PGS: a four-letter word?

Preimplantation genetic screening (PGS) involves the identification of chromosome abnormalities in IVF embryos (rather than targeting diagnosis to a specified gene). Chiefly employed for couples with advanced maternal age, recurrent miscarriage or recurrent IVF failure, it aims to improve IVF success, and reduce miscarriage and affected live birth rates. The process involves the sampling of cells by embryo biopsy, cytogenetic diagnosis, then selective transfer of an apparently chromosomally normal embryo in the hope of establishing a pregnancy. Although PGS is the most common variant of PGD (preimplantation genetic diagnosis), accounting for 80% of cases it has, from the outset, been one of the most controversial areas of reproductive medicine. The subject of intense debate, it attracts opinions ranging from recommendations that it should be applied in all IVF cases, through to the suggestion it should be discontinued completely. What do you think? Should it continue or not?

At its broadest, the term preimplantation genetic diagnosis (PGD) can be defined as the diagnosis of genetic disease or chromosomal abnormality in human IVF embryos. The starting point involves counseling for, and testing of, a couple at risk of transmitting a genetic disorder, or who have manifest a need for assisted reproduction. Standard IVF treatment then follows and the embryo (or sometimes the oocyte) is biopsied, i.e. material from it is removed for sampling. Genetic diagnosis on the biopsied piece can then be genic, (i.e. looking for a disease-causing mutation in a single gene) or chromosomal, (i.e. looking for extra or missing chromosomes [or parts of them]). This article will focus primarily on the latter, most commonly referred to as “PGS” (preimplantation genetic screening). The terms PGD for aneuploidy (PGD-A) and PGT-A (preimplantation genetic testing for aneuploidy) are accepted alternatives for the same suite of techniques, but for the purposes of this article we will stick to PGS.

PGS exists to try and improve IVF success rates, to reduce the incidence of spontaneous abortions and avoid the births that might be affected with chromosomal disorders. For reasons explained below, PGS remains a controversial area of reproductive medicine.

The problem with chromosomal disorders

For the most part, chromosome abnormalities do not end well. The vast majority of humans have 46 chromosomes in each cell; 23 pairs of autosomes numbered 1–22 then, in females, a final pair that we call “X” and, in males an “odd couple” – one of which is an X, and the other a tiny Y. Any deviation from that norm usually has a consequence, which can be as relatively mild as impaired fertility or as severe as the embryo not making it past the first few divisions. By far the best known of chromosome disorders involves three copies (trisomy) of the smallest autosome – number 21. Down syndrome (the most common cause of mental retardation in humans) frequently ensues, however, eight times out of ten, trisomy 21 leads to a first trimester pregnancy loss. Indeed, that is the fate of the vast majority of embryos with extra chromosomes, whereas those with missing chromosomes don’t even make it to the point of clinical recognition. Taken together, chromosome disorders are the leading cause of mental retardation, pregnancy loss and IVF failure. They are one of the leading causes of birth defects and stillbirths. They can lead to infertility, high birth weights, low birth weights, abnormal placentas and obstetric complications. On the face of it, you don't want to be knowingly transferring IVF embryos with chromosomal disorders.

Who potentially benefits from PGS?

Couples presenting for PGS generally do so for reasons of advanced maternal age, previous recurrent miscarriage, previous recurrent implantation failure or severe male infertility. All of these referral categories can typically carry a higher risk of producing chromosomally abnormal conceptuses. Global figures are becoming increasingly difficult to collate but a reasonable estimate is that around 80,000 PGS cycles have been performed worldwide since its inception, accounting for 4 out of 5 of all PGD cycles.

The theory is straightforward; if you detect a chromosome disorder, do not transfer that embryo. However, real life tends to be rather more complicated.

An overview of embryo biopsy for PGS

It is a fact of PGS that, if you want to do genetic diagnosis, you need genetic material and, for the most part, that
means accessing embryonic cells. Sampling cells from an embryo (or oocyte) is, of necessity, quite invasive. Herein lies one of the core problems of PGS; if you compromise, in any way, the future development of the embryo by performing the biopsy then you have defeated the object of doing PGS in the first place.

