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Theory and practice of preimplantation genetic screening (PGS)

Andreas G. Schmutzler[a,b,*]

[a] University Women's Hospital, Christian-Albrechts-University, Kiel, Germany
[b] gyn-medicum, Center for Reproductive Medicine, Goettingen, Germany

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ABSTRACT

Objective: In the context of artificial reproductive technology (ART) treatments with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), the purpose of genetic screening of oocytes and embryos in vitro prior to implantation (preimplantation genetic screening, PGS) is highly controversial. Therefore, an analysis of the following theoretical prerequisites is presented: the abstract investigation method and the medical diagnostic decision, indication and ethical acceptability. The first is a scientific task, while the other is a physician's task.
Theory of PGS: As the new term preimplantation genetic diagnosis for aneuploidies (PGT-A) does not sufficiently take into account probable future developments, PGS is retained here. In clinical practice, PGS refers to the biopsy of polar bodies, blastomeres or trophoblast cells with indication-dependent genetic analysis. Goals include increasing pregnancy rates and reducing abortion rates, multiple birth rates, malformation rates and pointless ART treatments. To improve the pregnancy rate, PGS makes no sense if a stochastic selection advantage is not to be expected. Patients may have to choose between the chance of rapid success with a first fresh embryo transfer of blastocysts and a possibly higher overall cumulative chance of pregnancy from fresh and thawed transfers of four-to eight-cell embryos. It is neither necessary nor useful to make every medical decision dependent on randomized controlled trials (RCTs). The randomization of patients is not indicated if observational studies have not shown a positive result. For a "proof-of-principle study", the numerator and denominator of the cascade of parameters for success must be close to each other and far apart in an "efficacy study". The randomization may only be performed before the biopsy.
Practice: Following the introduction of blastocyst biopsy and comprehensive chromosome screening (CCS) with, for example, aCGH and NGS, referred to as "PGS 2.0″, all RCTs since 2012 have found a positive effect.
Discussion: There is still disagreement about the interpretation of the results of PGS 2.0, but the overwhelming view in opinion publications seems to be that it works. This fits with the increasing global commitment to PGS 2.0.
Conclusion: PGS may be beneficial if used with strict indications, taking into account stochastics and the will of the patient. The task of the physician, similar to counselling in prenatal medicine, is as follows: present all methods of investigation and respect the will of the patient.

1. Objective

The usefulness of oocyte and embryo in vitro preimplantation genetic screening (PGS), in the context of medical treatment with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), is highly controversial. The "followers," namely, the physicians and scientists, who use the method and the "adversaries" who do not are sometimes immersed in bitter public international discussions. This dispute has been ongoing for years, and "pro and con discussions" are almost standard at international specialist conferences on this topic. The author of this report was allowed to participate in these discussions repeatedly, each of which were in the "pro camp".

To mediate the controversy, a detailed analysis of the theoretical assumptions on which medical examinations are based could help. We can distinguish four different steps. (1) The assessment of the abstract investigation methodology: Each investigation must have a sufficiently high chance of success, i.e., it leads with adequate probability to a sufficiently reliable result. This assessment of the methodology must be carried out according to the rules of "evidence-based medicine" (EBM). (2) The assessment of the medical diagnostic decision: Here, the specific properties of the medical case are to be assessed, i.e., the characteristics of the patients and possibilities for the treating physicians to subsume the case under the abstract assessment of the methodology. (3) The assessment of the indication of a medical examination: It must also be

* Corresponding author. Waldweg 5, gyn-medicum Kinderwunschzentrum, Göttingen, Germany.
E-mail address: schmutzler@email.uni-kiel.de.

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decided whether the result of the assessment can result in medical consequences because if all possible results lead to the same outcome, the investigation is not indicated. On the one hand, this is because every investigation involves a cost in terms of time, money, and "nerves," which must be justified by an improved decision-making ability based on the examination findings. Furthermore, there may also be a medical risk, which must also be justified by the prospect of improved decision-making about the therapy. (4) The assessment of the ethical acceptability of a medical examination: Finally, the decision for or against an examination must be subjected to an ethical review. Here, too, the objective of the investigation, the methodology and the possible therapeutic consequences must be checked for acceptability in accordance with the rules of medical ethics. The assessment of the abstract investigation method is a scientific task, and the assessment of the concrete diagnostics, indication and medical ethics is the physician's task.

