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Review

Preimplantation Genetic Screening

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Abstract

The main aim of PGS has always been to improve IVF outcome, especially in patient groups assumed to have higher rates of chromosomally abnormal embryos, such as patients of advanced maternal age. In that sense, PGS is quite different from other types of screening as discussed in other papers in this issue. Today it bears no doubt that blastocysts found to be uniformly aneuploid in a biopsy will fail to implant, or worse, will implant and lead to a pregnancy and birth carrying a major chromosomal abnormality, such as trisomy 21. However, it has been argued that a cohort of embryos cannot be improved, and that PGS is only a selection method for which efficiency has not been proven. PGS would never increase the live birth rate for that given cohort, even with a 100% efficiency rate of embryo cryopreservation. The current debate on whether PGS should be applied and to which patients it should be offered has shifted from the effect on live birth rates towards other outcomes such as the reduction of transfers and of miscarriages. Taking the undeniable higher cost of IVF into account when PGS is included, what is the benefit to the patient? The views on this question differ on whether PGS is an additional source of income for the IVF clinic and may or may not balance the extra cost for cryopreservation and embryo transfer for the patients, or whether society pays for IVF treatments and may decide not to want to invest in a medical act that does not improve the primary goal of IVF, i.e. having a healthy child. PGS is also often presented as diminishing patient anxiety and stress through decreasing unnecessary embryos transfers and miscarriages, although no data on this

assertion are available. Whether this emotional argument will show to be strong enough to add PGS as a routine part of an IVF treatment remains to be seen.

Keywords
Preimplantation genetic screening; preimplantation genetic diagnosis for aneuploidy; preimplantation genetic testing for aneuploidy; embryo biopsy; embryo mosaicism; aCGH; NGS

Introduction

The aim of preimplantation genetic screening (PGS) is to select against oocytes and/or preimplantation embryos, obtained during an IVF cycle, that carry chromosomal abnormalities, so that only euploid embryos with a higher chance of implantation are transferred [1]. PGS has been developed after it was found that more than half of embryos obtained during an IVF cycle display chromosomal abnormalities that are both meiotic and mitotic in origin and are incompatible with normal development [2, 3]. The main aim of PGS has always been to improve IVF outcome, especially in patient groups assumed to have higher rates of chromosomally abnormal embryos, such as patients of advanced maternal age (AMA), with repeated pregnancy loss (RPL) or repeated implantation failure (RIF) after transfer of embryos of good morphology [4]. In that sense, PGS is quite different from other types of screening as discussed in other papers in this issue. This is why PGS has gone by many names: some authors wanted to clearly differentiate PGS, applied to patients with a relatively low risk for a live birth with a chromosomal abnormality, from preimplantation genetic diagnosis (PGD) applied to patients at risk for a monogenic disease or carrying a chromosomal structural abnormality. Others deemed that a diagnosis was obtained from the embryo, as clearly non-viable embryos were identified and excluded from the cohort of transferable embryos, and therefore argued that the procedure should be called preimplantation genetic diagnosis for aneuploidy screening (PGD-AS) [5] or preimplantation genetic testing for aneuploidy (PGT-A) [4].

These two fundamentally different viewpoints explain the controversy that has surrounded PGS since its inception. PGS at day 3 of development counting 5 to 9 chromosomes with fluorescent in situ hybridization (FISH) in one biopsied cell knew a high flight in the nineties of last century, and the early years of this century [6] and was at some point more frequently performed than PGD. The practice of PGS was mainly supported by non-randomized studies with poor experimental design and inadequate control groups [7], and by 2010, 11 randomized controlled trials (RCTs) had been published that demonstrated that PGS did not increase, and in some cases even decreased, pregnancy rates [6]. With hindsight, that first version of PGS may not have worked because only a handful of chromosomes were counted, while all chromosomes seem to be affected in preimplantation embryos [8]. Moreover, day 3 of development seemed particularly affected by chromosomal abnormalities, often in mosaic form. Cleavage stage embryos can be mosaic for euploid and aneuploid lineages, and therefore the biopsy of one cell is not representative for the status and implantation potential of an embryo [9, 10]. The development of comprehensive chromosome screening (CCS) based first on single nucleotide polymorphism (SNP) arrays or array

comparative genomic hybridisation (CGH) and later on next generation sequencing (NGS), and the shift towards biopsy at a later stage of embryonic development, the blastocyst, heralded a renaissance in PGS. This was made possible by the introduction of a highly efficient type of cryopreservation, called vitrification, which allows recovery of nearly 100% of frozen blastocysts. Not only does vitrification allow the spreading of embryo transfer over several cycles avoiding the need for embryo selection in the fresh cycle, it also gives the molecular biologists extra time for the analysis of the samples [11]. However, history repeated and PGS2.0 was widely implemented without the support of well-designed RCTs [12].

