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REVIEW

The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists

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STUDY QUESTION: We wanted to probe the opinions and current practices on preimplantation genetic screening (PGS), and more specifically on PGS in its newest form: PGS 2.0?

STUDY FINDING: Consensus is lacking on which patient groups, if any at all, can benefit from PGS 2.0 and, a fortiori, whether all IVF patients should be offered PGS.

WHAT IS KNOWN ALREADY: It is clear from all experts that PGS 2.0 can be defined as biopsy at the blastocyst stage followed by comprehensive chromosome screening and possibly combined with vitrification. Most agree that mosaicism is less of an issue at the blastocyst stage than at the cleavage stage but whether mosaicism is no issue at all at the blastocyst stage is currently called into question.

STUDY DESIGN, SAMPLES/MATERIALS, METHODS: A questionnaire was developed on the three major aspects of PGS 2.0: the Why, with general questions such as PGS 2.0 indications; the How, specifically on genetic analysis methods; the When, on the ideal method and timing of embryo biopsy. Thirty-five colleagues have been selected to address these questions on the basis of their experience with PGS, and demonstrated by peer-reviewed publications, presentations at meetings and participation in the discussion. The first group of experts who were asked about 'The Why' comprised fertility experts, the second group of molecular biologists were asked about 'The How' and the third group of embryologists were asked about 'The When'. Furthermore, the geographical distribution of the experts has been taken into account. Thirty have filled in the questionnaire as well as actively participated in the redaction of the current paper.

MAIN RESULTS AND THE ROLE OF CHANCE: The 30 participants were from Europe (Belgium, Germany, Greece, Italy, Netherlands, Spain, UK) and the USA. Array comparative genome hybridization is the most widely used method amongst the participants, but it is slowly being replaced by massive parallel sequencing. Most participants offering PGS 2.0 to their patients prefer blastocyst biopsy. The high efficiency of vitrification of blastocysts has added a layer of complexity to the discussion, and it is not clear whether PGS in combination with vitrification, PGS alone, or vitrification alone, followed by serial thawing and eSET will be the favoured approach. The opinions range from in favour of the introduction of PGS 2.0 for all IVF patients, over the proposal to use PGS as a tool to rank embryos according to their implantation potential, to scepticism towards PGS pending a positive outcome of robust, reliable and large-scale RCTs in distinct patient groups.

LIMITATIONS, REASONS FOR CAUTION: Care was taken to obtain a wide spectrum of views from carefully chosen experts. However, not all invited experts agreed to participate, which explains a lack of geographical coverage in some areas, for example China. This paper is a collation of current practices and opinions, and it was outside the scope of this study to bring a scientific, once-and-for-all solution to the ongoing debate.

WIDER IMPLICATIONS OF THE FINDINGS: This paper is unique in that it brings together opinions on PGS 2.0 from all different perspectives and gives an overview of currently applied technologies as well as potential future developments. It will be a useful reference for fertility specialists with an expertise outside reproductive genetics.

LARGE SCALE DATA: none.

STUDY FUNDING AND COMPETING INTEREST(S): No specific funding was obtained to conduct this questionnaire.

Key words: preimplantation genetic screening / blastocyst biopsy / array comparative genomic hybridization / massive parallel sequencing / vitrification / chromosomal abnormalities / preimplantation embryo

Introduction

Preimplantation genetic screening (PGS) has been proposed since the early 1990s to improve IVF results. Since its early implementation, there has been a steady increase in the number of IVF cycles that were combined with PGS. The technology has undergone important developments, such as the use of fluorescence *in situ* hybridization in the early days to massive parallel sequencing (MPS) currently, but has also had its opponents. For more information on the different theoretical aspects of PGS, we refer to the sister paper in this issue (Geraedts and Sermon, 2016).

Methods

Three groups of experts have been interviewed on their current practices and opinions regarding PGS, and more specifically on PGS in its newest form: PGS2.0. K.Se. and J.Ge. collated three questionnaires especially designed for the three major aspects of PGS2.0 (why, how and when). About 10 colleagues have been selected per aspect as opinion leaders making use of the following criteria: experience with PGS, demonstrated by peer-reviewed publications and presentations at European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine meetings, and on their vocal participation in the discussion. Furthermore, geographical distribution has been taken into account. We also took care to select experts with differing opinions. The first group consisted of fertility experts, who were asked about 'The Why', the second group of molecular biologists were asked about 'The How' and the third group of embryologists were asked about 'The When'. The names of the respective experts, in what group they were interviewed and what initials were used to indicate particular experts throughout the paper are shown in Table I.

The Why

For the first part, we have tried to obtain a birds' eye view of how specialists in the field perceive the present and future of PGS as seen from their patients' perspective. Since this part is the least technical of the three, it leaves more room for expressing opinions and therefore the opinions voiced are more widely spread. We have selected thirteen fertility experts on the basis of their publication track record, or on their vocal participation in the discussion, of which 10 replied from 7 different countries. The questions and responses are described below.

> For which of the following patient groups is PGS indicated? Please explain your choice, including why you did not choose any of the other possibilities. Why do you think some indications are valid, and others not?

Repeated miscarriages (RM) and repeated implantation failure were the PGS indications that were most chosen by participants. This was followed by advanced maternal age (AMA), PGS in conjunction with PGD and single embryo transfer (SET), sometimes in young or good prognosis patients. Male factor infertility (MFI) and female infertility

Table I Experts who participated in a study on 'the why, the how and the when' of preimplantation genetic screening (PGS) 2.0.

Fertility specialists (initials used in text)		Molecular biologists (initials used in text)		Embryologists (initials used in text)	
J. Cohen (J.C.)	USA	A. Capalbo (A.C.)	Italy	A. De Vos (A.D.V.)	Belgium
J. Delhanty (J.D.)	UK	E. Coonen (E.C.)	The Netherlands	J. Harper (J.H.)	UK
N. Gleicher (N.G.)	USA	M. De Rycke (M.D.R.)	Belgium	G. Kokkali (G.K.)	Greece
G. Griesinger (G.G.)	Germany	F. Fiorentino (F.F.)	Italy	M. Meseguer (M.Me.)	Spain
S. Mastenbroek (S.Ma.)	The Netherlands	J. Grifo (J.Gr.)	USA	M. Montag (M.Mo.)	Germany
D. Meldrum (D.M.)	USA	A. Handyside (A.H.)	UK	L. Rienzi (L.R.)	Italy
R. Scott (R.S.)	USA	S. Munné (S.Mu.)	USA	K. Scott (K.Sc.)	USA
C. Simon (C.S.)	Spain	C. Rubio (C.R.)	Spain	J. Swain (J.S.)	USA
F. Ubaldi (F.U.)	Italy	N. Treff (N.T.)	USA		
R. Vassena (R.V.)	Spain	J.R. Vermeesch (J.R.V.)	Belgium		
W. Verpoest (W.V.)	Belgium	D. Wells (D.W.)	UK		

were least selected as appropriate indications for PGS. Two respondents (D.M. and F.U.) indicated that all IVF patients should be offered PGS. Other indications mentioned that were not included in the questionnaire were PGS in patients younger than 40 years (J.C.), in good prognosis patients (R.V.) and in egg donor cycles (W.V.). Finally, two respondents (N.G., S.Ma.) replied that there are no indications for PGS. The replies per respondent are given in Table II.