2. Theory of PGS

Historically, a distinction has been made between preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS). PGD describes a case group in which the indication for the investigation is "hereditary disease in future parents", while PGS describes a case group that has "suspected genetic disorders at the level of gametes and embryos". The terminology has recently been "officially" changed: PGD was changed to "structural rearrangement testing (PGT-S)" and "monogenetic disorder testing (PGT-M)" and PGS was changed to "aneuploidy testing (PGT-A)".

However, this classification does not take into account probable future developments that occur in two directions: on the one hand, PGD is routinely carried out in parallel with a PGS, and on the other hand, single-gene examinations will probably be carried out as soon as possible, together with aneuploidy screening, e.g., for the developmental competence of embryos.

For this reason, it still seems most appropriate to clearly distinguish the two methods, for event-related case groups, as is done in this report: if the cause of the investigation lies in a familial disease, PGD should be used; otherwise PGS should be used, i.e., in a complete discussion of the justification of the investigation, the justification should be primarily focused not on the scientific methodology but instead on the medical indication.

In general, PGD and PGS in routine clinical practice are 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.

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 other (Schmutzler, 2014a). The possible aims could be to (1) increase the pregnancy rate, (2) reduce the abortion rate, (3) reduce the multiple birth rate, (4) reduce the malformation rate and, importantly, (5) reduce of 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) and 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 it is not this easy.

2.1. First aim: to increase the pregnancy rate

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 are oocytes biopsied. 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, PGS makes no sense if a stochastic selective benefit is not expected. We can consider four examples. (1) "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. PGS makes sense. (2) "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. PGS does not make sense. (3) "The case of good genetic embryo quality": all three embryos are euploid. Then, the selection advantage in terms of the pregnancy rate is also zero. PGS does not make sense.

Furthermore, other factors influencing the chance of pregnancy should be considered. After the above, the chance of pregnancy with the first fresh transfer may be increased by PGS. However, considering the chance independent of time and adding the odds of fresh and further transfers after cryopreservation (cumulative pregnancy rate), this chance without PGS could be higher than that of a single fresh PGS transfer. This idea is highly controversial. On the one hand, the blastocyst culture per se could be disadvantageous if a longer culture time of five days instead of two to three days in vitro is worse than the "blastocyst culture in vivo" after transfer on day two or three after oocyte retrieval. On the other hand, the transfer of blastocysts on day five, the day on which implantation normally takes place physiologically, might improve implantation. Therefore, we analyse (4) "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, PGS makes sense.

2.2. Second aim: to decrease the miscarriage rate

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. (1) "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. PGS makes sense. (2) "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. PGS makes sense.

2.3. Third aim: to decrease the malformation rate

If the exploration of the patient's preference shows that her primary goal is to reduce the risk of miscarriage, then it makes sense to analyse a single existing blastocyst. This goal thus competes with the pregnancy rate. 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 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. PGS makes sense.

2.4. Fourth aim: to decrease the multiple pregnancy rate

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 fear 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. Thus, PGS makes sense. When both embryos are euploid, the selection advantage is zero, and one of the embryos would be frozen. If one is euploid, PGS also stochastically makes sense. If both are aneuploid, the treatment would be shortened because no second transfer would be performed after cryo-preservation.

2.5. Fifth aim: to decrease the incidence of pointless ART treatments

Finally, certain patients may be at 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. This is not unusual in ART. For example, if the patient has undergone multiple treatments with timed intercourse or artificial inseminations and has not achieved pregnancy, with a two-year waiting time, regular cycles, open tubes and normal sperm, IVF in parallel with ICSI may also be proposed for diagnostic reasons. If, in the end, very few or no eggs are fertilized by IVF, this "diagnostic" IVF procedure will lead to the continuation of therapy with ICSI.

The example is the "case of many therapies": 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 PGS reveal that nine of ten oocytes are aneuploid, and the euploid egg did not develop into a blastocyst. When PGS is repeated, all eight oocytes are aneuploid. The patient opts for egg donation. PGS makes sense.

3. PGS as evidence-based medicine

It is well known that evidence-based medicine (EBM) distinguishes three levels: "top" (level I) randomized controlled trials (RCTs) and their meta-analyses; "center" (level II) controlled/cohort/case-control studies; and "bottom" (level III) estimations of authorities based on experience or first principles. This ranking is widely acknowledged but predominantly in one direction only: top is higher in quality than below. However, the notion that "below" is sufficient is often overlooked (popular example: parachute). This is the case for the majority of medical decisions. Among other things, this is the reason that it is still necessary that a physician, after a length training time, makes the decision, based on experience, intuition and the principles of the art, rather than a robot, a computer or artificial intelligence. On the other hand, it is often not taken into account that when exploring new approaches, for ethical or logical reasons, the approach from bottom to top must be followed.