Technologies employed in PGS

In vitro preimplantation embryo development after IVF and ICSI

During an IVF cycle, the patient receives ovarian stimulation hormones to obtain as many oocytes as possible. Final maturation of the oocytes is induced with an hCG injection and 36 h later, the oocytes are retrieved by puncturing and aspirating the follicles under ultrasound guidance. Fertilisation can then be obtained by incubating the oocytes with sperm, allowing the sperm to penetrate the zona pellucida and the oolemma under their own power. This is what is usually described as IVF. Alternatively, the sperm can be injected into the oocyte using micromanipulation, a procedure called intracytoplasmic sperm injection or ICSI. ICSI was originally developed for patients with poor quality sperm that was not able to fertilise the oocyte. Nowadays, ICSI is used more frequently than IVF, even for patients with good sperm, with the argument that chances of fertilisation are maximized [13]. In case of PGD or PGS, ICSI is preferred because it avoids the risk of DNA contamination with sperm cells sticking to the zona pellucida [14], although this precaution is not necessary if the embryo biopsy is analysed by FISH.

In case of IVF, all oocytes retrieved are incubated with sperm, as it is not possible to evaluate the maturity of the oocytes because they are still surrounded by a layer of corona cells. Before ICSI, these cells are removed and only those oocytes that are in the metaphase II stage, i.e. that have extruded one polar body and display germinal vesicle breakdown, are selected for sperm injection. Fertilisation is assessed the next day by the extrusion of the second polar body, and the presence of two pronuclei at the so-called 2PN stage. The zygote then starts to cleave, and by day 3, the cleavage stage embryo should count about eight blastomeres of regular size, and few or no acellular fragments. By day 4, the cells of the embryo start to compact and the boundaries between the individual cells become indistinguishable. After this morula stage, a cavity is formed in the embryo, and the first differentiation into an inner cell mass (ICM), to become the embryo proper, and the trophectoderm cells, to become the placenta, takes place. Up to the morula stage, the embryo does not increase in volume, but with the formation of the blastocoele, the zona pellucida becomes too tight and the embryo hatches from the zona and is ready for implantation in the endometrium. Throughout the in vitro development of embryos in the IVF lab, the regularity of the consecutive cleavages, the even size of the blastomeres, and the number of acellular fragments are monitored. These parameters are predictive of the implantation potential of the embryo, which is why standardized grading is used in routine IVF care [15]. The different stages of preimplantation development, and how the quality of an embryo is assessed, are extensively described in the ESHRE atlas of human embryology (http://atlas.eshre.eu/).

Biopsy methods and timing

Polar body biopsy

The earliest time point possible to assess the chromosomal content of the oocyte, is after fertilisation, when two polar bodies have been extruded. These by-products of meiosis can be removed without further damage to the developing embryo, and mirror the chromosomal content of the oocyte. This is the main disadvantage of polar body biopsy (PBB): only the maternal contribution to the embryo is tested, and therefore PBB cannot be used for instance when the father carries a structural chromosomal aberration. Also, mosaicism occurring at later stages of development as mentioned in the previous paragraph cannot be assessed through PBB, although it can be argued that this is an advantage [7].

The pioneers of PBB were the late Yury Verlinsky and his colleagues, who were also the main practitioners. Originally, they proposed to only biopsy the first polar body, before fertilisation, as this is a true preconception diagnosis, but as this led to a number of misdiagnoses (mainly for monogenic diseases), the standard method became to analyse both first and second polar body [16].