Those respondents who are clearly in favour of PGS, having ticked four or more boxes in the questionnaire, list several arguments. There is the biological argument that some patient categories are more susceptible to produce aneuploid embryos (J.D.). F.U. takes the biological argument even further since even young and good prognosis patients produce aneuploid embryos. He states that 'there is no medical reason to transfer an aneuploid embryo when we can detect them'. Several respondents base their judgment on RCTs (Grifo *et al.*, 2014). J.C. mentions several small RCTs with promising results for blastocyst biopsy in conjunction with either array comparative hybridization (aCGH), quantitative PCR (qPCR) or MPS (Forman *et al.*, 2013b; Scott *et al.*, 2013a, b; Fiorentino *et al.*, 2014b). R.S. finds the rationale for PGS in a long series of RCTs providing class I data (Forman *et al.*, 2013b; Scott *et al.*, 2013a, b; Fiorentino *et al.*, 2014a; Chang *et al.*, 2016), as does S.Mu. C.S. refers to RCTs carried out by his group for AMA and MFI that demonstrate superiority in the PGS group (Rubio *et al.*, 2014, 2015, submitted) as well as data on RM using PGS 1.0 (Mastenbroek, 2013; Rubio *et al.*, 2013). Finally, D.M. offers PGS to all patients because pregnancy failure and particularly miscarriage causes couples to drop out and stop further treatment.

A smaller group of respondents (G.G., R.V., W.V.) is more cautious, and proposes PGS in no more than three indications. G.G. proposes PGS as an additional tool to select a euploid embryo in PGD cycles or the single embryo to be transferred in patients with a sufficient number of embryos. R.V. agrees with the argument in favour of the SET in good prognosis patients, arguing that in patients who produce many embryos, the embryo loss brought about by a combination of blastocyst culture, embryo freezing and thawing and the false positive rate of PGS should be compensated by a significantly shorter time to pregnancy. She also mentions patients with a long lasting infertility with no discernible cause or with RMs, possibly to offer the patients closure after one or two cycles with a negative PGS outcome and no transfer. In contrast, this respondent has a sceptical approach towards PGS for AMA: there are no RCTs in this population, and many patients of advanced age will produce very few blastocysts. Time to pregnancy, she argues, is not an issue for these patients who typically have only enough embryos for two transfers (Paulson, 2016). W.V. acknowledges that the risk of miscarriage or lack of implantation may be reduced per euploid embryo transfer, but nevertheless states that there is currently no evidence that in an unselected infertile patient population PGS is beneficial.

Finally, two respondents (N.G. and S.Ma.) state that there is currently insufficient evidence for the effectiveness of PGS (Gleicher *et al.*, 2014; Mastenbroek and Repping, 2014) and therefore PGS is currently not indicated in any patient group for routine clinical use. Although S.Ma. acknowledges that aneuploidy has been demonstrated in all IVF patient categories, he considers the biological argument insufficient grounds for routine clinical use which should be founded on evidence of effectiveness and safety (Dondorp and de Wert, 2011; Ethics Comittee of the American Society for Reproductive Medicine, 2015). Moreover, N.G. points out that the biological argument may be flawed as reports are starting to emerge showing that 'aneuploid' blastocysts can implant (Gleicher *et al.*, 2015; Greco *et al.*, 2015). Therefore we need rigorously designed clinical trials using appropriate outcome measures to support the hypothesis. For example, higher implantation rates after PGS are often used to claim that PGS increases overall success rates after IVF/ICSI, but this is incorrect as PGS is also associated with a lower chance of receiving a transfer and less embryos being available for transfer and/or cryopreservation (Mastenbroek *et al.*, 2007; Gleicher and Barad, 2012; Gleicher *et al.*, 2014; Mastenbroek and Repping, 2014; Griesinger, 2016).

> What is success?
>
> Please explain your choice, including why you did not choose any of the other possibilities. Why do you prefer one definition of success to the other?

An overview of answers can be found in Table II. Live birth rate and reduced time-to-pregnancy represent the best consensus on what is success. Clinical pregnancy rate is considered by fewer respondents,

Table II The Why. Replies to questions for fertility specialists.

Answers	J.C.	J.D.	N.G.	G.G.	S.Ma.	D.M.	R.S.	C.S.	F.U.	R.V.	W.V.
Q1: For which of the following patient groups is PGS indicated? *Other, see text											
Advanced maternal age		X				X	X	X	X		X
Female infertility						X	X		X		
Male factor infertility		X				X	X		X		
Repeated implantation failure	X	X				X	X	X	X	X	X
Repeated miscarriage	X	X				X	X	X	X	X	X
PGD	X			X		X	X	X	X		
Single embryo transfer—SET	X			X		X	X		X		
Other*	X					X			X	X	X
Q2: What is success? *Other, see text											
Clinical pregnancy	X	X			X	X	X	X	X		
Live birth	X	X	X	X	X	X	X	X	X		X
Less aneuploidy	X				X	X	X	X			X
Reduced time to pregnancy	X		X	X	X	X	X	X	X	X	X
Other*	X			X	X	X		X	X		
Q3: What is your preferred transfer policy after PGS? *Other: see text											
SET	X	X		X		X	X		X	X	X
Double embryo transfer			X								
Multiple embryo transfer			X								
Other*					X			X			
Q4: In which cycle do you preferably transfer?											
Fresh			X								
Frozen		X				X			X		X
Both	X			X	X		X	X		X	
Q5: Are you involved in one or more PGS trials? Type of trials are detailed in Table III.											
Yes	X			X			X	X	X		X

mostly because a clinical pregnancy not ending in a live birth is not considered success. Reduced rate of live birth with aneuploidy is considered by six respondents but mainly as a positive adjuvant to the other outcomes. However, two respondents (N.G., S.Ma.) call for caution here as PGS still shows false positives and negatives, and has a less than 100% accuracy, which can have far-reaching consequences in terms of pregnancy and live birth rates, but also the birth of trisomic children (Mastenbroek *et al.*, 2011; Scott *et al.*, 2012; Esfandiari *et al*, 2013; Gleicher *et al.*, 2015; Greco *et al.*, 2015). One respondent (D.M.) specifies that singleton live births should be preferred. A less obvious measure of success mentioned by C.S. is to use PGS as a diagnosis for patients who repeatedly produce aneuploid embryos only, and to offer these patients closure or other forms of treatment such as donation. F.U. sees PGS also as a means to reduce the two most important adverse effects in IVF treatments: miscarriages and multiple births, although the latter can be achieved through SET only (J.Ge.) (Hodes-Wertz *et al.*, 2012; Ubaldi *et al.*, 2015). Respondent G.G. specifies that the increased live birth rate should be related to the number of patients starting IVF treatment.