Level III: 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.

Level II: 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 just because level I theoretical considerations suggest this. This is especially true if there are numerous level II studies that have not provided proof. Instead, such an experiment (RCT) can only be carried out with patients if at least one observational study leads to a positive result. 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). To isolate the random factor, comparison groups are formed, and if necessary, cohort studies are conducted. 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.

Level I: The level I study, namely, the prospective randomized, usually blinded, controlled trial (RCT), is in contrast to the above. Only if the results of the level II studies have made the effectiveness of the method likely in terms of "proof of principle" is it ethically possible to randomize large groups of patients, possibly in a multi-centric way and, if necessary, internationally.

Here, the already proven effectiveness of the principle should be checked on a broader basis to determine whether the method only works for selected cases in the hands of a few practitioners or for a large case group with many different practitioners.

The research objectives are primarily the effectiveness of the method, i.e., the relation between the achieved and defined goal ("does it work?") and, secondarily, the efficiency, i.e., cost, such as the relation between the result and effort ("is it worth it?"). Only then can the method 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 important clinically. Second, the question of efficiency, i.e., cost, is addressed. However, cost depends on many factors outside of medicine and can ultimately be considered on an individual basis.

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. Furthermore, 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.

Quality control statistics are traditional and standard in reproductive medicine. For external control, there are numerous national and multiple international registers. Furthermore, quality is continuously monitored internally. This includes both the treatment of gametes and embryos in the reproductive biology laboratory as well as the upstream and downstream reproductive medical treatment of patients.

The core data are fertilization and pregnancy rates. However, unwanted effects, such as miscarriages, multiple births, overstimulation or treatment discontinuation by patients are of interest. Typically, success rates are expressed as fractions, with numerators and denominators selected from a cascade of decreasing bar graphs, according to the study objective. For example, 200 initial interviews could result in 100 oocyte retrievals with 1000 eggs, 600 fertilizations, 95 embryo transfers with 170 embryos, and 30 pregnancies, with 24 singletons and 6 twins.

If ten different oocyte retrievals result in one embryo transfer with a

Table 1
Quality control in PGS.

Nominators/Denominators	
I. Reproductive Medicine: Indication, COH and OR	
1	first contact
2	recommendation of therapy
3	start of stimulation
4	oocyte retrieval
5	oocytes retrieved
II. Embryology: Fertilization and Cultivation	
Day 0	
6	number of oocytes
7	mature oocyte
8	number of mature oocytes
Day 1	
9	fertilization
10	number of fertilized oocytes
11	regular fertilization
12	number of regular fertilized oocyte
Day 2 – 6, morphology	
13	division
14	number of regularly divided embryos
15	ideal division
16	number of ideally divided embryos
17	number of embryos frozen
III. Embryology and Genetics: PGS by Biopsy and CCS	
18	number of oocytes treated
19	number of oocytes successfully biopsied
20	number of oocytes diagnosed
21	number of euploid oocytes
22	number of embryos treated
23	number of embryos successfully biopsied
24	number of embryos diagnosed
25	number of euploid embryos
IV. Reproductive Medicine: Embryo Transfers	
Fresh embryo transfer	
26	embryo transfer
27	number of embryos transferred
28	number of ideal embryos transferred
Frozen embryo transfer # 1 – x	
29	embryos thawed
30	survival
31	number of surviving embryos
32	number of embryos transferred
33	number of ideal embryos transferred
V. Obstetrics: Pregnancy and Delivery	
Pregnancy	
34	biochemical pregnancy
35	clinical pregnancy
36	number of implanted embryos
37	number of foetuses
38	ongoing pregnancy
Delivery	
39	birth
40	birth at term
41	spontaneous birth
42	living children
43	children with normal birth weight
44	healthy children

consecutive pregnancy, the pregnancy rate per transfer is 100% and that per oocyte retrieval is 10%. This can be due to the age of the patient, the stimulation protocol, the embryo transfer by the physician or the fertilization rate by the embryologist. To capture this, the correct numerators and denominators must be selected from the cascade (Table 1).

As a rule of thumb, e.g., a high rate of multiple pregnancies at a low pregnancy rate, indicates a good laboratory and a poor embryo transfer technique (“ … the physician is to blame. If he carries out the transfer properly, embryos that are well-cultivated by the laboratory will implant … "). In addition, if there are many miscarriages, the in vitro culture must also be considered. If one intends to analyse this in the context of “intention to treat” (ITT) and relate broad starting points and endpoints to one other, namely, the number of births to the cycles started, the investigator would not grasp these problems.

