Scientific and Clinical Advances Advisory Committee Paper

Paper title	**Preimplantation genetic screening**
Paper number	SCAAC(06/15)07
Meeting date	10 June 2015
Agenda item	7
Author	Sarah Testori (Scientific and Clinical Policy Manager)
Information/decision	Decision
Resource implications	None
Implementation	Update to guidance on PGS in the Code of Practice. Any changes to the Code of Practice will need to be approved by the Authority and would come into effect in October 2015 or April 2016
Communication	Code of Practice changes will be communicated in Clinic Focus
Organisational risk	Low
Committee recommendation	Members are asked to: • Review the recent advances in technologies for PGS and consider whether these require changes to the guidance note on PGS in the Code of Practice.
Evaluation	Updates and further studies will be evaluated in annual horizon scanning
Annexes	Annex A – Current PGS Guidance note Annex B – February 2015 SCAAC PGS paper Annex C – Summary of embryo testing SCAAC focus meeting Annex D – Summary of current PGS technologies

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

1. Introduction

1.1. For an embryo to develop properly it must have the correct number of chromosomes. It is thought that the majority of unsuccessful IVF treatments are caused by chromosomal abnormalities known as aneuploidies[1], as they leads to implantation failure and miscarriage, as well as genetic conditions such as Down's syndrome (Hassold & Hunt 2001). These chromosomal abnormalities happen very frequently during normal human reproduction, and it is estimated that over two thirds of embryos produced, either through natural conception of IVF, are lost before birth due to aneuploidy.

1.2. In order to try and improve the success of IVF, a technique known as preimplantation genetic screening (PGS) has been developed. PGS describes the process of checking the chromosomal composition of embryos. This allows aneuploid embryos to be identified and removed prior to transfer and so only embryos with the correct chromosome complement are used in treatment.

2. How PGS is regulated

2.1. The Human Fertilisation and Embryology Act 1990 (as amended) (the Act) outlines the circumstances under which it is permissible to carry out embryo testing. Embryo testing may be used to establish whether an embryo has a gene, chromosome or mitochondrion abnormality that **may affect its capacity to result in a live birth,** or in cases where there is a 'particular risk'[2] that the embryo may have any gene, chromosome or mitochondrion abnormality, and for the purpose of determining whether that abnormality is present in an embryo. The use of PGS is permitted as it relates to the first circumstance, whereas the second circumstance applies to preimplantation genetic diagnosis (PGD).

2.2. The Act therefore sets out clear selection criteria providing a mechanism by which treatment of patients by PGD can be regulated (through the authorized list of genetic conditions). The same is not true for PGS. In the past the HFEA has regulated the provision of PGS to patients through guidance in the Code of Practice and licence conditions. More recently, guidance has placed the onus on clinics to justify their use of PGS for particular patient groups. However, this approach may no longer be appropriate.

2.3. Current guidance in the Code of Practice states that centres should provide information to patients including the risks associated with the procedure, and the unproven nature of the procedure. In addition centres are asked to "keep up to date with relevant literature and professional guidance in order to validate the use of PGS for each category of patient to whom they offer it."

2.4. In the intervening period since this PGS guidance note was written, new technologies for carrying out PGS have been developed. These are capable of generating far more accurate and detailed data on the genetic make-up of embryos and this has implications for the clinical applications of PGS and the way in which the HFEA may wish to issue guidance to clinics on their use. The

[1] Aneuploidy – The normal chromosome complement of a human cell is 23 pairs of chromosomes. A cell is described as aneuploid if it has a piece, entire chromosome, or number of chromosomes missing or additional to this compete complement.

[2] Practically 'particular risk' can be determined to be present in patients with a family history of monogenic disease, or by the identification of a specific genetic abnormality (for example by carrier testing) in one or both of the parents.

aim of this paper is to identify areas of the Code of Practice that require updating in the light of the most recent techniques and research into PGS[3].

3. The efficacy of PGS

3.1. In the first 15 years of its existence, the common methodology employed for PGS was blastomere[4] biopsy followed by fluorescence in situ hybridization (FISH) analysis (Mastenbroek & Repping 2014). However, several years after its initial introduction, a number of randomised controlled trials (RCTs) showed that far from leading to improved IVF outcomes, PGS significantly decreased chance of ongoing pregnancy in comparison with IVF without PGS (Mastenbroek et al. 2011). It is now widely accepted that PGS by FISH does not improve pregnancy outcomes[5] and that its premature introduction was a mistake.

Mosaicism

3.2. Mosaicism is the phenomenon whereby a single cell, or small group of cells, may not represent the chromosomal complement of the entire embryo (Taylor et al. 2014). This feature of biology is likely to reduce the accuracy of PGS, as by chance cell(s) removed for biopsy may have more or less aneuploidy than is present in the embryo as a whole.