Typically, there are three sources of cellular material available for biopsies prior to PGS: polar bodies (the by-products of maternal meiosis) from oocytes; single cells from cleavage-stage (roughly eight-cell, day-three) embryos, and trophectoderm cells from blastocyst-stage (day-five or six) embryos.
The polar bodies (PBs) are the consequence of asymmetric meiotic division in mammalian eggs and thus do not make a contribution to further embryonic development. There are usually two of them (although the first one can divide), the products of the first and second meiotic division. Polar body biopsy is less invasive than biopsying cells that the embryo might otherwise have used. The main drawback for PGS is that it can only detect those chromosome abnormalities that arise in maternal meiosis and, although this is actually the majority, some errors arise in the sperm, and others arise in the embryo after fertilization. The other issue is that randomized controlled trial (RCT, see later) data involving PGS by this approach does not appear to increase the likelihood of a live birth within one year compared to regular IVF, but it does appear to decrease the miscarriage rate. As a general rule, however, polar body biopsy has not been as widespread as cleavage-stage nor blastocyst-stage biopsy.

Cleavage-stage biopsy involves the removal of one or two cells (referred to as blastomeres) on embryos 3 days after fertilization, which, by then is usually around the six-to-ten cell stage. Surrounding the embryo is a glycoprotein layer called the zona pellucida and, in order to get to the cells, one needs to dissolve a little window of it with acid, or else make a hole with a laser. A biopsy pipette removes the cell or cells in question. This approach is the oldest and was, until recently, the most widely used approach for PGS. There is an inherent problem with cleavage-stage biopsy; remove only one cell and that is all you’ve got on which to make your diagnosis. Remove two or more, and you’re significantly reducing your embryonic volume, which can impair its future development. When it was first used for PGS, the signs were good, however the RCTs were not. Some very famous studies even suggested that it made things worse, i.e. it resulted in IVF success rates going down. Opponents of PGS argued that this was sufficient evidence to discontinue PGS completely, the proponents argued that the opponents were just doing it wrong, i.e. they were being too rough with the embryos. Back in 2007–2008, it is probably fair to say that the opponents won the day and PGS was in the doldrums.

At that stage, the possibility for blastocyst biopsy was already known. The reason why it did not immediately become popular was essentially that IVF in the nineties and noughties was not producing enough blastocyst embryos in culture. Blastulation is the first stage of embryonic differentiation where the inner cell mass (which will go on to form the foetus) and the trophectoderm (which will go on to form the placenta) are first seen as distinct structures. Removal of five to ten cells from the trophectoderm is therefore an attractive option (as the future foetus is left untouched), provided (as is now the case due to improved culture conditions) a sufficient number of blastocysts can be generated in each IVF cycle. With blastocyst (trophectoderm) biopsy you have a number of advantages. First, with >100 cells to go at, the removal of less than a tenth of them is less likely to harm the embryo. Second, with more cells on which to make the diagnosis, you can be more confident of your result. Third, the blastocyst seems to "sort itself out" somewhat chromosomally and day five embryos tend to be more chromosomally normal than day three embryos on average. Finally, there seems to be some evidence that this approach could be less operator-dependent and thus more reproducible between clinics.

Whereas RCTs for cleavage-stage biopsy have produced mixed results, some suggesting a benefit whereas others reporting that they can cause a reduced implantation rate, there seems to be no such problem after trophectoderm biopsy. Indeed, most RCTs demonstrating the benefits of PGS are after blastocyst biopsy.

Alternatives to embryo biopsy

Less invasive approaches that do not involve cell removal include blastocentesis, the aspiration of blastocoelic fluid (the cavity surrounded by the trophectoderm in the blastocyst embryo). The fluid certainly contains DNA, it has been found in over 80% of the samples tested and experiments have shown >97% concordance with results from trophectoderm biopsy. However, the confounding effects of both false-positive and false-negative outcomes suggest that it is a while before this will become mainstream. Analysis of spent culture medium for DNA is also a possibility, reportedly making the right diagnosis over 90% of the time in recent studies. This remains a little too inaccurate to enter mainstream diagnostics. Finally, time-lapse imaging and morphokinetics for
PGS has been looked at for some time. Time-lapse devices (basically a microscope and still camera inside an incubator) are very popular in IVF clinics as they can monitor embryos closely and minimize the need for embryologists to take the embryos out of the incubator quite so much. There are some suggestions that chromosomally abnormal embryos can display different kinetic parameters to chromosomally normal ones, with the latter following a more defined pattern. Definitive diagnosis is not clear, however, with many groups not showing significant differences. Thus, morphokinetic time-lapse monitoring alone is probably not sufficient to assess the chromosomal status of an embryo, but it may have a role in assessing implantation potential.