For PGS trials, this means that we have to distinguish two stages of the investigations. (1) 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 PGS group and non-PGS group as the control. (2) 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 ITT. However, the point at which randomization should occur is 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 PGS.

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.

4. Practice

The origin of PGS of human embryos is based on human PGD (Handyside, 1990). Subsequently, the method has been extrapolated for the screening of oocytes (Schmutzler, 1990; Verlinsky, 1990) 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 abovementioned 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 Geraedts, 2010). If the study design did not adequately align the biopsy with the stochastic criteria (see Mastenbroek, 2007), 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 (1999) but has often received little attention in discussions.

With the use of FISH, however, the notion that at least about half of 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 region, 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”.

The effectiveness of the method should be increased by applying strict standard operation procedures (Harton, 2011), 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 (Geraedts, 2011). Subsequently, an international multicentre RCT was launched to

Table 2
Results of PGS 2.0

Reference	Methods RCTs with PGS vs. Nothing	Results
Advanced Maternal Age		
Schoolcraft (2012)	> 35 years blastocyst biopsy aSNP	Implantation increased (71% vs. 46%)
Forman (2013)	35 years blastocyst biopsy single embryo transfer with PGS vs. double embryo transfer without PGS RT-PCR	Multiples reduced (0% vs. 65%) Same pregnancy rate (61% vs. 65%)
Rubio (2013)	43 years biopsy of eight-cell embryo FISH	Birth rate per cycle increased (24% vs. 11%)
Younger Patients		
Yang (2012)	32 years blastocyst biopsy aCGH	Pregnancy per embryo transfer increased (71% vs. 46%)
Scott (2013)	32 years blastocyst biopsy RT-PCR	Birth rate per cycle increased (85% vs. 68%)
Meta-Analysis		
Chen (2015)	7 trials (including 4 RCTs)	Implantation, clinical pregnancy, ongoing pregnancy rate and live birth rate increased; miscarriage and multiple pregnancy rates reduced
Dahdouh (2015)	8 trials (including 3 RCTs)	Implantation rate increased

RCT = randomized controlled trial, PGS = preimplantation genetic screening, aSNP = array single nucleotide polymorphism, RT-PCR = real-time polymerase chain reaction, FISH = fluorescence in situ hybridization, aCGH = array comparative genome hybridization.

investigate the increase in the pregnancy rates in the 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. For the first time, all RCTs found significant advantages for PGS (Table 2). After recruitment delays, the results of the ESHRE study were published in 2018 (Verpoest, 2018). The study goal was not achieved, but the implantation rate was increased by PGS.

5. Discussion

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 (Table 3). This fits with the increase in the use of the method around the world (Schmutzler, 2014b), especially in the USA (Table 4). The authors of the ESHRE RCT found that the results "point to a clinical benefit". The American Society of Reproductive Medicine (Practice Committees, 2018) 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 theories 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, this is not yet the case with PGS. 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.

Table 4
Global application of PGD and PGS.

Region Organization	Reference	Year investigated	Cycles PGD and PGS	Percentage of all ART-cycles
Europe EIM	Calhaz-Jorge (2017)	2013	9791	1%
USA CDC	CDC (2018)	2016	57,987	22%
Australia ANZARD	Fitzgerald (2018)	2016	7425	11%
World ICMART	Adamson et al., 2018	2011	12,614	1%

EIM = European IVF-monitoring Consortium, CDC = Centers for Disease Control and Prevention, ANZARD = Australia and New Zealand Assisted Reproduction Database, ICMART = International Committee for Monitoring Assisted Reproductive Technology.

However, several RCTs indicate that it is likely that PGS, 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 that of counselling specialists in prenatal medicine: present all methods of investigation of the embryo and respect the patient's preference.

Declaration

Because this investigation analysed theories and the literature and because new patient examinations were not conducted, ethical approval was not required.

Table 3
Opinions on PGS 2.0

Institution	Reference	Conclusions
IVF-Worldwide Survey	Weissman RBMO (2017)	Majority opinion: 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 cycles
Virtual Academy of Genetics	Fiorentino (2015)	PGS is not experimental, increases live birth rates, and reduces miscarriage and multiple birth rates
Experts	Sermon MHR (2016)	Majority opinion: PGS increases live birth rates and reduces time to pregnancy
Forum COGEN	Griffin BMJ (2017)	PGS is evidence-based medicine, a pragmatic approach is favoured

COGEN = Controversies in Genetics (= Series of international congresses), PGS = preimplantation genetic screening.