3.3. It has been estimated that of the levels of mosaicism in IVF cleavage stage embryos ranges from 10-30% (Munné et al. 1994; Delhanty et al. 1997), and this is thought to increase with maternal age (Munné et al. 2002). Mosaicism can also be detected in some blastocyst stage embryos, albeit at lower levels (Northrop et al. 2010; Fragouli, et al. 2011). It is still unclear what impact this has on the efficacy of PGS.

New technologies

3.4. The technology for carrying out PGS has progressed at a rapid rate over the past few years. It is now possible to carry out embryo biopsy at the blastocyst[6] stage

[3] SCAAC last gave dedicated consideration to the safety and efficacy of PGS in 2009 and has continued to monitor research through its horizon scanning function on an annual basis. Following on from the 2009 meeting, the guidance and licence conditions outlined in the Code of Practice were amended (See Annex A). SCAAC also gave consideration to the latest PGS clinical trials and advances in next-generation sequencing (NGS) technologies for use in PGS at its last meeting in February 2015 (See Annex B), and agreed that the patient information on the HFEA website should be updated. Subsequent to this meeting, an embryo testing SCAAC focus meeting was held in April 2015, the purpose of which was to seek opinions on embryo testing from industry experts (see Annex C for a summary of this meeting). Work is now underway to redraft the PGS patient information in light of these recent discussions, and those arising from this meeting.

[4] An embryo is referred to as cleavage stage on day 2 and 3 of development, during which time the cells of the embryo divide without increasing in size. The cells of a cleavage stage embryo are termed blastomeres. At the end of cleavage, the embryo comprises of 16 blastomeres.

[5] To a greater or lesser extent, this failure of PGS to improve IVF outcomes was attributed to the technique of FISH itself, which is subject to considerable interpretation errors and only enables analysis of limited number of chromosomes (Gleicher & Barad 2012), as well as different performance levels at different laboratories (Cohen et al. 2007).

[6] An embryo is referred to as blastocyst stage from day 5 of development onwards, until the process of implantation takes place. During this time cells differentiate into an outer layer of cells called the trophectoderm, which develops into the placenta, and an inner cell mass, which develop into the fetus proper.

embryo (trophectoderm biopsy or TE biopsy), or on metaphase II oocytes by removing the 1st and 2nd polar bodies[7] (polar body biopsy), in addition to cleavage stage[3] biopsy. (For a discussion of the merits of different biopsy stages see Annex 3, section 1).

3.5. However, even greater advances have been made in the development of techniques for genetic testing. Four methods are currently in use: comparative genomic hybridisation (aCGH), single nucleotide polymorphism array (SNP-array), quantitative polymerase chain reaction (qPCR), and next-generation sequencing (NGS). All four are far more accurate than FISH, with reported accuracy values at 95-98%, ~99%, ~98% and ~99%, respectively (Fragouli et al. 2010; Gutiérrez-Mateo et al. 2011; Treff et al. 2012; Wells et al. 2014; Treff et al. 2010).

3.6. aCGH, SNP-array and NGS can also interrogate chromosomes at high resolutions of ~10Mb (Munné 2012; Wells et al. 2014; Treff et al. 2010), meaning that duplications and deletions of small parts of chromosomes (known as segmental aneuploidies) and chromosome translocations[8] can be identified in addition to gross chromosomal abnormalities. All three methods are also capable of discriminating mosaic versus completely aneuploidy embryos.

3.7. In addition to aneuploidy screening SNP-array is also capable carrying out haplotyping,[9] a method that can test for specific genetic diseases (Harper & Harton 2010). NGS too has the potential to deliver accurate mutation detection as well as aneuploidy screening (Wells et al. 2014). These two techniques effectively enable PGD to be carried out by a methodology that has been designed primarily for PGS.

3.8. qPCR is quite different from these three techniques. It is not a high-resolution technology and cannot generate any information on segmental aneuploidies or single gene defects. Instead it has the advantage of being far quicker to perform, at approximately 4 hours compared to greater than 12 hours, and as such is compatible with fresh embryo transfer (Treff et al. 2012). (For further details about current PGS technologies see Annex 3, Sections 2-5)

Clinical trials

4. A growing number of clinical trials have shown that PGS using new technologies have clinical value (Schoolcraft et al. 2012; Yang et al. 2012; Scott, et al. 2013; Forman et al. 2013; Scott et al. 2012; Rubio et al. 2014)[10]. Indeed, in a recent systematic review designed to evaluate the effect of blastocyst biopsy and comprehensive chromosome screening (CCS) technologies on good prognosis patients, the authors found that PGS was associated with higher clinical implantation rates, and higher ongoing pregnancy rates. Although a number of

[7] The polar bodies are the two small cells that are produced and extruded from the oocyte during the first and second meiotic divisions.

