PGT-A also Known as PGS: The Indications

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Definitions

This chapter is dealing with the view of the physician, i.e. gynaecologist and specialist in reproductive medicine, on the genetic detection of presumed anomalies of chromosomes of oocytes and embryos as part of a treatment with artificial reproductive technologies (ART), i.e. IVF or ICSI. This in turn is done in the broadest sense as a treatment of sterility. The analysis is done after oocyte retrieval and before embryo transfer, for the detection of numerical pathologies, i.e. aneuploidies.

There are two important subgroups: the first subgroup, where one or both of the future parents are suffering from a hereditary disease or are carrier thereof, and the second subgroup, where both are healthy in this regard.

Historically, a distinction between these subgroups has been made by the terms preimplantation genetic diagnosis (PGD) for the first and preimplantation genetic screening (PGS) for the second.

To be more precise, PGD describes a case group in which the indication for the investigation is "hereditary disease in future parents", in order to avoid this in the offspring. And PGS describes a case group in which the indication is "suspected genetic disorders at the level of gametes and embryos", in order to increase the success rates of the treatment with in vitro fertilization.

This chapter is a newly edited, updated, partly shortened, partly extended textbook version of a journal article of the author [1].

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These success rates could be, from the perspective of the five main professionals involved

- Geneticist: quick, low-cost, simple, few investigations with precise reliable genetic results.
- Embryologist: quick, low-cost, simple treatments of few oocytes, sperms and embryos with a high rate of implantation followed by the birth of a healthy singleton child at term.
- Reproductive endocrinologist: short time to a healthy singleton child, few simple low-cost treatments with a low rate of complications, miscarriages and psychological stress to the patients.
- Obstetrician: healthy clinical and ongoing pregnancy, few non-invasive investigations, healthy spontaneous delivery at term.
- Neonatologist: healthy mature child with normal birth weight.

Historically, one would either try to detect a monogenetic disorder in one or two biopsied blastomeres from an eight-cell day 3 "embryo" (more precise pre-embryo, as trophoblast and embryoblast morphologically—if at all—are not yet distinguishable) by PCR. Or, one would try to detect an aneuploidy in five or some more chromosomes by FISH, but not both at the same time!

Nowadays, for the first subgroup ("hereditary") one could, in about five trophoblast cells of a day 5 or 6 blastocyst on the one hand, look for a monogenetic disorder and at the same time check for aneuploidies or, vice versa in a "non-hereditary" case, check for aneuploidies and at the same time for a panel of genes, e.g. for a carrier status, currently by NGS. Both approaches might be contested for ethical reasons. But it might be difficult to defend not to look for aneuploidies, when looking for monogenetic defects.

Finally, it is thinkable that in the future in both subgroups, one might look, when checking the health of the embryo in vitro, not only for aneuploidies but also for any other genetic or epigenetic defects, e.g. single genes steering the development in vitro or in vivo.

Now, the terminology in this field has recently been "officially" changed: PGD was changed to "structural rearrangement testing (PGT-SR)" and "monogenetic disorder testing (PGT-M)", and PGS was changed to "aneuploidy testing (PGT-A)". This classification takes the view of the laboratory and not of the gynaecologist treating the patients. It is focusing on the methods applied in the genetic laboratory and not on the medical indication to do so.

So, there are several considerations: the term "PGT-A" might get us to believe that this method is only indicated when treating non-hereditary cases. The term "PGT-SR" and "PGT-M" might make us believe that "PGT-A" is not indicated. The medical indication for any of these is not visible. The terms "screening" and "test" suggest different settings. In a screening the indication is not a pathological finding per se but risk factors, and one has to scan many in order to find a few pathologies. In a test the indication might or might not be a pathologic finding, and one expects a much higher yield. So, in this last regard, the change from screening as PGS to

PGT-A seems to be justified, as, dependent on age, in average at least half of all oocytes are aneuploid. Finally, probable future developments are not mirrored in that scheme: testing in parallel aneuploidy, single genes and other issues, like epigenetics, proteomics and metabolomics, to detect the developmental competence of embryos.

