cells should have herniated through the hole in the outer shell of

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the embryo and these cells can be collected for analysis. Approximately 5 trophectoderm cells are removed for genetic analysis. These cells are transferred to a small test tube for genetic testing.

#### It is important to note that:

- Embryos undergoing cell biopsy can be created using ICSI (preferred) or standard IVF insemination.
- Embryos need to be at a specific stage of development to be considered suitable for biopsy. Embryos that are too advanced, or less advanced, may not be able to be biopsied.
- A small proportion of embryos may be damaged by the biopsy process or may not survive the freeze/thaw process.
- The cell(s) taken at biopsy are assumed to represent the whole embryo. It is possible that the chromosome constitution of the biopsied cells differs from that of the remaining embryo.
- This testing is capable of detecting some deletions or duplications (i.e.: cases where part of a chromosome is extra or missing), depending on the size of the chromosome segment involved.
- This testing can detect some, but not all, cases of mosaicism (e.g.: the presence of both “normal” and abnormal cell lines in the embryo). The likelihood of detecting mosaicism will depend on the proportion of abnormal to “normal” cells in the biopsy sample, as well as the quality of the resulting data. Decisions relating to the fate of mosaic embryos will be made in accordance with the policy of the treating IVF clinic.

## 2. Cell Free PGT (cf-PGT) – Collecting DNA from the Culture media

This is a relatively new type of Preimplantation Genetic Testing where scientists take a sample of the leftover nutrient solution (culture media) that the embryo has been growing in while in the laboratory. This solution contains DNA produced by the embryo which can be used for genetic testing. As this procedure can safely test early blastocysts where the ICM is not clearly defined, it can be used for embryos that are not able to be biopsied by day 6. This technique is only used on embryos that are slower in their development.

#### It is important to note that:

- ICSI insemination **must be used** when performing cf-PGT.
- This technology is designed to be used in partnership with embryo biopsy, and the decision of whether to use cf-PGT is based on embryo quality. Advanced stage embryos on day 5 should be used on day 5: this means that they should either be transferred fresh on day 5 or frozen on day 5 with or without biopsy. There is no evidence to suggest that cf-PGT is advantageous on embryos of advanced stage (suitable for biopsy) on day 5.
- Not all embryos release enough DNA for a result to be obtained. To maximise the chance of having enough DNA to work with, embryos have to be cultured and remain viable until Day 6.
- A small proportion of embryos may not survive the freeze/thaw process.
- This screening test is not designed to detect deletions or duplications (i.e.: cases where part of a chromosome is extra or missing). If these are detected, they will be reported as an incidental finding.
- At the current time, cf-PGT cannot reliably quantify and report on mosaicism.

Published studies and our own results have demonstrated that cf-PGT results are identical to the cell biopsy PGT results in between 75-85% of the cases<sup>1</sup>.

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## Results

A Genetic Counsellor or a Scientist will contact you with your results at the completion of testing. This is usually 2 weeks after sample collection. Your fertility specialist will also discuss these results further with you at your next appointment. Table 1 summarises the expected outcomes following each method of PGT-A.

Table 1: Possible outcomes following PGT-A

	PGT using cell biopsy	cf-PGT
Embryos with a result following PGT-A*	92.4%	91.1%
Embryos with no result due to*: <ul style="list-style-type: none"> <li>Failed/low level DNA amplification (<i>insufficient embryonic DNA detected to proceed with PGT-A</i>)</li> <li>Inconclusive result (<i>PGT-A performed, but a conclusive result not obtained</i>)</li> </ul>	7.6%	8.9%
False Positive <sup>1,3</sup> ( <i>Chromosomally normal embryo incorrectly classified as abnormal</i> )	0.5%-10.9%	6.2%
False Negative <sup>1,2,3</sup> ( <i>Chromosomally abnormal embryo incorrectly classified as normal</i> )	1.9%-3.2%	9.4%

\*data obtained from in-house testing between 2019-2020

PGT-A is only designed to analyse chromosome copy number. The test does not give any information relating to other genetic conditions or abnormalities including chromosome rearrangements, single gene disorders or small duplication/deletions. There is a 3-5% background population risk for birth defects or genetic conditions in any pregnancy. PGT-A is only designed to detect birth defects caused by aneuploidy and not these other conditions.

## What are the expected outcomes?

If the embryo has “no abnormality detected” following PGT-A, it is considered genetically suitable for transfer and can be thawed for use in a frozen embryo transfer cycle.

If your embryo is classified as mosaic, there may be the possibility of considering this for transfer under select clinical circumstances. Your fertility specialist and a genetic counsellor/clinical geneticist will discuss with you what this result may mean for a pregnancy and whether this embryo could be considered for transfer.

It is well documented that the frequency of chromosomal aneuploidy increases with maternal age. Therefore, older women will be less likely to obtain a chromosomally normal embryo for transfer compared with younger women. Encouragingly, data indicates that once a chromosomally normal embryo is identified for transfer following PGT-A, the pregnancy rate in older women is not significantly different from that of younger women.

## Other important information

- Due to the complexity of PGT-A, it may not be possible to obtain a conclusive result for some or all embryos. In this case, the embryo/s can either be thawed and transferred without a genetic result, thawed and re-biopsied if it/they reach an appropriate stage of development, or thawed and allowed to succumb.
- It is possible that at the completion of the cycle there will be no embryos available for transfer. This may occur as a result of one of the following scenarios:
  - All embryo samples tested during an IVF cycle may be found to be aneuploid, meaning that no embryos are genetically suitable for transfer.

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- Embryos with “no abnormality detected” may not survive the freeze/thaw process and therefore may not be suitable for transfer.
- Embryos with “no abnormality detected” may survive the freeze/thaw process, but may not continue to develop normally and therefore may not be suitable for transfer.
- There are some rare chromosomal problems that cannot be tested for using PGT-A.
- This test cannot provide an absolute guarantee of the chromosome status of the embryo. In some embryos, the biopsied cell/s or culture media may not be representative of the whole embryo.

While every effort is made to ensure that the PGT-A test offered has the highest possible accuracy using the currently available technology, results are not 100% accurate. **Therefore, prenatal diagnosis is highly recommended in an ensuing pregnancy.**

### What are the costs?

Information relating to the cost of PGT-A is available from your IVF clinic.

### References

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2. Andrea R. Victor, Darren K. Griffin, Alan J. Brake, Jack C. Tyndall, Alex E. Murphy, Laura T. Lepkowsky, Archana Lal, Christo G. Zouves, Frank L. Barnes, Rajiv C. McCoy, and Manuel Viotti 2019. Assessment of aneuploidy concordance between clinical trophectoderm biopsy and blastocyst. *Human Reproduction*
3. Nidhee M. Sachdev, David H. McCulloh, Yael Kramer, David Keefe and James A. Grifo 2020 The reproducibility of trophectoderm biopsies in euploid, aneuploid, and mosaic embryos using independently verified next-generation sequencing (NGS): a pilot study. *Journal of Assisted Reproduction and Genetics*