[8] Chromosome translocations occur when pieces of chromosomes break off and reattached to other chromosomes.

[9] Haplotyping is a technique which can determine which parental chromatid has been inherited at a particular locus (chromosomal location).

[10] Four of these were presented to the Committee at the meeting in February 2015 (See Annex B for details) (Yang et al. 2012; Forman et al. 2013; Schoolcraft et al. 2012; Scott, et al. 2013), with two further studies by Scott et al. (2012)[A] and Rubio et al. (2014)[B] demonstrating the utility of PGS.

reviews recently presented to the Committee (See Annex B) have criticised these trials for shortcomings in their experimental design (Mastenbroek & Repping 2014; Gleicher & Barad 2012; Lee et al. 2015), it is notable that there is a growing body of clinical data showing a trend which points to PGS improving IVF outcomes.

5. Options for revising our guidance

5.1. The use of PGS is controversial. This is perhaps due in part to the fact that PGS by FISH was introduced into clinical practice before its efficacy had been demonstrated. With this in mind it is understandable that a number of scientists and clinicians working within the field of assisted reproduction have serious reservations about the use of PGS via newer techniques before they have been thoroughly assessed by robust randomised controlled trials (RCTs). However, the current data, while not conclusive, does broadly support the accuracy and efficacy of the newer PGS techniques to improve IVF outcomes.

5.2. The Act allows embryo testing for the purposes of determining whether an embryo has a genetic abnormality that might affect its capacity to result in a live birth. Given that most human embryos have abnormalities,[11] it could be argued that the legislation does not act as a barrier to any patient accessing PGS.

5.3. The Code of Practice must reflect both the current evidence and law while ensuring that patients receive proper information (particularly about the potential risks and benefits of proposed treatment), allowing them to make informed choice, and clinics adhere to best practice, to promote high quality care for everyone affected by assisted reproduction.

Which patient group?

5.4. Traditionally the clinical indications for PGS have been for patients of advanced maternal age, those who have had recurrent miscarriage or recurrent implantation failure, or those with severe male factor infertility caused by high levels of aneuploidy in sperm. This is because these are most likely to produce aneuploid embryos.

[A] Scott et al. (2012) have also published data in support of CCS in a recent prospective, blinded, non-selection study. The authors cultured and selected embryos for transfer without carrying out CCS analysis. Embryos were biopsied before transfer, including 113 blastomeres at the cleavage stage and 142 trophectoderm biopsies at the blastocyst stage, and CCS carried out to determine whether it was predictive of clinical outcome. Their results showed that CCS was highly predictive, with 96% of aneuploid predicted embryos failing to sustain implantation and 41% sustained implantation from embryos predicted to be euploid (Scott et al. 2012).

[B] Rubio et al. (2014) sought to assess the value of CCS by aCGH in severe male factor infertility patients, by conducting a prospective RCT. Patients were randomly allocated into two groups: conventional blastocyst transfer or CCS cycle. Data published in this preliminary study reported on results from 35 completed cycles with 33 transfers and 15 ongoing pregnancies (45.4 ongoing pregnancy rate per transfer and 42.8 per cycle) in the blastocyst transfer group and 33 cycles with 31 transfers and 22 ongoing pregnancies (71.0 ongoing pregnancy rate per transfer and 66.7 per cycle) in the CCS group. The authors commented that “embryo selection based in 24-chromosome aneuploidy screening could be considered as a valuable clinical tool to assess embryo viability in severe male factor patients” since the technique significantly improved clinical outcomes (Rubio et al. 2014).

[11] Chromosomal abnormalities are present embryos from assisted reproductive technology at levels ranging from 60% abnormal embryos in women younger than 35 years to 80% in women 41 years and older (Munné et al. 2007)

5.5. Many of the recent clinical trials that have investigated the efficacy of PGS have focussed on good prognosis patients, a fact that has been levelled as a criticism of the trials' design. However, at both the recent ESHRE conference, 'An update on preimplantation genetic screening (PGS)', held in Rome and at the embryo testing SCAAC meeting in April, a rebuttal to this has been given: IVF success rates are not high[12] and anything that can improve them is a positive thing. Data from these trials has shown that PGS improves IVF outcomes in the good prognosis patients group.