Taken together therefore, non-invasive approaches may well be promising, but they currently lack sufficient reducibility to replace standard PGS technology.

**FISHy beginnings**

Unlike use of PGD to screen for single gene mutations, detection of chromosome abnormalities (PGS) is less targeted. The aim is to try and detect as many chromosomes as possible. The first approach to be employed for this purpose was fluorescence in-situ hybridization (FISH). From the very early stages, PGS was both the most popular form of PGD, but was also the most controversial. With the stated aim to improve IVF success, decrease the time to pregnancy, reduce the risk of pregnancy loss and the birth of chromosomally abnormal children, PGS started out by screening a limited number of chromosomes. FISH is a multicolor technique directly on the nuclei of embryo blastomeres first used to sex embryos for families at risk of passing on sex-linked diseases like muscular dystrophy. Soon thereafter 5 to 12 colour FISH approaches were used. Nonetheless, from the outset, the approach had a number of drawbacks. Most significantly, not all chromosomes were screened. There was potential to generate both false-negative and false-positive results and these seemed to increase with the more chromosomes that were screened. Other problems included inter-centre variation in techniques such as embryo biopsy, single cell fixation to slides and analysis protocols. Around 2008–2010, following unfavorable RCT data, FISH was discontinued and replaced with whole genomic approaches.

**Whole genome analysis**

As the starting material for PGS is just a few cells (6 pg of DNA/cell), if whole genome analysis is going to be effective then the DNA needs to be amplified. Without going into specifics, there are an assortment of approaches, variously called degenerate oligonucleotide primed PCR (DOP-PCR), multiple displacement amplification (MDA) and MALBAC (multiple annealing and looping based amplification cycles). These methods can, if used correctly, be uniform, specific and reproducible.

Once amplified, two very accurate approaches for analyzing chromosome copy number for every chromosome has been described. The first, array-comparative genomic hybridization (a-CGH) is similar in some ways to FISH in that it involved DNA hybridization on a glass slide. Here, however, it co-hybridizes differentially-labelled test (amplified embryonic) and reference (normal) DNA on microarrays. Analysis with specialized software enables an automated ratio comparison of the ratio of red and green intensity information of fluorescent colours at each genetic locus along the chromosome. Chromosomal gains and losses are accurately detected as subtle swings towards the red or green. This approach has been applied successfully in polar body, cleavage-stage and blastocyst PGS but is limited in its ability to detect some abnormalities. Next Generation Sequencing (NGS) is now rapidly replacing a-CGH. This involves whole genome amplification, breaking down to small fragments, parallel DNA sequencing (low coverage) then “binning” to represent the number of sequence reads per chromosome. Using this approach, multiple samples can be processed simultaneously, thereby, reducing cost and workload. An added advantage of this approach is that it can also be used to detect copy number of mitochondrial DNA which is proposed as a biomarker for the estimation of the viability of the embryo (this is, in fact, another controversial area we won’t expand upon here). Real time quantitative PCR (RT-qPCR) can be robust, rapid, accurate and cost-effective however there are a number of types of abnormalities that could be missed by this system such as mosaicism (see below) and abnormalities caused by parts of, not whole, chromosomes.
**Simultaneous PGD and PGS (Karyomapping)**

Certain types of microarrays (called SNP [single nucleotide polymorphism] chips) detect genetic variation across each chromosome and have been applied to embryo biopsies. By using the SNP chip output for each parent plus a genetic relative of known disease status (this is usually an affected child), four distinct sets of markers can be identified. These basically represent each of the parental chromosomes. An analysis protocol called “Karyomapping” essentially detects the inheritance of these blocks of chromosomes. By comparison with the relative of known disease status (affected child), the inheritance of a disease gene can be detected, as can the presence of extra or missing chromosomes. The test is therefore relatively universal, being able to detect any single gene disorder and every chromosome disorder simultaneously. This is now a relatively mainstream approach, at time of writing, used to treat 6,000 patients.

**To freeze or not to freeze?**

Traditionally, those clinics performing PGD and PGS were in a race against time to perform the diagnosis within a 24 hour (or ideally same day) time scale so that the embryo could get transferred before blastulation happened (the point being that blastocyst transfer success rates were historically low compared to cleavage stage). Given that success rates for frozen embryos were also lower compared to fresh, the motivation to freeze PGS embryo was low. However, concurrent development of enhanced culture conditions and much improved fast-freezing (vitrification) approaches has led to a general acceptance of freezing as part of the PGS procedure. Indeed, more recently, strategies that involve freezing all embryos are becoming popular, with embryo transfer in the later menstrual cycles, giving the opportunity for the endometrium to be more physiologically receptive to the embryo.