Four respondents consider all measures of success listed in the questionnaire to be appropriate, although there is considerable disagreement on how this can be achieved. S.Ma. argues that although PGS could in theory be used to improve all outcomes listed, this is not self-evident, because of possible harm of the biopsy (De Vos and Van Steirteghem, 2001; Scott *et al.*, 2013b), mosaicism (van Echten-Arends *et al.*, 2011), the already mentioned less than 100% accuracy of PGS (Mastenbroek *et al.*, 2011; Scott *et al.*, 2012; Esfandiari *et al*, 2013; Gleicher *et al.*, 2015; Greco *et al.*, 2015) and the lack of proper clinical evidence (Mastenbroek and Repping, 2014). This is also argued by respondent W.V., who emphasizes that success is only achieved if PGS is not inferior in live birth rate as compared with IVF without PGS, and prevents implantation failure, miscarriage and aneuploid births. Moreover, S.Ma. and R.V. argue that improved results of cryopreservation programmes allowing the consecutive transfer of cryopreserved embryos without compromising pregnancy chances could lead to cumulative pregnancy rates that are higher than with PGS (Mastenbroek *et al.*, 2011; Wong *et al.*, 2014). R.V. adds that PGS cannot improve the intrinsic quality of the cohort of embryos at hand, but allows for ranking of the embryos based on their chromosomal quality. Respondent J.C. disagrees and is uncomfortable with the 'reduced time to pregnancy' debate: 'the discussion that further PGS selection 'does not matter' because of vitrification, shows little understanding on the part of some practitioners to weigh emotional and economic hardship. This attitude suggests that the practice of transferring undiagnosed embryos (many of which are abnormal) one at a time

Table III List of PGS RCTs the respondents are involved in.

Trial number	Trial title	Fertility specialists	Molecular biologists	Embryologists
ISRCTN81216689	Trial evaluating the influence of morphology and developmental rate on euploid blastocysts ongoing implantation rate	F.U.	A.C.	L.R.
NCT01219283	Study of the Efficacy of 24 Chromosome Preimplantation Genetic Diagnosis (PGD)	R.S.		K.Sc.
NCT01408433	Single Embryo Transfer of a Euploid Embryo Versus Double Embryo Transfer	R.S.	N.T.	K.Sc.
NCT01532284	ESHRE Study into The Evaluation of oocyte Euploidy by Microarray analysis (ESTEEM)	W.V., G.G.	M.D.R.	A.D.V., G.K.
NCT01571076	Preimplantation Genetic Screening (PGS) in Advanced Female Age and Male Severe Factor	C.S.	C.R.	
NCT01917240	Sequential versus Monophasic Media Impact Trial (SuMMIT)		N.T.	
NCT01946945	Comparison of Standard ART Practice versus Trophectoderm Biopsy and Whole Chromosome Analysis	J.C.	S.Mu., D.W.	
NCT01977144	Screening of Low Responders for Aneuploidy to Improve Reproductive Efficiency (Solaire)		N.T.	
NCT02000349	Comparison of Frozen-thawed Embryo Transfers and Fresh Embryo Transfers With Whole Chromosome Analysis Using Next Generation Sequencing	J.C.	S.Mu., D.W.	
NCT02032264	Next Generation Sequencing Screening for Embryonic Ploidy Status (nexgen)	R.S.	N.T.	K.Sc.
NCT02268786	Single Embryo TrAnsfeR of Euploid Embryo (STAR)	J.C.	S.Mu., D.W.	

is acceptable. To me this is not appropriate medical practice'. This opinion is shared by respondents J.Gr. and F.U.: 'Success means to quickly reach the goal ... and aneuploidies in embryos are the single most important factor that relates with spontaneous pregnancy termination and implantation failure'. N.G. argues that reduced time to pregnancy may represent success as long as it can be shown to be cost-effective.

What is your preferred transfer policy after PGS? Please explain your choice. Are there legal or other restrictions, or rationale for your choice?

The replies of the respondents are summarized in Table II and are largely in agreement. Most respondents are in favour of elective SET (eSET) or deferred eSET in cryo cycles (J.Gr., W.V.), and consider it standard of care and good medical practice (Grifo *et al.*, 2013). Also S.Ma. reports that in their IVF/ICSI programme SET is performed in about 70% of their cycles, even though PGS is not included. After PGS, arguments in favour of eSET include higher implantation rates (J.C., J.D., R.S.), high live birth rate (F.U.), avoiding multiple pregnancies (D.M., F.U.) or legal restrictions (in the UK, J.D.). Respondent J.C. adds: 'Frankly, with next generation sequencing and good internal quality control and a serious reduction of error rate, there is not a single group of patients where double embryo transfer (DET) is necessary.' W.V. is of the opinion that: 'There is no evidence at all that higher order transfer has any benefit in times of PGS and vitrification.' Respondent C.S. uses a proprietary algorithm to personalize treatment and to choose between eSET and DET according to the clinical history, age, embryo quality and personal preference of the couple after the prediction of twin pregnancy is calculated. Respondent N.G. makes similar considerations to choose between DET or multiple embryo transfer, but in contrast to most respondents, does not consider twin pregnancies an adverse effect.

In which cycle do you preferably transfer? Please explain your choice. If you have ticked 'Both', what are the reasons to choose one or the other time of transfer?

Our respondents show a spectrum, ranging from a more conservative preference for fresh transfer over a pragmatic approach choosing fresh or frozen transfer according to circumstances, to the transfer of frozen-warmed embryos only. The only proponent of fresh transfer (N.G.) states that frozen transfer is unsupported by credible evidence but that on the contrary, data suggest that fresh is almost always better than frozen (Kushnir *et al.*, 2015). Respondents choosing fresh or frozen transfer according to circumstances, would do so for instance to avoid the risk of ovarian hyperstimulation syndrome (OHSS) in fresh cycles (G.G., C.S.), depending on endometrium receptivity (R.S., C.S.), or depending on the moment of biopsy whereby Day 3 biopsy allows for fresh transfer, while Day 5 biopsy necessitates frozen-warmed transfer (R.V.). S.Ma. uses fresh and frozen transfer sequentially. Both respondents J.C. and S.Ma. state that it is not known yet what is best (Mastenbroek *et al.*, 2011; Maheshwari and Bhattacharya, 2013; Wong *et al.*, 2014) but mention that RCTs to show the benefit of fresh versus frozen embryo transfer are currently underway; J.C. adds that with more data in the near future, fresh transfer may be a thing of the past. Those respondents preferring transfer of frozen-thawed embryos only do so because there is more time for a PGS diagnosis (J.D., D.M., F.U.), reduced risk for OHSS (F.U., W.V.), transfer in a more physiological endometrium and gain of implantation in a natural cycle (J.D., D.M., F.U., W.V.) (Shapiro *et al.*, 2013, 2011).