5.6. There has been a shift in our understanding of the role of PGS. It should no longer be considered as a means for increasing the pregnancy rate per cycle, particularly in women of advanced maternal age, who are likely to have no normal embryos to transfer after PGS. The current view is that PGS should not be considered as a means of deselecting aneuploid embryos, but rather for ranking embryos in order of quality and using this information to prioritise embryo transfer[13]. The end goal for fertility treatment of achieving a pregnancy is not the only marker of success. Achieving a pregnancy in the shortest possible time, with the fewest number of miscarriages is also highly desirable and PGS may help in this regard.

5.7. Currently the guidance in the Code of Practice states that centres must "validate the use of PGS for each category of patient to which they offer it." This was written at a time when PGS was conducted by FISH and is out of keeping with current clinical practice and science.

5.8. Perhaps a more pertinent requirement already exists in the form of licence condition T49, which states, "The clinician responsible for the patient must document the justification for the use of their gametes or embryos created with their gametes in treatment, based on the patient's medical history and therapeutic indications."

Embryo biopsy competency

5.9. An issue that was raised at the embryo testing SCAAC meeting was that samples received by embryo testing laboratories vary considerably in quality[14]. This is primarily due to variation in biopsy competency, which goes hand-in-hand with a centre's ability to freeze embryos. To address this, it was suggested that embryo biopsy and freezing practitioners should demonstrate their competency on inspections; ongoing key performance indicators should be monitored that include misdiagnosis rate and inconclusive results rate; and that patients should have access to a counsellor pre- and post-testing (See Annex C).

5.10. However, measures are already in place in the Code of Practice Staff guidance note, which states that the "competence of each person performing micromanipulation procedures should be evaluated at intervals specified in the quality management system" and "retraining should be given when required."

5.11. Furthermore, due to the small number of PGS cycles per year and limited data

[12] In the UK, in 2013, the pregnancy rate per embryo transferred was 35.5% (HFEA Fertility treatment in 2013: Trends and figures report)

[13] This view was presented at both ESHRE's PGS conference and at the embryo testing SCAAC focus meeting.

[14] It was noted that around 3-5% of PGS cycles produce inconclusive test results.

collected on the HFEA register, the organisation is not best placed to set the bar for acceptable PGS test failure rates. In order to ensure that clinics are meeting KPIs they should engage in a dialogue with their third party embryo testing laboratories, and perhaps this could be included in the Third party agreement Guidance Note.

Additional genetic data from PGS tests

5.12. As discussed earlier, many of the newer PGS technologies are able to generate very detailed genetic information, including the presence of segmental aneuploidies as small as 14Mb in size. However, for findings of this nature it will often be unclear a) whether the genetic abnormality would affect an embryo's capacity to result in a live birth, and b) what the clinical relevance would be (ie whether or not it would cause a genetic disease). As such, this raises both legal and ethical concerns.

5.13. The Executive has sought legal advice on this matter and has been advised that as the additional data created by some newer PGS techniques is a by-product of the primary test, it cannot be said that they constitute a breach of the requirements set out in the Act. However, concerns still remain. Why would clinics choose to carry out tests that could produce data that are currently impossible to interpret? Is it proper that clinical decisions are made on data of this sort, and when they are, can clinics be considered to be subverting the legislation?

5.14. Leaving aside the ethics of carrying out genetic testing which may generate uninterpretable data, practically what should clinics do with such results? The current practice of embryo testing laboratories is to flag all anomalies to the requesting centre and to highlight when there are abnormalities present for which clinical significance cannot be determined. It is then the decision of the treating clinician, genetic counsellor or other qualified professional to determine whether the embryo should be transferred in accordance with the law/with the aid of a local ethics board[15]. In the view of the Executive, it is only acceptable to carry out genetic tests that can generate data of unknown clinical significance if patients receive patient information and are offered counselling prior to PGS, which sufficiently explains what the potential finding may be and what they mean.

Patient information and counselling

5.15. This issue of counselling was discussed an Authority paper presented at the meeting in May 2015, entitled "Embryo testing: Testing for more than one condition or abnormality at a time". The paper says that, due to the complexity of embryo testing and the factors involved, counselling should be offered to patients both before and after testing. This is so patients fully understand the information it might reveal – both positive and negative – and are given sufficient time to consider the implications and think about what information they want to receive. Furthermore, during discussions feeding into the paper, stakeholders suggested that any unanticipated genetic information that could be obtained – be they incidental findings or additional testing – should be explained during counselling.

5.16. The Code of Practice currently states that centres should ensure that before people seeking treatment give consent to PGS for aneuploidy, they are given specific information (see Annex A). However, in the light of the new PGS

[15] This information was obtained at the embryo testing SCAAC focus meeting.

technologies that are in use and the additional data they are capable of generating, the Executive feels it is advisable to update this list. The Code also states that centres should ensure that people seeking treatment involving genetic testing have access to clinical geneticists and genetic counsellors. However, this only requires clinics to provide access and therefore does not ensure that patients do receive counselling, nor does it advise clinics to ensure access to counselling before and after treatment.