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References

Adamson, G.D., de Mouzon, J., Chambers, G.M., Zegers-Hochschild, F., Mansour, R., Ishihara, O., Banker, M., Dyer, S., 2018 Nov. International committee for monitoring assisted reproductive technology: world report on assisted reproductive technology, 2011. Fertil. Steril. 110 (6), 1067–1080.

Calhaz-Jorge, C., De Geyter, C., Kupka, M.S., de Mouzon, J., Erb, K., Mocanu, E., Motrenko, T., Scaravelli, G., Wyns, C., Goossens, V., 2017 Oct 1. The european IVF-monitoring consortium (EIM) for the european society of human reproduction and Embryology (ESHRE). Assisted reproductive technology in europe, 2013: results generated from european registers by ESHRE. Hum. Reprod. 32 (10), 1957–1973.

CDC, Centers for Disease Control and Prevention, December 2018. National Center for Chronic Disease Prevention and Health Promotion, Division of Reproductive Health. 2016 Assisted Reproductive Technologies. National Summary Report, Atlanta, GA, USA.

Chen, M., Wei, S., Hu, J., Quan, S., 2015 Oct 15. Can comprehensive chromosome screening technology improve IVF/ICSI outcomes? A meta-analysis. PLoS One 10 (10) e0140779.

Dahdouh, E.M., Balayla, J., García-Velasco, J.A., 2015 Dec. Comprehensive chromosome screening improves embryo selection: a meta-analysis. Fertil. Steril. 104 (6), 1503–1512.

Fiorentino, F., Fishel, S., Franasiak, F., Gianaroli, L., Gordon, T., Griffin, D., Grifo, J., Hamamah, S., Handyside, A., Horowitz, A., Hughes, M., Leong, M., Munne, S., Nánássy, L., Schmutzler, A., Scott, R., Shoham, Z., Shulman, L., Vereczkey, A., Weissman, A., Wells, D., Yaron, Y., 2015. In: A Statement on the Use of Preimplantation Genetic Screening (PGS) of Chromosomes for IVF Patients, vol. 27 Virtual Academy of Genetics, Paris 9. https://ivf-worldwide.com.

Fitzgerald, O., Paul, R.C., Harris, K., Chambers, G.M., 2018. Assisted Reproductive Technology in Australia and New Zealand 2016. National Perinatal Epidemiology and Statistics Unit, the University of New South Wales Sydney, Sydney.

Forman, E.J., Hong, K.H., Ferry, K.M., Tao, X., Taylor, D., Levy, B., Treff, N.R., Scott Jr., R.T., 2013 Jul. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil. Steril. 100 (1), 100–107.

Geraedts, J., Collins, J., Gianaroli, L., Goossens, V., Handyside, A., Harper, J., Montag, M., Repping, S., Schmutzler, A., 2010. What next for preimplantation genetic screening? A polar body approach!. Hum. Reprod. 25, 575–577.

Geraedts, J., Montag, M., Magli, M.C., Repping, S., Handyside, A., Staessen, C., Harper, J., Schmutzler, A., Collins, J., Goossens, V., van der Ven, H., Vesela, K., Gianaroli, L., 2011. Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results. Hum. Reprod. 26, 3173–3180.

Griffin, D.K., Fishel, S., Gordon, T., Yaron, Y., Grifo, J., Hourvitz, A., Rechitsky, S., Elson, J., Blazek, J., Fiorentino, F., Treff, N., Munne, S., Leong, M., Schmutzler, A., Vereczkey, A., Ghobara, T., Nánássy, L., Large, M., Hamamah, S., Anderson, R., Gianaroli, L., Wells, D., 2017 Feb 14. Continuing to deliver: the evidence base for pre-implantation genetic screening. BMJ 356.

Handyside, A.H., Kontogianni, E.H., Hardy, K., Winston, R.M., 1990. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 344, 768–770.

Harton, G., Braude, P., Lashwood, A., Schmutzler, A., Traeger-Synodinos, J., Wilton, L., Harper, J.C., 2011. ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening. Hum. Reprod. 26, 14–24.