Are you involved in one or more PGS trials?

Table III shows which RCTs the respondents are involved in. As this question was asked to all three groups, and some respondents are from the same IVF centre, all replies are discussed in this paragraph. Two of the

trials (NCT01219283 and NCT01408433) have been completed. However, not all the other trials are actively recruiting patients at the moment. In most cases there is a comparison between a PGS arm, where genetic analysis is carried out, and a control arm, where embryos are selected on morphological criteria only and are not analysed genetically. In all except one, the ESHRE ESTEEM (ESHRE Study into The Evaluation of oocyte Euploidy by Microarray analysis) study (NCT01532284), in which polar body (PB) biopsy is performed, the intervention is performed after trophectoderm biopsy. In almost all cases infertility cases of a wide age group are included.

What is according to you the best strategy for PGS? Is this strategy different from the one currently used in your centre?

Many respondents consider the best strategy at this moment is to perform blastocyst biopsy (J.D., D.M., R.S., C.S., F.U., W.V.) followed by comprehensive chromosome screening (CCS), which can be either aCGH (J.D., R.S., C.S., W.V.) or qPCR (F.U.), and frozen eSET (D.M., R.S.) in a natural cycle. R.V. specifies that blastocyst biopsy should be reserved for patients predicted to have at least four to five testable embryos. Two respondents (N.G., S.Ma.) argue that currently the level of evidence of published trials is insufficient to justify dissemination into clinical practice (Gleicher *et al.*, 2014; Mastenbroek and Repping, 2014), and N.G. deplores that despite this fact, PGS 2.0 is widely propagated. Four respondents (J.C., S.Ma., R.V., W.V.) call for solid RCTs, for each of the subsets of patients currently considered for PGS. MPS is seen as not fully validated yet, but certainly in the pipeline (R.S., F.U., R.V.).

Would you consider changing your strategy in the future and why?

As befits clinicians guided by evidence-based medicine, most respondents would adapt their current strategy if solid data would indicate that changes would benefit their patients. These solid data include validation and minimal error rates for different assays such as MPS (J.C., J.D., F.U.) and the extent to which mosaicism in human embryos affects both PGS diagnosis and the ability to give rise to a healthy pregnancy (R.V.). There is also a general call for additional large-scale RCTs for different patient populations showing clear clinical benefits for PGS outweighing the downsides and additional costs to the patients (N.G., S.Ma., R.S., F.U., R.V.) (Geraedts and Sermon, 2016). The financial implications are indeed also mentioned by three respondents (J.D., N.G., C.S.) and may be a decisive element to expand the patient groups to whom PGS is offered. C.S. for instance would expand PGS to all IVF patients when it becomes affordable. This is also a future possibility for W.V. who would propose deferred eSET of blastocysts after PGS for all types of infertility, including donor embryos or embryos obtained from donor oocytes. One respondent (D.M.) considers that their current practice (PGS on blastocysts and eSET) avoids twins and miscarriages and should therefore not be changed.

The How

In this part, we asked molecular biologists what methods they used for chromosome analysis in embryos. Of the twelve experts asked to participate, eleven replied. Most respondents use these methods for PGS, except E.C. and J.R.V. who only perform PGD for structural chromosomal abnormalities such as translocations. Both these respondents however also take into account the ploidy status of the chromosomes other than the ones involved in the structural chromosome abnormality.

Which amplification method do you use? Please explain why you prefer this method, including why you prefer not to use other available methods.

Most respondents use whole genome amplification (WGA) methods as a first step in CCS. Two respondents use qPCR primarily in preference to WGA (A.C., N.T.) for CCS and one respondent (D.W.) uses qPCR in specific circumstances.

There are several advantages of using WGA, either PCR-library based WGA methods such as Sureplex (Rubicon Genomics technology), used by E.C., M.D.R. and A.H. before aCGH, and by A.H. and D.W. before MPS, GenomePlex (Sigma Aldrich), used by N.T. for translocations, or multiple displacement amplification, used by A.H. before single nucleotide polymorphism (SNP) array. WGA yields more and highly reliable information both on full and segmental aneuploidies (C.R., J.Gr., S.Mu., D.W.) and for instance allows the combination of a PGD for a monogenic disease or structural chromosomal aberration together with a CCS (F.F., E.C., J.R.V., S.Mu.), it leaves archived material for re-testing in case of misdiagnosis, or the need for additional information (A.H., D.W.), and finally has been widely validated (M.D.R., F.F., A.H.) (Fiorentino *et al.*, 2011; Gutiérrez-Mateo *et al.*, 2011; Schoolcraft *et al.*, 2012; Scott *et al.*, 2012). Storage of amplified material is now part of the accreditation process of many (commercial) labs (A.H.). Two respondents (A.C., N.T.) use WGA followed by SNP array exclusively for structural chromosomal abnormalities where resolution below the whole chromosome, and therefore SNP array, is necessary.

Which method do you use for comprehensive chromosome screening?

A.C. exclusively uses qPCR for conventional PGS cases because it is extensively validated (Treff *et al.*, 2012; Forman *et al.*, 2013b; Scott *et al.*, 2013a, b; Capalbo *et al.*, 2015a), flexible, faster and less expensive. For instance, specific primers can be added to the qPCR mix when a monogenic disease needs to be diagnosed in conjunction with the PGS (Zimmerman *et al.*, 2016). This respondent also mentions chromosome-specific amplification bias introduced as a disadvantage of WGA (Capalbo *et al.*, 2015a), and claims that WGA methods are not properly validated, which is contradicted by several other respondents (M.D.R., F.F.). N.T. also prefers qPCR for the same reasons of speed, reliability and low cost. He and his group have validated the method extensively (Forman *et al.*, 2013a; Scott *et al.*, 2013a, b). D.W. uses qPCR in a minority of cases where the clinic wishes to perform trophectoderm biopsy followed by fresh transfer early in the morning of Day 6 and where the quick turn-around time and the lower cost are of essence. qPCR however only allows for the analyses of whole chromosomes and not for segmental abnormalities, but this is mentioned as an advantage by respondent A.C. rather than a disadvantage.

Most respondents use aCGH as a very robust and well-validated gold standard (Jacobs *et al.*, 2014), which however is quickly being overtaken by MPS methods (Fiorentino, 2012; Deleye *et al.*, 2015). Array-CGH allows analysis of all chromosomes as well as segmental abnormalities of certain sizes (see also below on segmental abnormalities) and can be applied both at the single cell level, such as on PBs and cleavage-stage blastomeres, as well as multiple cells obtained at the blastocyst stage. It has a quick turn-around time of about 24 h and can therefore be used in a fresh cycle. However, it is less efficient at detecting mosaicism in blastocyst biopsies and is quickly becoming more expensive than MPS (D.W.).