6. Recommendations

6.1. Members are asked to:

- Consider whether any points in the Code of Practice PGS guidance note (Annex A) should be amended and, if so, provide comments to the Executive regarding possible amendments.
- In particular consider:
  - whether the Committee agrees with the Executive's recommendation to remove the requirement for clinics to validate the use of PGS for each category of patient to which they offer it from the Code of Practice, and to replace this by highlighting licence condition T49;
  - whether the current Code is comprehensive enough in detailing the information patients should be given prior to treatment, in particular whether information should be provided on uninterpretable results and on the stage and risk of embryo biopsy;
  - whether the Code should specifically require clinics to provide genetic counselling prior to and after treatment, or whether providing patient information is sufficient;
  - whether we should ask to centres to go beyond providing information on the lack of efficacy of FISH, and ask them to specifically justify their use of this technique over one of the newer ones;
  - whether clinics should be encouraged to assess key performances, including misdiagnosis rate and inconclusive results rate, in collaboration with their embryo testing laboratory (either through an update to the PGS or Third party agreement guidance notes, or through means of a clinic focus article).
- Consider whether the HFEA has any role to play in facilitating or encouraging PGS RCTs to take place and trials in other relevant areas.

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ANNEX A: Current HFEA Code of Practice guidance note on PGS

Mandatory requirements

Human Fertilisation and Embryology (HFE) Act 1990 (as amended)

Schedule 2

Licences for treatment

1 (1) A licence under this paragraph may authorise any of the following in the course of providing treatment services –
...(b) procuring, keeping, testing, processing or distributing embryos…

Embryo testing

1ZA (1) A licence under paragraph 1 cannot authorise the testing of an embryo, except for one or more of the following purposes -

(a) establishing whether the embryo has a gene, chromosome or mitochondrion abnormality that may affect its capacity to result in a live birth,

(b) in a case where there is a particular risk that the embryo may have any gene, chromosome or mitochondrion abnormality, establishing whether it has that abnormality or any other gene, chromosome or mitochondrion abnormality,

Licence conditions

T88 With respect to any embryo testing programme involving biopsy the centre must ensure that:

a. no embryo is transferred to a woman where that embryo or any material removed from it or from the gametes that produced it, has been subject to a test that supplies genetic information about the embryo, unless the test has been expressly authorised by the Authority, and

b. any information derived from tests on an embryo, or any material removed from it or from the gametes that produced it, is not used to select embryos of a particular sex for social reasons.

T89 With respect to any embryo testing programme the centre must ensure that embryo testing is only being carried out for those genetic conditions that are expressly authorised by the Authority.

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HFEA guidance

The use of PGS

Interpretation of mandatory requirements **9A**

An embryo may be tested to establish whether it has a particular chromosomal abnormality only if:

a) that abnormality may affect its capacity to result in a live birth, or

b) there is a particular risk that it has that abnormality, and where the Authority is satisfied that there is a significant risk that a person with that abnormality will have or develop a serious medical condition.

9.1 The centre should ensure that before people seeking treatment give consent to PGS for aneuploidy, they are given information explaining:

(a) the risks associated with the procedure

(b) the unproven nature of the procedure, in particular that:

(i) more robust clinical and laboratory trials are needed to assess whether or not PGS can significantly increase live birth rates

(ii) the method of fluorescent in situ hybridisation (FISH) on embryos, using a limited number of chromosomes, is not effective at increasing live birth rates

(c) that embryos biopsied may not be available for cryopreservation and for use in subsequent treatment cycles

(d) the misdiagnosis rates associated with PGS for aneuploidy, including the fact that false results can be positive or negative

(e) that the more chromosome tests are carried out, the higher the possibility of the test not working and the lower the chance of finding suitable embryos for transfer

(f) that there is no guarantee against a miscarriage occurring, despite PGS for aneuploidy being performed, and

(g) the financial and emotional costs where treatment fails and there is no live birth following PGS for aneuploidy.

9.2 Embryos from which biopsies have been taken, or resulting from gametes from which biopsies have been taken, should not be transferred with any other (non-biopsied) embryos in the same treatment cycle.

9.3 Centres should ensure that they keep up to date with relevant literature and professional guidance in order to validate the use of PGS for each category of patient to which they offer it. Validation should also be based on data from previously published studies and retrospective evaluation of the clinic's own data.