For the physicians' decision to advise for a screening or a test, there are two major considerations: reliability of the diagnostic method and its indication. In the field of PGT primarily the method gets discussed, by the scientists, whereas the normally also important indication, not, may be due to the often not present physicians. In specialized meetings in the field, they are in the vast minority.

As many of the PGT tests are reliable now, the primary medical focus now should be laid on the indication. This is even more true, as the methods were and are—fortunately—pushed by scientists in the lab and unfortunately in the beginning, and still in many countries, mostly ignored by the physicians. But also, the history of studies in PGT-A points in this direction: some primarily designed with a focus on the laboratory evaluation of the method, and thus sometimes ignoring indications, led to sometimes wrong clinical conclusions; see below.

For this reason, for discussing indications, one has to distinguish the two major case groups, event-related case groups, as is done in this report: if the cause of the investigation lies in a disease in the family, PGD could be used, done by one or some of the PGTs (-SR, -M and -A). Otherwise, at the moment for the lack of anything better, PGS is also still being used by IVF registries.

One argument to use only PGT could be that ultimately there is no such thing as complete genetic normality in any human being. So, it would be discussed to test for a panel of frequent and less frequent deviations. And it will be discussed, if some would signify just a quantitative or also a qualitative difference. But also there, one should concede that in a complete discussion of the justification of any investigation, the justification should be not solely focused on the scientific methodology but also on the medical indication.

In general, PGT in routine clinical practice is understood to be invasive diagnostics with a biopsy of polar bodies of oocytes, blastomeres of eight-cell embryos and trophoblast cells of blastocysts, followed by indication-dependent relevant genetic analysis, as performed in this report. Experimentally, "semi-invasive" (aspiration of blastocoel fluid) and non-invasive (analysis of the culture medium) methods have been proposed. These genetic and embryology lab aspects of PGT will not be evaluated here and also not the indications for PGT-SR and PGT-M. The focus of this chapter is on the primary medical point of view, the indication of PGT-A also known as PGS.

Indications

First, a distinction must be made between goals and indications. In contrast to popular belief, the "pregnancy rate" is not the only aim. Instead, there are five distinct aims that partly compete with one another [2]. The possible aims could be to:

- Increase the pregnancy rate.
- Reduce the miscarriage rate.
- Reduce the multiple birth rate.
- Reduce the malformation rate.
- Reduce the rate of pointless treatments with artificial reproductive technology (ART, i.e. IVF or ICSI).

Furthermore, various indications were discussed, such as:
- Advanced maternal age (AMA).
- Repeated implantation failure (RIF).
- Repeated miscarriage (RM).
- Severe male factor (SMF) infertility.

The basic idea is that if one tries to implant only euploid embryos into the uterus, one can improve the patient's situation. However, experience and general principles show that this is not that easy [2].

Pregnancy

Experience with the eight-cell embryo biopsy showed that the intervention had a negative effect on pregnancy rates to some extent. For this reason, this approach was ultimately abandoned for PGS after a long dispute. To lessen the trauma to the embryo, blastocysts are being biopsied. Only in countries where this is legally problematic, oocytes are biopsied for analysis of polar bodies. Based on this experience, hardly anyone claims that there are no effects of these biopsies on embryo development. Thus, if the primary goal is to improve the pregnancy rate, PGT-A makes sense if a stochastic selective benefit is to be expected.

We can consider different settings: the indication "to increase the chance of pregnancy" is there, if the probability to transfer an euploid embryo theoretically might be increased. The real benefit of the test depends in a retrospective view on the outcome of the test.

> "The *normal* case": we receive ten oocytes. Six of them are fertilized, and three develop to blastocysts on day 5, two of which are euploid. If one only intends to transfer one blastocyst into the uterus, the selection advantage can be calculated as 100% euploid after diagnosis and a 67% chance of a euploid blastocyst without PGS, so there is a 50% selection advantage (from 67 to 100%). This advantage will most likely outweigh the disadvantage of biopsy trauma. There is an indication and a benefit to do PGT-A.

> "The case of *good genetic* embryo quality": all three embryos are euploid. Then, the selection advantage in terms of the pregnancy rate is zero. An indication was there, but retrospectively no benefit.