MPS has already been introduced by a number of respondents (Fiorentino *et al.*, 2014a, b, 2015; Wells *et al.*, 2014; Bono *et al.*, 2015; Kung

Table IV The How. Replies to questions for molecular biologists.

Answer	A.C.	E.C.	M.D.R.	F.F.	J.Gr.	A.H.	S.Mu.	C.R.	N.T.	J.R.V.	D.W.
Q1: Which amplification method do you use?											
Whole genome amplification		X	X	X	X	X	X	X		X	X
Quantitative PCR—qPCR	X								X		X
Q2: Which method do you use for comprehensive chromosome screening? CGH: comparative genome hybridization											
Fluorescence *in situ* hybridization	–	–	–	–	–	–	–	–	–	–	–
Metaphase-CGH	–	–	–	–	–	–	–	–	–	–	–
Array-CGH—aCGH	(X)	X	X	X	X	X	X	X			X
Single Nucleotide Polymorphism					X	X	X		X	X	X
Massive parallel sequencing—MPS				X	X	X	X	X			X
Q3: Which abnormalities do you score?											
Whole chromosome	X	X	X	X	X	X	X	X	X	X	X
Mosaicism			X	X	X	X	X			X	X
Segmentals		X	X	X	X	X	X	X		X	X
Q4: If partial deletions and duplications are detected: which is the minimal size? * in PGD for translocations, $^{+}$ *de novo*, $^{\circ}$ aCGH, $^{\$}$ MPS											
1–5 Mb					X$^{\circ}$		X$^{\circ}$				X
>5 Mb					X$^{\$}$		X$^{\$}$		X*	X*	
>10 Mb		X*						X*			
>20 Mb		X^{+}	X^{+}					X^{+}			
Q5: How many hours after obtaining the sample are your results available? * after qPCR, $^{+}$ after aCGH, $^{\circ}$ after MPS											
4 h*	X								X		X
12–24 h				X		X	X				X^{+}
24–48 h		X						X		X	
Freeze all			X		X						X$^{\circ}$
Q6: Are you involved in one or more PGS trial? Type of trials are detailed in Table III.											
Yes	X		X				X	X	X		X

et al., 2015; Vera-Rodríguez *et al.*, 2016) while several other respondents report that they are in the process of introducing it in their centres (M.D.R., E.C., J.R.V.). The cost of MPS is dropping quickly as the method allows for far-reaching automation and high-throughput. Moreover, the level of detection is such that in one run whole chromosome abnormalities, segmental abnormalities, (un)balanced translocations, single gene disorders and even mitochondrial disorders can potentially be diagnosed. Four respondents mention it as the only method that will enable detection of low-grade mosaicism in blastocyst biopsies (F.F., J.Gr., S.Mu., D.W.); however respondent J.R.V. adds that haplarithmisis, a method for concurrent aneuploidy screening and genotyping using SNP arrays (Zamani Esteki *et al.*, 2015), is also able to do so. MPS can be performed within the time frame of a blastocyst biopsy and fresh transfer; however mostly in order to obtain cost savings, such as the need for full capacity runs of the sequencer, MPS is usually proposed in frozen cycles (D.W.).

Four participants have indicated that they use SNP arrays either for karyomapping (A.H., J.Gr., S.Mu., D.W.) (Konstantinidis *et al.*, 2015) or for haplarithmisis (Zamani Esteki *et al.*, 2015) (J.R.V.). However both A.H. as D.W. have indicated that they consider MPS to be the better option, and two other respondents (E.C., M.D.R.) mention that they have considered SNP analysis, but will move directly to MPS because of the higher financial implications for the implementation of SNP analysis and because karyomapping has limitations in the detection of copy number aberrations (M.D.R.).

> Which abnormalities do you score?
>
> Especially if you score segmental abnormalities, please explain why you have chosen to score (or not) these abnormalities. What are the consequences for diagnosis of the embryo? Is it scored abnormal, or would you still consider transfer?

Unsurprisingly, all respondents answered that they score whole chromosome abnormalities. However, while a majority score mosaics, few report it back (S.Mu., D.W.) while others will categorize the mosaic embryo as either normal or abnormal depending on the level of mosaicism. Segmental abnormalities are scored by even more respondents, including those stating that they only use array-based assays for PGD for structural abnormalities (Table IV).

There is significant variation in opinion on whether mosaic embryos should be transferred or not. Firstly, detection levels vary significantly between technologies and it is clear that aCGH is less suitable for establishing exact levels of (low) mosaicism than MPS (F.F., J.Gr., S.Mu., D.W.) (Munné *et al.*, 2016). Secondly, the true clinical significance of mosaicism is still unclear. Two respondents, F.F. and N.G., demonstrated that mosaic embryos can grow into healthy euploid newborns (Gleicher *et al.*, 2015; Greco *et al.*, 2015). Moreover, respondent N.T. states

Table V The When. Replies to questions for embryologists.

Answer	A.D.V.	G.K.	J.H.	M.Me.	M.Mo.	L.R.	K.Sc.	J.S.
Q1: Which type of DNA source material do you use for PGS?								
Polar body	X	X			X			
Blastomere				X				
Trophectoderm	X	X	X	X		X	X	X
Blastocoelic fluid	–	–	–	–	–	–	–	–
Q2: If applicable, which method of biopsying both polar bodies, do you prefer?								
Simultaneous	X	X			X			
Sequential			X				X	
Q3: At what time after ICSI do you perform the biopsy(ies)?								
Polar body	9 h	9–10 h	12–16 h		7–9 h			
Blastomere	70 h	64–72 h	64–72 h	68–70 h				
Trophectoderm	D5, D6	106–110 h	115 h	116–120 h		D5–7	112–150 h	118–142 h
Blastocoelic fluid	–	–	–	–	–	–	–	–
Q4: Which method do you use for zona breaching?								
Laser	X	X	X	X	X	X	X	X
Mechanical	–	–	–	–	–	–	–	–
Acid Tyrode's	–	–	–	–	–	–	–	–
Q5: In which culture medium do you biopsy? * With HEPES added; ° Only for blastomere biopsy								
Standard culture medium	X	X	X*	X	X*	X	X	X
Ca^{2+}-Mg^{2-}-free°	X	X	X	X				
Q7: What do you do with undiagnosed embryos? See text for more details								
Discard	–	–	–	–	–	–	–	–
Transfer	X				X			
Rebiopsy		X	X	X		X	X	X
Q8: Are you involved in one or more PGS trial? Type of trials are detailed in Table III.								
Yes	X	X				X	X	

that mosaicism can only be established after two separate biopsies, as the frequency of mosaicism due to technical artefacts can easily be overestimated.