Annex B – February 2015 SCAAC PGS paper

1. Introduction

1.1. Preimplantation genetic screening (PGS) describes the process of checking the chromosomal composition of embryos. The rationale for this is based on the fact that human reproduction is highly inefficient, producing high percentages of aneuploid embryos even at young ages (Kuliev et al, 2005; Fragouli et al, 2011). These aneuploid embryos are frequently non-viable, leading to implantation failure, miscarriages, or congenital abnormalities (Hassold and Hunt, 2001). PGS is meant to identify aneuploid embryos prior to embryo transfer, allowing the choice of embryos free from aneuploidies and thereby increasing the likelihood of pregnancy and reducing miscarriage rates.

1.2. Based on this rationale, PGS was widely adopted in IVF treatment. However, several years after its initial introduction, a number of randomised controlled trials (RCTs) showed that far from leading to improved IVF outcomes, PGS significantly decreased chance of ongoing pregnancy in comparison with IVF without PGS (Mastenbroek et al, 2011). (Although a recent prospective RCT demonstrated an increase in the live birth rate in older women after PGS using FISH (Rubio et al, 2013)).

1.3. The common methodology employed for PGS in the first 15 years of its existence was blastomere aspiration of embryos on the third day after fertilization, followed by fluorescence in situ hybridization (FISH) analysis of the aspirated blastomere(s) (Mastenbroek and Repping, 2014). To a greater or lesser extent, the failure of PGS to improve IVF outcomes was attributed to, the technique of FISH itself, which is subject to considerable interpretation errors and only enables analysis of limited number of chromosomes (Gleicher and Barad, 2012), as well as different performance levels at different laboratories (Cohen et al, 2007),

1.4. In addition to this, there is some concern that other factors may diminish the accuracy with which PGS can operate. Mosaicism is the phenomenon whereby a single cell, or small group of cells, may not represent the chromosomal complement of the entire embryo (Taylor et al, 2014). Therefore by chance a biopsy taken for the purpose of PGS may give a result of more or less aneuploidy than is present in the embryo as a whole. It is also believed that embryos may have a repair and/or exclusion mechanism to deal with aneuploid blastomeres (Bazrgar et al, 2013).

1.5. This has led to the development of techniques such as comparative genomic hybridization arrays (aCGH) and single nucleotide polymorphisms (SNP) arrays, which allow the analysis of all 24 chromosomes with a greater accuracy than FISH (Wells et al, 2008). These are now the methods of choice for most clinics undertaking PGS.

2. Background

2.1. The HFEA publishes information for patients about PGS on its website. SCAAC last gave dedicated consideration to the safety and efficacy of PGS in 2009 and has continued to monitor research through its horizon scanning function on an annual basis.

2.2. Following on from SCAAC's meeting in 2009 HFEA guidance and licence conditions outlined in the Code of Practice was amended. (See Annex B for the current Code of Practice guidance note on PGS).

2.3. The new guidance states that, “Centres should ensure that they keep up to date with relevant literature and professional guidance in order to validate the use of PGS for each category of patient to whom they offer it. Validation should also be based on data from previously published studies and retrospective evaluation of the clinic’s own data,” and that, centres should provide information to patients considering PGS, about the risks associated with the procedure, and the unproven nature of the procedure.

2.4. There are conflicting views about the value of PGS in IVF treatment. While it is now widely acknowledged that PGS, using FISH on a limited number of chromosomes, confers no advantage and may even be detrimental to overall success in achieving a live birth, there is no consensus on the impact of the newer techniques of aCGH and SNP arrays on IVF outcomes (Gleicher and Barad, 2012; (Lee et al, 2015; (Fragouli and Wells, 2012; Wells et al, 2014).

2.5. A number of clinical trials, including RCTs, have been recently carried out to assess the effectiveness of these newer techniques. This paper presents the conclusions of these trials and subsequent critical reviews of their data. Also presented are developments from the recent literature regarding the application of next-generation sequencing in PGS. Further to this the Executive has recommended consideration to updating the patient information on the website and the guidance in the Code of Practice where appropriate.

3. Research developments

3.1. There is an ongoing debate as to the clinical utility of PGS. Its proponents (Wells et al, 2014; Fiorentino et al, 2014a) cite a growing number of RCTs which have produced clinical data supporting the hypothesis that screening of embryos for aneuploidy can improve IVF outcomes. While its detractors assert that significant shortcomings of the same RCTs significantly downgrade the level of evidence they provide, and point to concerns regarding safety and efficacy of PGS in general (Mastenbroek and Repping, 2014; Gleicher and Barad, 2012).