**The issue of mosaics**

It would be very wrong to imagine that all cells in a human embryo are uniformly normal or abnormal. Mosaicism is a frequently observed phenomenon, where more than one chromosomal constitution is observed in the same embryo. Obviously, this presents a challenge for PGS given that the diagnosis is made on just a small sample of cells. Mosaicism can arise with an embryo that was previously normal, undergoing a post-zygotic chromosome segregation error or an originally abnormal embryo that underwent “correction” in a proportion of the cells. There are a variety of mechanisms that have been proposed to explain mosaicism – all or most probably occur to a greater or lesser extent. The number of human IVF embryos that are mosaic to some degree is not completely established but even conservative estimates suggest that the number is around 75%. Indeed, as mentioned, cleavage-stage embryos are thought to be more chromosomally abnormal (and hence more prone to mosaicism) on average than trophectoderm-stage embryos. Moreover, while the incidence of chromosomal abnormalities in general tends to be associated with maternal age, this does not appear to apply to mosaicism per se. Other factors may be involved, such as ovarian stimulation protocols, embryo culture conditions, genetic background and environmental pollutants.

The medical outcomes of mosaicism are dependent upon many factors, such as the timing of the error, the proportion of the embryo that is involved and which chromosome is affected. As a general rule, an embryo would most likely be more severely affected when the error occurs at earlier stages of development, or in meiosis, as a greater number of cells is likely to be affected. Sadly, nobody has a definitive answer to the incidence of mosaicism in IVF embryos and, in fact, it tends to be reported only when it is identified. As mentioned, these days this is usually from a five to ten cell biopsy of an embryo that has about 50–150 cells in it. Of all the available techniques, NGS is really the only one that can accurately detect mosaicism from a trophectoderm biopsy and, of course, this depends on whether a mixture of normal and abnormal cells is actually present in the sample taken. In an ideal world, a rigorous “cell by cell” approach to assess the relative incidence of chromosome abnormalities in trophectoderm and inner cell mass in a large cohort of embryos should be performed. To the best of our knowledge however, this has yet to happen. Whether diagnosis of a sample of trophectoderm (which will go on to form the placenta) is, by and large, representative of the inner cell mass (which will go on to form the baby) is still an open question but a body of evidence is now pointing to reasonable concordance (about 95% of the time when all studies are taken into account). The issue of whether or not mosaic embryos (as defined by a least one variant cell being detected in the trophectoderm biopsy) should (or could) be transferred (especially when there are no “normal” diagnoses) is a subject of much discussion. Indeed, at least one recent study has reported unaffected live births following the transfer of mosaic embryos. The debate continues and guidelines evolve. However, whether or not PGS should be performed at all remains a bone of contention.

**What is the argument about?**

There seems to be little doubt that a combination of a widespread move to trophectoderm biopsy plus, for diagnosis, aCGH (and later NGS) coupled with the
greater willingness to adopt freezing strategies has improved the situation. PGS is certainly a lot more satisfactory than in the days of cleavage-stage biopsy and FISH. There is now a growing body of evidence that PGS can be beneficial, in the right hands, and under certain circumstances. Some studies including meta-analysis and systematic reviews have demonstrated some improvements in pregnancy rate, live births, and miscarriage rate. However, the most recent trial [Single Embryo TrAnsFeR of Euploid Embryo - STAR Trial (NCT02268786)] gave encouragement to both supporters and opponents of PGS, reporting a small, but not statistically significant, overall IVF success rate, but nonetheless significant improvements in the advanced maternal age category.

In essence, the debate revolves around what we consider to be a sufficient body of evidence-based medicine. If you Google “evidence-based medicine” (EBM) you will find a definition along the lines of “an approach to medical practice intended to optimize decision making by emphasizing the use of evidence from well designed and conducted research.” Nobody would argue with that. But the questions remain, what do we mean by “well-designed” and “well-conducted”? The problem with reproductive medicine in general (not just PGS) is that, conceptually, it is unlike any other areas of medicine for the following reasons. Firstly, patients undergo pretty radical treatments with an intention that does not always involve benefiting their own health. Secondly, there are few, if any other, medical disciplines where so many different academic fields combine. Thirdly, reproductive medicine is a field in which barely perceptible “good gardening” skills are so crucial. Technical ability, for example, in performing embryo biopsy and general handling of embryos, can have a significant impact on outcomes. Fourthly, it is the only form of treatment where the physiologies of two individuals combine (sometimes “couples” don’t even meet in the case of donor eggs or sperm) and where the sole intention is to produce another human being.