Segmental aneuploidies are even more the subject of debate. Respondent A.C., who does not score segmental abnormalities, states that the role and incidence in preimplantation embryos is still not well established and remains controversial. Uncertainty of diagnosis and difficulties in clinical management remain until positive and negative clinical predictive values can be established, with a risk that reproductively competent embryos are erroneously discarded owing to false aneuploidy calls. N.T. shares this opinion: 'it should be considered unethical to use a test which results in discarding an embryo without having first proven that it has true predictive value for a negative clinical outcome' (Treff *et al.*, 2010, 2012; Mertzanidou *et al.*, 2013b; Chow *et al.*, 2014). J.R.V., who performs PGD for structural aberrations on blastomere biopsies, would transfer embryos with segmental anomalies unrelated to the question. However, S.Mu. argues that if these methods are used to detect unbalanced translocations, those found '*de novo*' should have the same risk of producing abnormal babies and reduced viability as those resulting from translocations. It could be considered that '*de novo*' segmental abnormality identification has been previously validated during the work done on translocations. It should however also be noted that inherited translocations are present in all cells, while postzygotic segmental abnormalities may be present in a small percentage of the embryo's cells (K.Se.) (Mertzanidou *et al.*, 2013a).

Given these uncertainties that are clearly acknowledged by all, most respondents will use a pragmatic approach in dealing with segmental aneuploidies. Some will only recommend not to transfer embryos with very clear and large abnormalities, involving for example a whole chromosome arm, or defects >15 Mb (C.R.). Some will use the information to rank the embryos (J.R.V., E.C.) or will take into consideration the size, type of copy number variant (CNV) and gene content (F.F.). Viable aneuploidies, such as those involving chromosomes 13, 18 and 21, are treated with extreme caution (E.C., F.F.). Finally, several respondents include genetic counselling to help patients reach a decision (F.F., M.D.R., A.H., S.Mu., D.W.) and in case of transfer, let patients sign an informed consent (M.D.R.) and/or strongly recommend prenatal diagnosis (A.H., D.W.).

Is there a general policy (e.g. for prenatal samples) for segmental abnormalities in your centre?

Only five respondents mention that they have a specific policy for segmental abnormalities identified during prenatal diagnosis (E.C., M.D.R., F.F., C.R., J.R.V.). E.C., F.F., M.D.R. and J.R.V. evaluate the risk case by

case according to the size of the CNV, whether it is a loss or gain, and the gene content, and M.D.R. and J.R.V. follow guidelines as used by Belgian genetic centres (Vanakker *et al.*, 2014). C.R. only carries out prenatal diagnosis using arrays if there are specific ultrasound findings. Other respondents do not offer prenatal diagnosis.

If partial deletions and duplications are detected: which is the minimal size?

The complete answers can be found in Table IV. Generally, the minimal size is smaller for known segmental aberrations such as translocations. The sizes range between >1 Mb (S.Mu.) and >20 Mb (E.C., M.D.R.). For translocations, >5 Mb is a recurring figure (C.R., N.T., J.R.V., D.W.).

How many hours after obtaining the sample are your results available?

An overview of the answers can be found in Table IV. Respondents using qPCR need less than 4 h to have results (A.C., N.T., D.W.). Respondents using aCGH need somewhere between 12 and 24 h (F.F., A.H., S.Mu., D.W.) or 24–48 h (E.C., C.R., J.R.V.). MPS requires even longer (48 h or more) and is therefore typically used in conjunction with frozen cycles. M.D.R. specifies that they analyse many samples together for reasons of economy, cryopreserve the embryos, and inform the patients that the results will be ready in 2 weeks, in time for a warming cycle.

The When

Nine clinical embryologists with expertise in embryo biopsy were asked about their views on current practices; eight, working in seven different countries, replied. PGS is offered in all IVF centres where the respondents are based.

Which type of material do you use for PGS? Please explain why you prefer this method, including why you prefer not to use other available methods.

PB biopsy is only carried out by three respondents: A.D.V. and G.K. use it within the framework of the ESTEEM RCT only (see Table II), while M.Mo. is limited by legal restrictions, as trophectoderm biopsy is only allowed in certified centres in Germany. PB biopsy is less invasive than cleavage stage biopsy and avoids the issue of mosaicism (G.K.). As a main disadvantage of PB biopsy, several respondents (M.Me., K.Sc., J.S.) mention the fact that no paternal information is obtained. Other disadvantages mentioned are the cost (J.H.), fragmentation of the PBs at biopsy (J.S.) and small amount of genetic material for analysis (J.S.).

M.Me. is the only respondent who still performs blastomere biopsy, although he mentions that his centre is moving toward trophectoderm biopsy. This respondent claims to have similar results with both blastomere and trophectoderm biopsy, and with more chances for transfer with the former. However, this respondent agrees that the efficiency is greater with trophectoderm biopsy as fewer embryos need to be analysed that have a greater chance to be euploid. Other respondents consider that the disadvantages of blastomere biopsy outweigh the advantages: mosaicism present at the cleavage stage (G.K., A.D.V., J.H., J.S.) (Mertzanidou *et al.*, 2013b) and lower implantation rates (K.Sc.) (Scott *et al.*, 2013a, b). One respondent (J.S.) considers that the disadvantages of blastomere biopsy are sufficient and critical and that blastomere biopsy at Day 3 should be abandoned (Table V).

Most respondents prefer blastocyst biopsy for the following reasons: (i) it is less damaging to the embryo than blastomere biopsy (A.D.V., G.K., L.R., K.Sc., J.S.) (Scott *et al.*, 2013b), (ii) yields more material for genetic analysis (A.D.V., G.K., M.Me., L.R., J.S.) (Jones *et al.*, 2008; Capalbo *et al.*, 2015b), thereby also (iii) avoiding or decreasing issues with mosaicism (A.D.V., G.K., J.H., L.R., J.S.) (Capalbo *et al.*, 2013). Moreover, only viable embryos are analysed (L.R.). All these elements taken together leads to an increased predictive positive value for blastocyst biopsy (Scott *et al.*, 2012). L.R. concludes that one RCT (Lee *et al.*, 2015) and one meta-analysis (Dahdouh *et al.*, 2015) have recently demonstrated the effectiveness of this approach, and believes that to date this is the only validated approach.

Blastocoele fluid (BF) biopsy is mostly considered too new and not validated (J.H., G.K., L.R., K.Sc., J.S.). Respondent J.S.: 'while a few preliminary studies and at least one published study (Tobler *et al.*, 2015) point to the presence of genomic DNA in the fluid, there appear to be problems in terms of correlation of the blastocoele DNA and the chromosome status of the embryo'. During the writing of this manuscript, results from a longitudinal cohort study comparing aCGH on cell biopsies and BF were published, concluding that if the proportion of clinically useful BFs is improved, blastocentesis could become the preferred source of DNA for chromosomal testing (Magli *et al.*, 2015).