Polar body biopsy is carried out typically 6 to 12h after fertilisation, after breaching the zona pellucida by mechanical means or using a laser. A biopsy pipette is inserted into the hole and the PBs are retrieved, either sequentially or simultaneously. The simultaneous biopsy has as advantage that the oocyte needs to be manipulated only once; however, it takes some skill and training to differentiate the first from the second polar body [17]. This is important to obtain an accurate diagnosis: the first polar body normally contains 23 chromosomes and 46 chromatids, but can display abnormalities both at the level of the chromosomes, caused by non-disjunction, as of the chromatids, caused by premature predivision, while the second polar body is a fully haploid cell with 23 chromosomes/chromatids [18].

Cleavage stage biopsy

Until very recently, cleavage stage biopsy was the most popular time point at which to biopsy the embryo. At day 3 of development, a normally developing embryo contains eight blastomeres. Either one or two blastomeres can be biopsied at this stage, and the choice of how many to biopsy depends on several factors. Although the best developing cleavage stage embryos can survive the biopsy of two cells and develop into a normal baby, overall two-cell biopsy has been shown to be more damaging to the embryo than one-cell biopsy [19] Therefore, two-cell biopsy was only performed in PGD for monogenic diseases, where the risk for an affected result was 25 or 50% and misdiagnosis carries dire consequences [20]. For PGS, most groups would biopsy only one cell [21].

Technically, cleavage stage biopsy is very similar to PBB. First, a hole is created in the zona pellucida using either acid Tyrode's solution or laser, although the latter has become the preferred method as it is much more accurate, leaves smaller holes of controllable size in the zona and requires much less training and skill to perform [6]. Second, a biopsy pipette is inserted in the embryo and one or two blastomeres are gently aspirated [22].

Blastocyst biopsy

Blastocyst biopsy was described already in 1990 [23], but has only recently known a high flight and is now introduced in a growing number of PGD centres [11, 24]. The advent of better-adapted culture systems yielding more blastocysts per treatment cycle paved the way for this type of biopsy. Blastocyst biopsy has many advantages: as five to ten cells are biopsied, the genetic diagnosis is more accurate. Moreover, an embryo selection already took place during the culture, as only about half of fertilized embryos reach the blastocyst stage [25], which means that fewer analyses need to be carried out on embryos that have shown to have a better implantation potential. It is also believed that blastocyst biopsy is less detrimental to the embryo [26]. Finally, blastocysts are less subject to chromosomal mosaicism than cleavage stage embryos, although the debate on the biological and clinical significance of this is still ongoing [27].

Capalbo et al. have given an extensive description of blastocyst biopsy in [28]. Biopsy is performed on Day 5, 6 or 7 of preimplantation development on good quality blastocysts. A hole is made in the zona pellucida opposite the site of the ICM. Some medium is then blown through the hole to detach the TE from the zona and to induce collapse. Three to ten TE cells are then gently aspirated, and separated from the rest of the embryo by laser pulses.

An important factor to facilitate the introduction and widespread use of blastocyst biopsy is the vitrification of blastocysts. Genetic analysis as described below is time consuming and the high costs can be decreased by pooling samples from different patient cycles. By vitrifying all blastocysts after biopsy, the genetic lab has ample time to complete the analysis and the embryology lab to rank the embryos and thaw and transfer them accordingly [29].

For the sake of completeness, I also mention here blastocentesis, whereby blastocoel fluid is aspirated from the blastocyst and is then analysed using NGS [30, 31]. Whether the blastocoele fluid truly represents the chromosome complement of the whole embryo still needs to be demonstrated; however, this is an interesting research avenue as blastocysts completely recover and regain their original morphology after biopsy, and blastocoele aspiration is therefore considered to be less invasive than blastocyst biopsy [32]. Analysis of the culture medium in which the embryo was cultured is non-invasive, although here there are reasonable doubts whether the DNA present in the medium is coming from the embryo [33-35].