3.2. <u>Clinical trials supporting the effectiveness of newer techniques for PGS</u>

3.2.1. In a recent study, Yang et al (2012) compared the effect of PGS via aCGH versus embryo selection on the basis of morphology on IVF outcomes. First-time IVF patients with a good prognosis (age <35, no prior miscarriage) and normal karyotype seeking elective single embryo transfer (SET) were prospectively randomized into two groups: In Group A (n=55), embryos were selected on the basis of morphology and comprehensive chromosomal screening via aCGH (from day 5 trophectoderm biopsy) while Group B embryos were assessed by morphology only. In Group A, a total of 425 blastocysts were biopsied and analysed via aCGH and aneuploidy was detected in 44.9% of blastocysts. A total of 389 blastocysts were microscopically examined from Group B. The authors found that embryos randomized to the aCGH group implanted with greater efficiency, resulted in ongoing pregnancy more often (69.1% in Group A versus 41.7% in Group B), and yielded a lower miscarriage rate than those selected without aCGH.

3.2.2. A recent study (Forman et al, 2013) carried out a randomized trial aimed at determining whether performing PGS (via rapid, real-time PCR on trophectoderm biopsies of day 5 embryos) and transferring a single euploid blastocyst can result in an ongoing pregnancy rate that is equivalent to transferring two untested blastocysts while reducing the risk of multiple gestation. A total of 205 infertile couples (with a female partner less than 43 years old and with a serum anti-Müllerian hormone level ≥1.2 ng/mL and day 3 FSH <12 IU/L and with at least two blastocysts on day 6) were

randomised into two groups. From the study group (n=89) all viable blastocysts were biopsied for real-time, PCR–based PGS and single euploid blastocysts were transferred. Patients from the control group (n=86) had their two best-quality, untested blastocysts transferred. The ongoing pregnancy rate per randomized patient after the first ET was similar between the groups (60-65%), with singleton pregnancies in 100% of the study group, compared to 53.4% multiple pregnancies in the control group.

3.2.3. A recent study (Schoolcraft et al, 2012) carried out a RCT to evaluate the clinical efficacy of PGS (via SNP microarray on with trophectoderm biopsies, with all blastocysts subsequently vitrified) in IVF patients of advanced maternal age. Infertile patients of maternal age >35 years were randomized at egg retrieval into two groups. In Group A (n=30) fresh blastocyst transfer was carried out on embryos selected by morphology alone. Patients in Group B (n=30) underwent frozen blastocyst transfer with only euploid embryos tested by PGS. Infertile advanced maternal age patients had higher ongoing implantation rates (A=40.9%, B=60.8%, P<0.05) and fewer first trimester pregnancy losses (A=20%, B=0%, P<0.05) following a frozen blastocyst transfer with screened euploid embryos, when compared to routine fresh blastocyst transfer based on embryo morphology alone.

3.2.4. In a recent study by Scott et al (2013) carried out a RCT to determine whether rapid quantitative real-time PCR (qPCR)–based PGS (on day 5 trophectoderm blastocyst biopsies) improves IVF implantation and delivery rates. Infertile couples (n=155), in whom the female partner (or egg donor) was between the ages of 21 and 42 years, were included in the trial. A total of 134 blastocysts were transferred to 72 patients in the study (PGS) group and 163 blastocysts to 83 patients in the control group. PGS resulted in statistically significantly improved IVF outcomes, as evidenced by meaningful increases in sustained implantation (PGS=79.8%, control=63.2%, P=0.002) and delivery rates (PGS=66.4%, control=47.9%, P=0.001).

3.3. <u>Reviews criticising recent PGS trials</u>

3.3.1. A number of recent reviews have questioned the extent to which these and other trials demonstrate the ability of PGS to improve IVF outcomes (Mastenbroek and Repping, 2014; Gleicher and Barad, 2012; Lee et al, 2015). The authors of these reviews point to a litany of shortcomings which include: small sample size, lack of blinding, power calculations, suitable controls and generalisability, suboptimal primary end-points (implantation rate, instead of ongoing pregnancy rate or live birth rate) and a failure to consider subsequent frozen transfer cycle in control groups and the resulting cumulative delivery rates.

3.3.2. Despite this, Lee et al (2014) considered that data from clinical trials “did reveal potential benefits of using PGD-A [PGS] techniques over morphology-based selection of embryos” while Gleicher et al, (2012) were “convinced that PGS in properly selected patients…improves IVF pregnancy and, likely, also reduces miscarriage rates.”

3.3.3. Mastenbroek et al (2014) were far more scathing in their conclusions. They point to the finanical aspects of PGS, which is “commercialy very attractive as it can significantly increase the turnover of a clinic”, and suggest that, “the medical professionals offering PGS either are unaware of the true value of the available data or are driven by other motives.”

3.3.4. A number of other issues more general to PGS are also raised in these reviews:

- The exact prevalence of mosaicism between cells at the blastocyst stage using the new methods for analysis is as yet unknown. Any mosaicism would decrease the accuracy with which an embryo could be selected as euploid or aneuploidy, and could result in healthy embryos being destroyed and unhealthy embryos being transferred.