If applicable, which method of biopsying both polar bodies, do you prefer? Please explain why you prefer this method, including why you prefer not to use other available methods.

Three respondents did not reply to this question as they indicated that they did not perform PB biopsy. Three more respondents indicated that they performed simultaneous PBI and II biopsy, because it was specified in the ESTEEM study (see Table III) (A.D.V., G.K.), and because simultaneous biopsy can be carried out in a minimum of time with a minimum of manipulation (G.K., M.Mo.). J.H. and K.Sc. would prefer sequential biopsy, although neither uses PB biopsy routinely, because the risk for degradation of the DNA of the first PB is lower (K.Sc.) and because it takes less time (J.H.). M.Mo. notes that sequential PB biopsy can be preferred when a highly fragmented PB1 is already present at time of ICSI.

At what time after ICSI do you perform the biopsy(ies)?

The ESTEEM study requires that the PB biopsy is carried out around 9 h post ICSI, which is why this is the preferred time for A.D.V. and G.K. M.Mo. extends this to an earlier timing, from 7–9 h post ICSI. Four respondents indicated the timing of blastomere biopsy, usually at Day 3 between 64 and 72 h. The timing for blastocyst biopsy varies widely, from early day 5 (115–116 h) to day 5 and 6 (up to 142–150 h) and even day 7.

Which method do you use for zona breaching?

Please explain why you prefer this method, including why you prefer not to use other available methods:

Zona breaching using the laser is preferred by all respondents who offer an extensive list of advantages. Laser zona breaching is fast and allows making small holes in a controlled and standardized manner. This is particularly important for blastocyst biopsy where a small hole is made on Day 3 or 4, allowing the embryo to herniate about 2 days later without completely going out of the zona. Inner call mass (ICM) herniation is not considered an issue, as it occurs in less than 10% of biopsied blastocysts (A.D.V.) and in any case can be solved by making a second zona opening on the opposite site of the herniating ICM (A.D.V., J.S.). One respondent (L.R.) has developed a biopsy method with simultaneous zona breaching and trophectoderm aspiration and biopsy (Capalbo *et al.*, 2014). It is also safer for

the embryo because the time out of the incubator is limited (K.Sc., G.K., J.S.) and because it avoids the possible damage and even embryo rupture that can be caused by acid Tyrode's zona breaching (M.Mo., J.S.). Finally, it is technically less demanding and requires less training (J.S.). For those using PB biopsy (G.K., M.Mo.), using acid Tyrode's is out of the question, and mechanical zona breaching takes more time to perform. Although laser zona breaching can be considered safe since many children have been born after its use (A.D.V.) (Desmyttere *et al.*, 2012), there has been at least one report of negative effects (Honguntikar *et al.*, 2015). However, respondents do not refer to these negative reports and seem to accept zona breaching with laser as the safest method available.

> In which culture medium do you biopsy?
>
> Please explain why you prefer this method, including why you prefer not to use other available methods.

Both PB and blastocyst biopsy is carried out in standard culture medium. This is considered least detrimental to the embryo, avoiding an additional stressor (L.R., K.Sc., J.S.) and it also avoids additional variables that would need to be assessed for quality control (J.S.). All respondents indicating that they use, or have used, blastomere biopsy responded that they use $Ca^{2+}Mg^{2+}$-free medium to loosen cell-to-cell contacts in the cleavage stage embryo. Some (J.H., M.Mo.) buffer standard culture media with HEPES to allow for better pH control during manipulation.

> Would you consider alternative biopsy types (e.g. if you do cleavage stage biopsy, would you change to blastocyst)? If not, why not (legal restrictions, logistic problems, cost. . .)?

Three respondents are in transition from cleavage stage biopsy to blastocyst biopsy: A.D.V. for PGD for monogenic diseases, and M.Me. and M.Mo. for PGS. Blastomere biopsy is considered 'going back' (J.S.). Two respondents (L.R., K.Sc.) who use blastocyst biopsy exclusively would consider blastocoele biopsy on the condition that strong evidence in favour is available (L.R.) because it is less invasive for the embryo (K.Sc.). J.S. proposes a two pronuclei (2PN) banking approach to compile a sufficient number of 2PNs to grow to blastocysts for biopsy.

> What do you do with undiagnosed embryos? Please explain why you choose this course of action? Are there legal or other restrictions that determine your decision-making process?

One respondent (M.Mo.) transfers undiagnosed embryos after obtaining informed patient consent. Most respondents indicate that they re-biopsy the embryo and try to get a diagnosis from the second sample. For one respondent (A.D.V.) this is a rare occurrence as their chosen course of action after PGS is to transfer undiagnosed embryos after genetic counselling and obtaining informed consent from the patient. The majority of respondents however routinely rebiopsy embryos, if necessary followed by cryopreservation (J.H., G.K., M.Me., L.R., J.S.). One respondent (L.R.) indicated that qPCR is used on frozen-thawed undiagnosed blastocysts for a quick turn-around time. If after re-analysis there is still no diagnosis or patients refuse a second biopsy, G.K., M.Me. and J.S. would transfer the undiagnosed embryos, after consultation with the patients and their doctor (M.Me.).

The future of PGS

All three groups were asked what they considered would be the next major breakthroughs, and how PGS would develop in the future. These technological developments could lead to new applications such as non-invasive PGS through spent media assessment (J.C., C.S.) or disease susceptibility screening of embryos, as is currently carried out in newborns, e.g. inborn errors of metabolism such as phenylketonuria, and hypothyroidism (G.G.). New ways to assess the embryo that go beyond genomics, such as transcriptomics (G.K.) (Jones *et al.*, 2008), as yet undetermined metabolomics biomarkers (M.Me., L.R., K.Sc., R.S.), epigenomics (R.V.) or mitochondrial content, constitution, structure and function (G.K., M.Me., C.S., K.Sc., R.V.) (Diez-Juan *et al.*, 2015; Fragouli *et al.*, 2015), will be developed. Of course, these will need extensive validation prior to clinical application (K.Sc.).

These could then help to add nuances to our evaluation of embryo fitness, and they might help in refining our current embryo ranking strategy (R.V.) and to identify the euploid embryo most likely to achieve a viable pregnancy (D.M.).

However, several respondents add a cautionary note to these innovations. S.Ma. calls for rigorously designed trials on the new PGS technologies, a position endorsed by A.D.V., G.K., M.Me. and R.V. S.Ma.: '. . .stakeholders with potential commercial interests (are known to) have an effect on the design and reporting of clinical studies with the aim to (mis)use science for marketing purposes (Gøtzsche *et al.*, 2007; Psaty and Kronmal, 2008; Ross *et al.*, 2008; Macleod *et al.*, 2014). Clinicians should no longer leave the liability in PGS to the 'demand of the patient', but take up the challenge of determining by proper science the true value of PGS before introducing the technique in routine clinical practice.' M.Me. adds a nuance to this position: 'more RCTs would be ideal, but, most of them (well designed) are logistically impossible (a huge number of patients are needed) or unethical (existing retrospective evidence makes it difficult to allocate patients in the control group knowing that they would have lower chances to become pregnant. . .)'.