There is no indication "pregnancy increase", if the probability to transfer an euploid embryo definitely cannot be increased.

"The case of *poor development*": only one blastocyst develops. Then, the selection advantage is zero, and the primary goal of increasing the pregnancy rate may be slightly endangered. There is no indication to do PGT-A.

But there is an indication "pregnancy increase", if a fast success is desired. So, other factors influencing the chance of pregnancy must be considered. After the above, the chance of pregnancy with the first fresh transfer may be increased by PGT-A. However, considering the chance independent of time and adding the odds of fresh and further transfers after cryopreservation (cumulative pregnancy rate), this chance without PGT-A could be higher than that of a single fresh PGS transfer. This idea is highly controversial. In younger patients with good blastocyst morphology, PGT-A could not improve the cumulative pregnancy rate ([3], s. Table 15.1).

On the one hand, the blastocyst culture per se could be disadvantageous if a longer culture time of 5 days instead of 2–3 days in vitro is worse than the "blastocyst

Table 15.1 RCTs with PGT-A

First author	Methods	Clinical results
Schoolcraft et al. [4]	>35 years, blastocyst biopsy, aSNP	Implantation increased (71% vs. 46%)
Yang et al. [5]	32 years, blastocyst biopsy, aCGH	Pregnancy per embryo transfer increased (71% vs. 46%)
Forman et al. [6]	35 years, blastocyst biopsy, single embryo transfer with PGS vs. double embryo transfer without PGT-A, RT-PCR	Multiples reduced (0% vs. 65%), pregnancy same (61% vs. 65%)
Rubio et al. [7]	43 years, biopsy of eight-cell embryo, FISH	Birth per cycle increased (24% vs. 11%)
Scott Jr et al. [8]	32 years, blastocyst biopsy, RT-PCR	Birth per cycle increased (85% vs. 68%)
Chen et al. [9]	7 trials (including 4 RCTs)	Implantation, clinical pregnancy, ongoing pregnancy, live birth increased; miscarriage, multiples reduced
Dahdouh et al. [10]	8 trials (including 3 RCTs)	Implantation increased
Verpoest et al. [11]	36–40 years, polar bodies, aCGH	Implantation increased, miscarriages decreased, less interventions
Munné et al. [12]	25–40 years, frozen-thawed SET, NGS	35–40 years ongoing pregnancy per transfer increased
Simopoulou et al. [13]	11 RCTs	>35 years live birth increased
Yan et al. [3]	20–37 years, ≥3 good blastocysts, SET	Miscarriage decreased, cumulated live birth not better
Shi et al. [14]	9 RCTs, AMA	Live birth increased

RCT randomized controlled trial, *PGT-A* preimplantation genetic testing for aneuploidies, *aSNP* array single nucleotide polymorphism, *RT-PCR* real-time polymerase chain reaction, *FISH* fluorescence in situ hybridization, *aCGH* array comparative genome hybridization, *SET* single embryo transfer, *NGS* next-generation sequencing, *AMA* advanced maternal age

culture in vivo" after transfer on day 2 or 3 after oocyte retrieval. On the other hand, the transfer of blastocysts on day 5, the day on which implantation takes place physiologically, might improve implantation.

> "The case of *fast success*": the patient, who is 36 years old, has many oocytes. For her, it is more important to have a higher chance of success in the first transfer than to take the time to "blindly" undergo a fresh transfer first and cryo-transfers later on. Then, there is an indication to do PGT-A.

Miscarriage

It is known that the rate of miscarriages increases with age and that the dominant cause is the aneuploidy of embryos. The increase in the aneuploidy rate of the oocytes matches with increasing age. Similarly, the pregnancy rate drops drastically after 40 years. There are two different cases that can be considered.

There is an indication "decrease of miscarriage" after several miscarriages. Even if the patient has a chance to get a child after zero, one or several further miscarriages, the risk of miscarriage is decreased for the next pregnancy if done with PGT-A. The recommendation of the German Society of Gynaecology and Obstetrics to not apply PGT-A in these cases because of a long-time positive prognosis of recurrent miscarriages appears to be cruel.