Methods for genetic analysis of embryo biopsy samples, and the insights they brought us

Fluorescent in situ hybridisation

The earliest method used in PGS was fluorescent in situ hybridisation (FISH), because it could be applied to interphase nuclei from blastomeres from which obtaining a metaphase plate is very inefficient. FISH is now considered obsolete as the number of chromosomes that could reliably be counted are limited, due to the fact that only five fluorochomes are available and that only so many dots can be counted in one nucleus before overlaps make correct interpretation impossible. Nevertheless, it was the first method that yielded some insight into the cytogenetics of preimplantation embryos, and that drew our attention to the fact that more than half of cleavage stage embryos are chromosomally abnormal. Kuliev et al. analysed 3953 PGS cycles in which the polar bodies of 20,986 oocytes were analysed by FISH for chromosomes 13, 16, 18, 21, and 22. Forty-seven per cent of the oocytes were shown to be abnormal, with a clear correlation between

age of the patient and chromosomal abnormality [16]. Investigating 20 abnormally and 10 normally developing, normospermic embryos for chromosomes X, Y, 18, 13 and 21, Munné et al demonstrated that only 6/20 of abnormally developing embryos were euploid for the chromosomes tested, while only 3/10 of the normally developing embryos obtained from patients with a mean age of 40 years were euploid [2]. Five embryos in each group showed the same abnormality in every cell, which were therefore most likely of meiotic origin. Delhanty and colleagues investigated normally fertilised cleavage stage embryos from fertile patients undergoing PGD for monogenic diseases. Although they only investigated chromosomes 1, X and Y in spreads of donated embryos, they found that 48/93 embryos (52%) were chromosomally abnormal, of which 24 were chaotic, i.e. with nuclei showing randomly different chromosome complements [36]. These findings led to the introduction of PGS in the clinic, and in 1999 a large retrospective study including 117 women older than 35 years who were matched to 117 control patients was reported. The authors stated that PGS reduced embryo wastage and increased implantation rates [37]. In order to assess the level of mosaicism at different preimplantation development stages, Bielanska and colleagues applied FISH for chromosomes X, Y, 2, 7, 13, 16, 18, 21 and 22 on 59 2-to-4 cell embryos, 81 at the 5-8 cell stage, 43 morulae and 33 blastocysts or 216 in total [38]. Twenty-nine percent were normal, while 22.2 % were abnormal in all cells, either uniformly aneuploid or chaotic with every cell carrying different chromosome abnormalities. Forty-eight were mosaic for euploid and aneuploid cells. Remarkably, although overall the mosaicism rate increased to reach 90.9% at the blastocyst stage, the number of embryos with chaotic cells decreased significantly between the cleavage and blastocyst stages while the most common form of mosaicism at the blastocyst stage was euploid/polyploid. This led the authors to conclude that the embryo may tolerate chromosomally chaotic cells up to a certain point, possibly coincident with the embryonic genome activation between the four and the eight-cell stage, after which they are eliminated, while polyploidy at the blastocyst stage may be a physiological step in placentation.

Array comparative genomic hybridisation (aCGH)

As whole genome amplification (WGA) methods with high reproducibility, yield and coverage became available, the use of technologies that require a high DNA input such as DNA arrays at the single cell level became a reality. In the early years, a combination of degenerate oligonucleotide primed PCR (DOP–PCR) and metaphase comparative genomic hybridisation (mCGH) was used, yielding results on all chromosomes over their complete lengths with a resolution higher than in G-banding [39]. The method however was cumbersome and took several days, which is why it was only applied on polar bodies and on cleavage stage embryos, which were cryopreserved after the biopsy procedure. It quickly evolved when improved WGA methods became available, such as the DNA polymerase Φ29, in combination with array-based CGH (aCGH) [40]. With the development of a complete platform including library-based WGA such as Sureplex® in combination with arrays especially developed for single cell work [17, 41, 42], PGS with aCGH became accessible to most PGS centres. Handyside et al. [18] examined 105 aCGH sets of two polar bodies and the corresponding zygote resulting from a pilot study to evaluate the feasibility of a randomized controlled trial for PGS on polar bodies in patients with AMA [17, 43]. During the pilot study, it was found that 72% of the oocytes analysed had one or more aneuploidies in either one or both polar