R.V. also cautions that a more generalized switch to genome sequencing and an increased resolution of genome readings will bring about a significant increase in the acquisition of genomic data of unknown significance with respect to embryonic health (Peters *et al.*, 2015). She therefore calls for continued basic research in the genetics of development and implantation, a position endorsed by E.C., J.Gr., C.S., R.S., C.R. and J.R.V.

N.G. looks at the future from a completely different angle: 'PGS is, of course, an embryo selection method. In my opinion, it is time to challenge the whole concept of embryo selection. . . for two reasons: one, it biologically makes little sense, considering that the embryos we select from are the product of up to 4 months of follicle maturation, and egg quality represents ca. 95% of embryo quality. . . . If we want to have a real impact, we have to start intervening in early stages of follicle maturation to really affect egg and embryo quality; Two, every embryo selection method in the literature, . . .(have). . . outcome data almost exclusively reported with as a reference point embryo transfer, rather than by "intent to treat" (cycle start). When outcomes are generalized in this fashion, especially poor prognosis patients are usually outright harmed, and that applies to all embryo selection methods, including PGS (Griesinger, 2016).'

Conclusion

The initiators of this questionnaire, K.Se. and J.Ge., were pleasantly surprised by the positive response from those invited to participate: of the 35 experts invited, 30 responded. Of note is that no Chinese experts replied to our questionnaire, although two were invited.

Consensus is lacking on which patient groups, if any at all, can benefit from PGS 2.0, and a fortiori whether all IVF patients should be offered PGS. The high efficiency of vitrification of blastocysts has added a layer of complexity to the discussion, and it is not clear whether it will be a story of PGS in combination with vitrification, or of PGS alone, or vitrification alone, followed by serial thawing and eSET. The opinions range from in favour of the introduction of PGS 2.0 for all IVF patients, over the proposal to use PGS as a tool to rank embryos according to their implantation potential, to scepticism towards PGS pending a positive outcome of robust, reliable and large-scale RCTs in distinct patient groups. This latter group is not against PGS per se, but they oppose routine clinical introduction of a technique of as yet unknown efficacy. However, although a number of respondents have called for solid RCTs, separately for each of the subsets of patients currently considered for PGS, these are not underway. In ongoing RCTs, mostly all types of infertility and all age groups are included. Furthermore, the variation of molecular methods used is limited and all except one RCT use trophectoderm biopsies.

Therefore, although it is clear from all three groups of experts that PGS 2.0 can be defined as biopsy at the blastocyst stage followed by CCS and possibly combined with vitrification, experimental evidence for the safety and efficacy of PGS 2.0 needs to be awaited. There is agreement on the fact that mosaicism is less of an issue at the blastocyst stage than at the cleavage stage but whether mosaicism is no issue at all at the blastocyst stage is currently called into question. BF biopsy seems an attractive alternative as it is deemed less invasive, although experts are awaiting validation and translation to the clinic. The CCS method however is in full evolution: while aCGH is the most widely used method amongst our participants, combining ease-of-use with high information content, it is slowly being replaced by MPS (De Rycke *et al.*, 2015). SNP arrays and qPCR, both with their specific advantages and disadvantages, will follow suit.

Finally, our expert group is also looking to the future, and most see CCS as only one stop on the road to fully understanding the biology of the human preimplantation embryo. This understanding could be broadened by the analysis of the transcriptome, the epigenome, the metabolome and the mitochondrial function of embryos. A combination of all these elements may in the future allow fertility specialists to predict which embryo in a cohort will have the highest chance of implantation—or even predict with absolute accuracy whether an embryo will implant or not. This evolution would greatly benefit our patients, for whom the physical, emotional, financial burden and time spent will be reduced to the minimum.

Authors' roles

K.Se.: contributed to conception and design, acquisition of data, and analysis and interpretation of data; drafted the article and revised it critically for important intellectual content and approved of the version to be published. A.C., J.C., E.C., M.D.R., A.D.V., J.D., F.F., N.G., G.G., J.Gr., A.H., J.H., G.K., S.Ma., D.M., M.Me., M.Mo., S.Mu., L.R., C.R., K.Sc., R.S., C.S., J.S., N.T., F.U., R.V., J.R.V., W.V., D.W.: contributed to acquisition of data, and analysis and interpretation of data; drafted the article and revised it critically for important intellectual content and approved of the version to be published. J.Ge.: contributed to conception and design, acquisition of data, and analysis and interpretation of data; drafted the article and revised it critically for important intellectual content and approved of the version to be published.

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Conflict of interest

K.Se., E.C., M.D.R., A.D.V., J.D., N.G., J.Gr., J.Ge., J.H., G.K., S.Ma., D.M., M.Me., C.R., K.Sc., R.S., J.S., N.T., R.V. and W.V. declare to have no conflict of interest. A.C. declares to be shareholder of a Genetic Lab (GENETYX srl). J.C. declares to be a consultant for Reprogenetics. He is a product developer for Life Global as well as for Althea Science. F.F. declares to be a shareholder in Genoma, laboratories providing genetic testing services that include PGS. G.G. has received consultant/honorarium fees from MSD, Ferring, Glycotope, Serono, Finox, Vitrolife, Marckryl Pharma and IBSA and has served on speaker bureaus for MSD, Ferring, Serono, Vitrolife, Marckryl Pharma and IBSA. A.H. declares part time employment and stock holdings in Illumina, San Diego, CA, USA, which manufactures equipment and reagents for preimplantation genetic diagnostics. M.Mo. declares the following conflict of interest: CEO of ilabcomm GmbH, a consultancy company that also consults on laboratory practice in conjunction with PGS. S.Mu. has received stock options from Cooper Surgical, a company providing PGS services through Reprogenetics. L.R. declares to be shareholder of IVF centres (GENERA) and of a Genetic Lab (GENETYX srl). C.S. is stakeholder of Igenomix. F.U. declares to be shareholder of IVF centres (GENERA) and of a Genetic Lab (GENETYX srl). J.R.V. holds the following patents: ZL9 1 0050-PCT/EP20 11 /06021 1- WO/20 11 /1 57846 'Methods for haplotyping single cells' and ZL91 3096-PCT/EP201 4/06831 s-WO/20 1 5/028576 'Haplotyping and copy-number typing using polymorphic variant allelic frequencies'. He is a co-inventor on patent application ZL91 2076-PCT/EP20 1 3/070858 'High throughput genotyping by sequencing.' D.W. holds shares in Reprogenetics UK and receives financial compensation from Reprogenetics LLC, laboratories providing genetic testing services that include PGS.

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