Mastenbroek, S., Twisk, M., van Echten-Arends, J., Sikkema-Raddatz, B., Korevaar, J.C., Verhoeve, H.R., Vogel, N.E., Arts, E.G., de Vries, J.W., Bossuyt, P.M., Buys, C.H., Heineman, M.J., Repping, S., van der Veen, F., 2007 Jul 5. In vitro fertilization with preimplantation genetic screening. N. Engl. J. Med. 357 (1), 9–17 Epub 2007 Jul 4.

Munne, S., Magli, C., Cohen, J., Morton, P., Sadowy, S., Gianaroli, L., Tucker, M., Márquez, C., Sable, D., Ferraretti, A.P., Massey, J.B., Scott, R., 1999. Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. Hum. Reprod. 14, 2191–2199.

Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2018 Mar. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. Fertil. Steril. 109 (3), 429–436.

Rubio, C., Bellver, J., Rodrigo, L., Bosch, E., Mercader, A., Vidal, C., De los Santos, M.J., Giles, J., Labarta, E., Domingo, J., Crespo, J., Remohí, J., Pellicer, A., Simón, C., 2013. Preimplantation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: two randomized trials. Fertil. Steril. 99, 1400–1407.

Schmutzler, A.G., Schmutzler, R.K., Krebs, D., Gordon, J.W., 1990 Aug. Polar Body and Blastomere Biopsy in the Mouse, Second Joint Meeting of the European Society of Human Reproduction and Embryology and the European Sterility Congress Organization. Human Reprod Programme and Abstracts, Milan, Italy, pp. 7–8.

Schmutzler, A.G., Filges, I., Al-Hasani, S., Diedrich, K., Miny, P., 2014a. The future of aneuploidy screening. Diagnosis first! German; English Abstract. Gynäkologe 47 (4), 263–270.

Schmutzler, A.G., von Otte, S., Tobler, M., Filges, I., Eckmann-Scholz, C., Miny, P., 2014b. Global state of preimplantation genetic diagnosis. Frequency of application and indications. German; English Abstract. Gynäkologe 47 (8), 571–576.

Schoolcraft, W.B., Surrey, E., Minjarez, D., Gustofson, R.L., Scott Jr., R.T., Katz-Jaffe, M.G., 2012. Comprehensive chromosome screening (CCS) with vitrification results in improved clinical outcome in women > 35 years: a randomized control trial. Fertil. Steril. 98 (3), 1.

Scott Jr., R.T., Upham, K.M., Forman, E.J., Hong, K.H., Scott, K.L., Taylor, D., Tao, X., Treff, N.R., 2013 Sep. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil. Steril. 100 (3), 697–703.

Sermon, K., Capalbo, A., Cohen, J., Coonen, E., De Rycke, M., De Vos, A., Delhanty, J., Fiorentino, F., Gleicher, N., Griesinger, G., Grifo, J., Handyside, A., Harper, J., Kokkali, G., Mastenbroek, S., Meldrum, D., Meseguer, M., Montag, M., Munné, S., Rienzi, L., Rubio, C., Scott, K., Scott, R., Simon, C., Swain, J., Treff, N., Ubaldi, F., Vassena, R., Vermeesch, J.R., Verpoest, W., Wells, D., Geraedts, J., 2016 Aug. The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists. Mol. Hum. Reprod. 22 (8), 845–857.

Verlinsky, Y., Ginsberg, N., Lifchez, A., Valle, J., Moise, J., Strom, C.M., 1990. Analysis of the first polar body: preconception genetic diagnosis. Hum. Reprod. 7 (Oct), 826–829.

Verpoest, W., Staessen, C., Bossuyt, P.M., Goossens, V., Altarescu, G., Bonduelle, M., Devesa, M., Eldar-Geva, T., Gianaroli, L., Griesinger, G., Kakourou, G., Kokkali, G., Liebenthron, J., Magli, M.C., Parriego, M., Schmutzler, A.G., Tobler, M., van der Ven, K., Geraedts, J., Sermon, K., Reprod, Hum, 2018 Sep 1. In: Preimplantation Genetic Testing for Aneuploidy by Microarray Analysis of Polar Bodies in Advanced Maternal Age: a Randomized Clinical Trial, vol. 33. pp. 1767–1776 (9).

Weissman, A., Shoham, G., Shoham, Z., Fishel, S., Leong, M., Yaron, Y., 2017 Dec. Preimplantation genetic screening: results of a worldwide web-based survey. Reprod. Biomed. Online 35 (6), 693–700.

Yang, Z., Liu, J., Collins, G.S., Salem, S.A., Liu, X., Lyle, S.S., Peck, A.C., Sills, E.S., Salem, R.D., 2012. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol. Cytogenet. 5, 24.