bodies. For these abnormal as well as a small number with euploid polar bodies, the corresponding zygotes were then analysed. They found that almost all meiosis I errors were caused by premature predivision of sister chromatids, followed by random segregation of single chromatids at meiosis II to either the PB2 or the zygote, so that only 34% of abnormalities in the zygote originated in meiosis I. There were however more meiosis II-derived maternal aneuploidies in the zygote (45%), while the remainder (21%) were assumed to be paternal in origin or due to anaphase lag. Furthermore, over half of the zygotes examined had multiple aneuploidies. Array CGH was also an important tool to start to understand the origin of post-zygotic chromosomal abnormalities in embryos. By arraying every cell of cleavage stage embryos, it was found that the majority of cleavage stage embryos were chromosomally abnormal [9, 10, 44-46]. Mertzanidou et al. found that 3/27 (11%) of cleavage stage embryos carried a meiotic abnormality, a figure that is in line with other researchers, for instance Chavez et al. [45] who found 9/45 four-cell embryos to carry a meiotic abnormality. The majority of abnormalities found were therefore of post-zygotic and mosaic in origin: between 46% [45] and 87% [10] seem to be mitotic abnormalities. Mitotic non-disjunction seemed to be less frequent than previously assumed, and endoreduplication followed by a cellular division with multipolar spindles was proposed as a mechanism leading to chaotic karyotypes [9]. Array CGH was also widely applied in PGS at the blastocyst stage, and it appeared that blastocysts may carry less aneuploidies than earlier stages. Fragouli et al. found in a series of 1290 trophectoderm biopsies, that 25% abnormalities were purely meiotic in origin, 6% were mosaic for a meiotic and one or more mitotic errors, 27% or the blastocysts showed mitotic errors and the remaining 42% were euploid [47].

Single nucleotide polymorphism (SNP) arrays

Although aCGH has turned out to be a powerful tool for the karyotyping of single cells, it fails to detect the parental origin of the abnormality, and for instance does not detect uniparental disomy (UPD). It is also not applicable for monogenic diseases. Single nucleotide polymorphism arrays (SNP arrays) can be used after WGA at the single cell level to trace the parental origin of a missing or extra chromosome, to differentiate between a trisomy of meiotic or mitotic origin, and has been used to assess the frequency of uniparental disomy in embryos [48, 49]. It has also been used for extensive haplotyping around the site of mutations causing monogenic disease, and is therefore quite suitable to combine the diagnosis of monogenic diseases in embryos with PGS [50-52]. Using SNP arrays to obtain this level of information requires the additional SNP analysis of the couple undergoing IVF, and entail a high level of bioinformatics analysis. The groups who developed this type of technology gave their particular method specific names, such as “Parental support” [48, 53], “Karyomapping” [51] or “siChilds” [50]. The rates of meiotic and mitotic abnormalities found using SNP arrays were in concordance with those found with aCGH: Johnson et al. found 9/26 cleavage stage embryos to carry a meiotic abnormality while 15/26 carried a mitotic abnormality while they found no evidence in their data set of uniparental disomy [48]. Zaman-Esteki analysed one or two blastomeres from cleavage stage embryos, and therefore could not render a complete view of the abnormalities they found, but could still establish that 2 of the 40 embryos they analysed carried meiotic abnormalities, while of the 60 blastomeres they analysed, 63% carried a chromosomal abnormality. At the level of blastocysts, Johnson et al. found in 51 blastocysts a high level of euploidy (around 80%) while nine out of ten trisomies were

meiotic in origin [53]. Interestingly, they analysed the ICM and trophectoderm in three separate samples and found concordance in 49/51 embryos (96%). Given that several cells were analysed per sample, the level of mosaicism within one sample could not be assessed, and therefore, these authors were very cautious in determining mosaicism levels in the same embryo tissue, but concluded that it could not be high in their sample. Northrop et al. [49] analysed 50 blastocysts that had received a diagnosis of aneuploidy using FISH at the cleavage stage and that had developed further. Surprisingly, 29 (58%) of these blastocysts were euploid, while 13 carried a meiotic abnormality, 12 carried a mitotic mosaic abnormality and 4 carried both a mitotic and a meiotic mosaic abnormality. None of the above-mentioned reports identified UPD.

Next generation sequencing

The introduction of NGS was a logical next step: the resolution is higher than for aCGH, and therefore smaller segmental aberrations could be detected and mosaicism could be assessed more accurately, it is amenable to high-throughput analysis, and it can be used in conjunction with the detection of SNVs or single gene mutations [4]. NGS was adapted to PGS by developing pipelines that analysed samples pre-amplified with MDA or PCR-based methods, followed by low-pass sequencing of less than 0.1 % of the genome and bioinformatics analysis using specially developed algorithms that take into account bias introduced by the pre-amplification method. NGS for PGS was validated on different platforms, for instance the Ion Torrent platform [54] or the Illumina HiSeq platform [55, 56]. NGS is also amenable to combination with PGD. Wells et al. demonstrated that an aliquot of the pre-amplified material can be subjected to PCR for the detection of a particular mutation, after which both the pre-amplified material and the PCR product can be subjected to NGS analysis [54].