> "The case of *recurrent miscarriages*": the patient, who is 37 years old, has one child, has a normal ovarian reserve and has had three miscarriages. She wants a second child but, even more importantly, primarily no further miscarriages. There is an indication for PGT-A.

There is an indication "decrease of miscarriage" also in cases where the risk of a miscarriage because of elevated maternal age is high, even if not yet realized, also in combination with the indication "pregnancy increase" in order to shorten time to pregnancy. A sterility treatment with untested embryos might lead to several embryo transfers, fresh and frozen-thawed, plus miscarriages with curettage and waiting time before starting a new therapy—at a precious time when the pregnancy chance comes to its end.

> "The case of *advanced maternal age*": the patient, who is 41 years old, has had no pregnancies, has a slightly reduced ovarian reserve, and is afraid of taking too much time, especially due to miscarriages and the associated loss of time endangering her likelihood of having children. There is an indication for PGT-A.

Malformation

If the exploration of the patient's preference shows that her primary goal is to reduce the risk of another abortion with a medical indication, then it makes sense to analyse a single existing blastocyst. This goal thus competes with the pregnancy chance. This is the textbook example that the indication of PGT-A needs to be detected by

exploration of the will of the patient by the treating physician, i.e. the specialist in reproductive medicine, and cannot be detected theoretically or be done by a decision at the "green table" in the lab.

> The example is the "case of *trauma interruption*": the patient is 40 years old, has no children, has a reduced ovarian reserve and has had a medically induced termination of one pregnancy due to trisomy 21. The doctor recommends that the biopsy of the only embryo does not take place for the sake of safety so as not to jeopardize the chance of pregnancy. The patient explains after the conflicting goals are clarified: reducing the risk of re-interruption is more important to her than increasing the chance of pregnancy. So there is an indication for PGT-A.

Multiples

Similarly, an exploration may indicate that the patient does not want to have multiple children per birth, also at the risk of reducing the chance of pregnancy.

> This is the "case of being *afraid of multiples*": the patient, who is 38 years old, has three children from her first marriage, including twins, and two blastocysts have developed; however, she does not want to have both transferred. At the same time, she wants to increase the chance of quick success. When both embryos are euploid, the selection advantage is zero, and one of the embryos would be frozen. There is an indication for PGT-A, but no benefit.
>
> If only one is euploid, the test also stochastically makes sense. So, there would be an indication for PGT-A and additionally also a benefit in terms of an increase of the pregnancy chance.
>
> If both are aneuploid, the treatment would be shortened because no cryopreservation and second transfer would be performed. So, there is an indication for PGT-A and additionally a benefit by shortening the time to pregnancy.

Pointless ART Treatment

Finally, certain patients may be at an increased risk for an unusually low rate of euploid oocytes and embryos. The expectation values are approximately 50% for patients under 35 years of age, 33% for patients between 35 and 40 years of age, 25% for patients who are 40 years of age and below 25% for patients over 40 years of age.

PGS for this indication converts a therapeutic procedure, IVF, to a diagnostic procedure.

> The example is the "case of *many treatments*": the patient, who is 32 years old, has had no pregnancies, had three oocyte retrievals and had six embryo transfers, which were fresh or cryo-transfers. She wants to know if continuation of therapy makes sense. Polar body biopsy results of the first PGT-A reveal that nine of ten oocytes are aneuploid, and the euploid egg did not develop into a blastocyst. When PGT-A is repeated, all eight oocytes are aneuploid. The patient opts for egg donation. So, there is an indication for PGT-A.

Advanced Maternal Age (AMA), Repeated Miscarriage (RM), Repeated Implantation Failure (RIF) and Severe Male Factor (SMF)

There are positive findings for AMA (implantation, ongoing pregnancy, birth, live birth, miscarriage and interventions) and also several for RM (both see Table 15.1). These findings must be combined with the need of an individual indication as described above. There are no RCTs for RIF and SMF yet. As always, all study findings are a basis for the treating physician for the decision, common with the couple, about the presence of an individual indication, i.e. make it more or less likely.