So, at what stage is the evidence-base sufficient enough for everyone to agree that PGS is a good thing? Opponents of PGS base their argument on the assertion that any intervention should only be introduced into the clinic after at least one favourable double-blind randomized clinical trial. But, in PGS, it’s hard to imagine how we could introduce a placebo. Also, a poor embryologist could single-handedly ruin an RCT by inadvertently performing the embryo biopsy badly. So, here is the paradox: for PGS, are retrospective, single-centre studies equally important as randomized trials to the evidence base? Indeed, a meta-analysis of multiple centres could mask especially good (or bad) practice of specific clinics. An RCT can be beautifully designed but badly performed. What single-centre retrospective studies lack in prospective design, they could compensate for due to the rigour with which they are performed. Opponents of PGS argue that even the most recent studies are not sufficiently robust since clinics are motivated by the need to be seen to be innovating (and possibly by the money that comes in by billing patients for “the latest” therapy). A perception that a clinic is “cutting edge” can be crucial to their survival. Arguments in favour of PGS (and to be fair, these are the views of most of the IVF community) say that there is enough EBM to support PGS in that there are few areas of reproductive medicine where we can wait for RCTs.

Quite a number of widely used procedures have never been subject to an RCT, yet their benefits are obvious without one. The best example would be intracytoplasmic sperm injection (ICSI), the most common treatment for male factor infertility. If you want an insight into how a relatively impartial observer sees the argument between the proponents and opponents of PGS written by one of the current authors (Griffin) and Sally Sheldon of the Kent Law School (see further reading below). In this article “Jacob and Giuseppe” are introduced as imaginary scientists created to represent the extreme sides of the PGS argument. The exposition is a little “tongue in cheek” but paints a picture of two individuals, increasingly more polarized and entrenched in their own opinion, regardless of how much evidence appears. In this context, the authors ask the reader to consider the couple’s perspective: if they are looking to have a child by IVF (and PGS) they would perhaps choose a clinic that is dedicated to making that treatment work, not one that has an open mind based on the result of an RCT. If this were you and your partner, would you not want to find out the success rates of that particular clinic, rather than an RCT? From an ethico-legal perspective: what are the implications of not putting PGS into practice? What about the harm (both physically and psychologically) that could be inflicted on a couple who had a pregnancy (or a baby), assuming that they could, and would, have chosen to avoid this if they had been given the option of PGS? These questions are not easily answered.
Conclusions

In our opinion, nobody should ever take the extreme view of either a “Jacob” or a “Giuseppe.” Whichever way one’s opinion leans, more research needs to be done. PGS will never be perfect, but we can make it better. Conceptually it should work but an RCT involving many centres of varying quality may not necessarily show that. Through more research and more quality control, however, the benefits could ultimately be realized. New technologies should always be considered and the only way to get better at something is to practice it.

Additionally, study of IVF embryos can tell us so much about our early development, information that is also relevant to naturally conceived pregnancies. For instance, chromosome mosaicism can have severe clinical consequences, and there is a great opportunity to study this phenomenon in far more depth than was previously possible. Interested readers are directed to our article in the journal Reproduction for a list of biological questions that we might answered via study of this material (see further reading).

As noted earlier, PGS is controversial, but it’s unlikely to go away. Study of model organisms (e.g. pig and cattle embryos) and conduct of basic research will add to our understanding. Put bluntly, both “Jacob” and “Giuseppe” need to grow up and listen more to one another. We need to appreciate that the evidence base will never be perfect but to be honest with patients about where it is and where it might be, if only we carry on working on it.

So, here’s a final plea (and apologies for repetition from our previous article). Companies, governments, research councils and charities: please work together to generate more funding for basic research into the chromosomes of IVF embryos. Everyone will benefit. For the furtherance of medical research, a greater understanding of chromosome abnormality in general and mosaicism in particular will lead to improved patient care. As the mean age of families get older, this is one of the most important challenges in the medical world.

### Further reading


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