As the introduction of NGS in PGS was concurrent with, or even slightly later than, the switch to blastocyst biopsy, it is not surprising that most data found in the literature are on aneuploidy and mosaicism at the blastocyst stage. Wells et al. found 24/32 (75%) of blastocyst biopsies to be abnormal in a population of AMA. In their validation study, Ruttanijit et al. analysed 399 blastocysts and found that 48.9% were euploid, 38.1 % were aneuploid and 13% were mosaic, either diploid/aneuploid (8.5%) or aneuploid (4.5%). Since array technology is equally capable of detecting fully aneuploid samples, much of the current research is focused on what NGS can unveil on the problem of chromosomal mosaicism in blastocysts. It has been estimated that NGS detects mosaicism in 29% of blastocysts, while aCGH only detects 5% [57, 58]. In a views and reviews paper, Munné and Wells [59] report aneuploidy and mosaicism rates in blastocysts, based mostly on their own unpublished data, and estimate that 21% of blastocysts would be euploid/aneuploid mosaic, another 10% would be aneuploid mosaic, and the level of mosaicism would vary between 20 and 80%. This last figure cannot be narrowed down, because of the limitation of the NGS sensitivity and because usually only 5 cells are biopsied: below 20%, an embryo can be considered as euploid, and above 80%, it can be considered uniformly aneuploid. Moreover, a small TE biopsy of about 5 cells does not give a full view of the exact number of aneuploid cells in a blastocyst, or of their distribution.

Efficacy of PGS

Biological and technical variables influencing PGS efficacy

Today it bears no doubt that blastocysts found to be uniformly aneuploid in a TE biopsy will fail to implant, or worse, will implant and lead to a pregnancy and birth carrying a major chromosomal abnormality, such as trisomy 21. Studies looking into the concordance of results between the trophectoderm and the ICM have found identical results in both TE and ICM in overwhelming number of cases with full aneuploidy [60, 61]. It therefore seems to make clinical sense not to transfer these embryos. A larger problem however seems to be caused by the approximately 20% euploid/aneuploid embryos, of which about 40% may be able to implant and lead to live births [59, 62, 63]. Not transferring these embryos may reduce the chances for a patient of a healthy pregnancy. Disturbingly, in one study in which 15 mosaic euploid/aneuploid embryos were re-biopsied, 8/15 (53%) had a normal ICM, and 30/59 (51%) of the trophectoderm samples were normal [61]. This demonstrates that about half of the embryos diagnosed as mosaic could be considered euploid, and would be so if the biopsy had been of another site of the trophectoderm, and are therefore lost for the patient. Another study, analysing 15 embryos of which 8 were donated for research and not previously analysed and 7 were diagnosed as abnormal after PGS, showed only 73.3% overall concordance [64].

Another worrying finding is that factors unrelated to the patient, such as stimulation protocols or embryo culture systems, have an impact on the level of aneuploidy in blastocysts. In a large report testing 13 282 blastocyst biopsies from oocyte donors, who represent a young, healthy and presumed homogenous population, the euploidy rates per centre varied between 39.5 to 82.5% [65]. Although the authors did not attempt to correlate euploidy rate to other IVF-related factors, they offer hypotheses that both stimulation regimen, oocyte pick-up method and culture systems may affect embryo aneuploidy [65].

Finally, it has been argued that a cohort of embryos cannot be improved, and that PGS is only a selection method for which efficiency has not been proven. With the current efficiency of cryopreservation protocols reaching near 100% survival, it is very well possible to freeze all blastocysts obtained in an IVF cycle, and to subsequently transfer them one by one in ulterior unstimulated cycles. The single embryo transfer (SET) would avoid iatrogenic multiple pregnancies, and all morphologically good quality embryos would be transferred, and allowed to implant. It would be the uterus to make the ultimate selection, and not PGS. Because PGS is only a selection method, it would never increase the live birth rate for that given cohort [66]. Proponents of PGS argue that PGS is able to decrease the time to pregnancy, as no time is lost in transferring embryos with no chance of implantation, and that it will decrease miscarriage rates [66].