Ethics

Evidence-Based Medicine (EBM)

Evidence-based medicine distinguishes three levels

- "Top" (level I) randomized controlled trials (RCTs) and their meta-analyses
- "Centre" (level II) controlled, cohort or case-control studies
- "Bottom" (level III) estimations of authorities based on experience or first principles

When exploring new approaches, for ethical or logical reasons, the approach from bottom to top must be followed. When first principles, such as mathematics, e.g. stochastics, do not allow an advantage of a method, it is pointless and unethical to conduct further studies on this. If there is no single study that has provided proof of a principle to date, it is unethical to randomize patients to prove this principle.

Observational studies are usually conducted with "favourable cases", i.e. with patients for whom a benefit appears most likely. The patients who are individually selected for the purpose of a healing attempt are usually in a serious situation, and there is a suspicion of the chance of a cure by the new treatment method (diagnosis or therapy). These studies must have an ambitious goal, i.e. a high benefit because if the benefit is low, it is to be expected that when widely used, the benefit will disappear.

If the results of these studies have made the effectiveness of the method likely in terms of "proof of principle", it is ethically possible to randomize large groups of patients. On a broader basis, it must be determined whether the method only works for selected cases in the hands of a few specialists or for a large case group with many different practitioners. Only then the method can be recommended to the general public outside of studies for proven indications. For this reason, the goal of such a study may be significantly smaller than that of a study of the principle because small improvements are usually clinically important.

Design of Studies

These considerations are significant in the design of a level I (RCT) study. On the one hand, one should not withhold even small advances from the general population. On the other hand, the smaller the progress being studied, the more complex the investigation will be; that is, the investigation will be more expensive and time-consuming.

Statistically, more patients are needed to prove small differences, which increases costs. Additionally, if necessary, the duration is extended, as more patients must be recruited. Both can lead to the investigation not being carried out, either because the study is too expensive or pointless because one can expect that newer methods will be introduced after the investigation has ended.

Reflecting this, the decision not to investigate would possibly hurt less than the decision to perform a study that restricts the number of patients only due to a lack of financial resources and thus sets very high targets, in order to correctly claim that the high goal was not achieved. At the same time, however, there is a great danger that, because of the high quality of a level I study, the audience will draw the wrong conclusion that the method is ineffective. This may deprive the general public of a minor but clinically significant advance.

Design of PGT-A Studies

For PGS trials, this means that we have to distinguish two stages of the investigations

- The first is the "proof of principle". If one intends to investigate if PGS works at all, the numerator and denominator in the cascade must be close to one other, preferably the number of biochemical pregnancies to the number of embryo transfers or the number of transferred embryos to the number of implanted embryos. The closer the examination points are to each other, the lower the number of cases will be needed for the detection of statistically significant differences, and the smaller the detectable differences will be between the PGT group and the non-PGT group as the control.
- The second is the "efficacy study". If one intends to determine if a large group of patients benefits from the care of multiple physicians and in multiple settings, one should use an RCT with the starting point "intention to treat" (ITT). However, the point at which randomization occurs is most important. It makes no sense to use the first contact as the starting point and the birth of a healthy child as the endpoint because other factors, such as financial costs, might play a greater role than the effectiveness of PGT.

Likewise, the use of the start of ovarian stimulation as a starting point is not indicated because at that time, it is still unclear how many oocytes, fertilizations,

embryos and blastocysts will be present. If this is disregarded, there is a risk that biopsy will occur according to the protocol, without a stochastic selection advantage being present. This may result in a reduction rather than an increase in the pregnancy rate, and the method would be discredited falsely.

Therefore, the patient must be informed of the method twice, namely, at the start of the stimulation and immediately before the biopsy, to consider the possible advantages or disadvantages. Thus, an embryo biopsy according to the protocol, with no consideration of the number of embryos and the desired number of embryos to be transferred and with the sole aim of increasing the pregnancy rate, appears to be unethical.

PGT-A Studies

The origin of PGS of human embryos is based on human PGD [15]. Subsequently, the method has been extrapolated for the screening of oocytes [16, 17] and embryos. In oocytes, the first and second polar bodies, in embryos, from one to two cells of an eight-cell embryo, are examined by FISH with five to nine probes.

The above-mentioned goals and indications were developed until approximately 2010. Numerous studies (EBM levels II and I) have been performed to attempt to prove the effectiveness of the method. The goal of increasing the pregnancy rate could not be demonstrated, although more than ten level I studies were also carried out for this purpose (see [18]).

If the study design did not adequately align the biopsy with the stochastic criteria (see [19]), the pregnancy rate was, as expected, even lower. The goal of reducing the miscarriage rate has been pursued since 1999 in numerous publications of level II studies by Munne et al. [20] but has often received little attention in discussions. With the use of FISH, however, the notion that at least about half of the oocytes in humans are aneuploid has been undisputed.

Thus, the reason for the lack of success of the methodology has been unclear. Unusually, due to the importance of the issue, the largest European professional society in the field, namely, the European Society of Human Reproduction and Embryology (ESHRE), decided to solve this puzzle by sponsoring studies, together with the company Blue Gnome, later bought by Illumina. This new approach, by ESHRE and others, was later called the onset of “PGS 2.0”.

The effectiveness of the method should be increased by applying strict standard operation procedures [21], reducing trauma and increasing the analysis, i.e. by advancing the biopsy to the oocyte and analysing all chromosomes with aCGH. The pilot study showed the high effectiveness of the chips. It also showed that in 40-year-old women, on average, only one in four oocytes is euploid [22]. Subsequently, an international multicentre RCT was launched to investigate the increase in the pregnancy rates in AMA. The available resources allowed the randomization of 600 patients, resulting in a 15%-point study goal of increasing the pregnancy rates.

At the same time, beginning in 2012, the first RCTs appeared, which also used comprehensive chromosome screening (CCS) and, to reduce trauma, postponed the

biopsy to the blastocyst stage. Only now, for the first time, all RCTs found significant advantages for PGS (Table 15.1).

After recruitment delays, the results of the ESHRE study were published in 2018 [11]. The study goal was not achieved, but it showed that the implantation rate was increased by PGS.

PGT-A: Use and Opinions

Use

The method is increasingly used around the world (compare to [23]), especially in the USA. Last data show that in Europe, it is done in 4% percent of all ART treatments [24], in the USA in 44% ([25] for 2019), in Australia in 13% (ANZARD, [26]) and globally in 4% (ICMART, [27]).

Opinions

There is still disagreement about the interpretation of the PGS 2.0 results. However, when examining the opinion publications regarding this purpose, one finds that it is striking that the effectiveness is predominantly assumed:

- In an international survey study, the majority opinion was that PGS is evidence-based medicine, increases live birth rates, reduces miscarriage rates and should be performed with an indication, primarily for repeated implantation failure, in less than 20% of the cycles (IVF-Worldwide Survey, [28]).
- The "Virtual Academy of Genetics" stated that PGS is not experimental, increases live birth rates and reduces miscarriage and multiple birth rates [29].
- In an expert's opinion paper, the majority thinks that PGS increases live birth rates and reduces the time to pregnancy [30].
- The forum COGEN (Controversies in Genetics, a Series of international congresses) stated that PGS is evidence-based medicine and that a pragmatic approach is favoured [31].
- The authors of the ESHRE RCT found that the results "point to a clinical benefit".
- The American Society of Reproductive Medicine [32] stated that PGS "will likely be part of a future multidimensional approach", but it does not recommend "routine use of blastocyst biopsy with aneuploidy testing in all infertile patients". This is in accordance with the indications examined here.

When does a physician change his current treatment routine? Presumably, when a meta-analysis with enough RCTs suggests that another approach is more successful or when the vast majority of his colleagues change their treatment approaches. Thus far, globally this is not yet the case with PGT-A.

However, every physician specialist in reproductive medicine must address this topic. Ultimately, an ethically justifiable decision does not require a meta-analysis, an RCT or any other trial. However, several RCTs indicate that it is likely that PGT-A, if strictly indicated, while taking into account stochastics and patient preference, can benefit the patient.

Therefore, the task of treatment specialists in reproductive medicine is similar to the counselling of specialists in prenatal medicine: present all methods of investigation of the embryo and respect the patient's preference.

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