PGS and its troubled relationship with evidence-based medicine

In a context of evidence-based medicine, the only way to ascertain these hypotheses is to conduct well-designed randomized controlled trials (RCT) yielding robust data. Several authors have argued however that RCTs are inappropriate in PGS, as there is already sufficient evidence from retrospective studies, and because RCTs are expensive and time consuming to conduct (Griffin and Sheldon in Focus on Reproduction, January 2017). Also, many patients would refuse to be randomized in a trial, knowing (or having been informed by the clinician) that the treatment

arm is more efficient. Finally, in a rapidly evolving field, it is of the highest importance to innovate quickly, if necessary without waiting for strong evidence, especially in private clinics depending on a sufficient patient flow to survive. These arguments were categorically rebutted by the editor-in-chief of Human Reproduction, Hans Evers, who demonstrated that in IVF as in other fields of medicine, RCTs are highly needed. He also warned for the untested introduction of new technologies, the so-called add-ons to IVF, many of which are abandoned for lack of effect after having been widely applied in the clinic [67].

As all experts in the field now agree that PGS at the cleavage stage using FISH will not increase pregnancy rates, I will focus on the RCTs performed so far using comprehensive chromosome screening, either by aCGH or SNP arrays. The three earliest RCTs which are often cited as sound evidence in favour of PGS [68-70] have however been heavily criticized [12]. The main criticisms are the small size of the study [68], the fact that transfer of cryopreserved embryos which could have been higher in the control group was not taken into account and could have led to additional pregnancies [68, 70], the inclusion of good prognosis patients only with at least a number of analysable embryos, the difference in number of embryos transferred between the two study groups and finally the use of implantation rate as outcome measure [69, 70]. These trial characteristics lead to a distortion of the real a priori benefit for patients, as they do not represent those patients that for instance do not obtain blastocysts for analysis, or only have abnormal embryos and therefore do not even reach embryo transfer. Although these three RCTs were on specific patient categories, they are often cited as demonstrating PGS efficacy for all IVF patients [62, 66, 71, 72].

In a much more robust RCT, Rubio and colleagues compared live birth rates in 105 patients of AMA receiving PGS at the cleavage stage using aCGH with 100 patients undergoing IVF without PGS. They found no difference in cumulative live birth rates when including cryocycles: 37% in the PGS group vs 33.3% in the control group. There were however significant differences in the number of embryo transfers performed and in the miscarriage rate, which was extremely low in the PGS group (only one) versus 21 in the control group.

Another well-designed RCT is the ESTEEM (ESHRE study into the evaluation of oocyte euploidy by microarray analysis) study in an AMA population, testing aCGH in first and second polar body biopsies. The first results of this RCT were presented at the most recent meeting of the European Society for Human Reproduction and Embryology (ESHRE), and again showed no differences in live birth rates (20% in the PGS group vs 22% in the control group), although the number of embryo transfers here too was lower in the study group as well as the miscarriage rate. The STAR trial is another RCT for which the results are much awaited, although as in previous RCTs, randomization of patients is only performed after the patients had obtained at least 2 analysable blastocysts.

The current debate on whether PGS should be applied and to which patients it should be offered does not concern its effect on cumulative live birth rates anymore. Rather, the debate has shifted towards other outcomes such as the reduction of the number of transfers and the number of miscarriages. Taking the undeniable higher cost of IVF into account when PGS is included, what is the benefit to the patient? The views on this question differ on whether health care is considered a commodity in a free economy, where PGS is an additional source of income for the IVF clinic and may or may not balance the extra cost for cryopreservation and embryo transfer for the patients, or whether society pays for IVF treatments and may decide not to want to invest in a medical act that does not improve the primary goal of IVF, i.e. having a healthy child. PGS is also

often presented as diminishing patient anxiety and stress through decreasing unnecessary embryos transfers and miscarriages, although no data on this assertion are available.

Whether this emotional argument will show to be strong enough to add PGS as a routine part of an IVF treatment remains to be seen.

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Competing Interests

The authors have declared that no competing interests exist.

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