- It cannot be fully excluded that harm is caused to the embryo during the biopsy procedure. While follow-up studies to assess the impact of PGS have been undertaken, they have involved very small sample sizes. (One recent study found no statistically significant differences in major or minor anomalies between children conceived after IVF/ICSI with or without PGS (Beukers et al, 2013), while another found that the neurodevelopmental outcome of PGS children aged four was similar to that of controls (Schendelaar et al, 2013).

- These techniques favour day 5/6 transfer. However, there is some data to suggest that this results in less favourable live birth rates, compared with a day 2/3 transfer when frozen cycles are included (Blake et al, 2007).

- Aueuploidy increases with increased maternal age, and consequently PGS is sometimes recommended for patients of advanced maternal age. However, advanced age also results in diminished ovarian reserves, with only small egg and embryo numbers of poor quality. PGS could reduce the number of transferable embryos (potentially to zero), reducing the rate of live births per cycle. It might be more appropriate to use PGS in patients with recurrent implantation failure or recurrent miscarriages (or good prognosis patients) but this is questionable.

- There is accumulating evidence that freeze-all cycles can be used without impairing, and maybe even improving, the cumulative pregnancy rate of that IVF cycle. Therefore no selection method would ever lead to improved live birth rates. The purpose of embryo selection should therefore be restricted to determining the order in which the embryos will be transferred, but not to select out embryos (assuming embryo biopsy does no harm).

- It has not been determined whether PGS represents a cost-effective approach to IVF for patients.

- All the authors agreed that there was a need for more, well-designed RCTs to test the efficacy and cost-effectiveness of PGS, and that these are well overdue.

3.4. Next-generation sequencing for use in PGS

3.4.1. Advances in next generation sequencing (NGS) technology have provided new tools for detecting DNA mutations and/or chromosome aberrations for research and diagnosis purposes. Combining this technology with whole genome amplification (WGA), a technique whereby the entire genome is copied many times, has enabled the detection of copy number variations (CNV) in single cells (Navin et al, 2011). This has paved the way for the development of WGA and NGS protocols for use in PGS (See Annex C for details).

3.4.2. In a recent study Fiorentino et al (2014b) validated a NGS-based protocol for 24-chrmosome screening of embryos. In their study, karyotypically defined single-cells derived from cultured amniotic fluids or products of conception, or single blastomeres biopsied from embryos produced in 68 clinical PGS cycles, were analysed by NGS. The results were compared either to conventional karyotyping of single cells or aCGH

diagnoses of single blastomeres. The results demonstrated 100% consistency with conventional karyotyping and 99.8% chromosome copy number assignment consistency with aCGH. The authors noted that their protocol demonstrated the ability to accurately detect "segmental changes (as small as 14 megabases in size), indicating that diagnosis of partial aneuploidies is well within the ability of this technology."

3.4.3. Another recent study (Wells et al, 2014) aimed to develop a rapid, scalable, cost-effective method for the genetic analysis of single cells (blastomeres) or trophectoderm biopsies derived from human preimplantation embryos, using low-pass NGS (which provides less than 0.1% genomic coverage). Their data confirmed that highly accurate detection of aneuploidy could be achieved in single cells from embryos using their methodology. The authors assert that this technique can be carried out at a speed (potentially within 8 hours), throughput (at least 32 samples in one run) and cost (more than a third less than with the most widely used microarray-based approaches), appropriate for use in conjunction with standard embryo biopsy and transfer protocols. In addition this authors also demonstrate that NGS has the potential to carry out diagnosis of single gene mutations simultaneous to chromosomal analysis, and that it can be used to acquire quantitative data on mtDNA copy number and mutation load.

3.4.4. Following on from their previous work, Fiorentino et al (2014b) carried out a double blinded clinical study to determine whether NGS techniques can be used reliably for comprehensive aneuploidy screening of human embryos from patients undergoing IVF treatments, with the purpose of identifying and selecting chromosomally normal embryos for transfer. Fifty-five patients undergoing PGS were enrolled in the study, 192 blastocysts were obtained, and trophectoderm biopsies were performed on day 5 or day 6/7 for slower growing embryos. The consistency of NGS-based aneuploidy detection was assessed by matching the results obtained with aCGH-based diagnoses. Their results demonstrated that NGS was able to determine aneuploidy with 99.98% specificity and with 100% sensitivity. Following transfer of 50 euploid embryos in 47 women, 34 women became pregnant (based on positive hCG levels, resulting in the birth of 31 healthy babies (with 3 biochemical pregnancies and 1 miscarriage) (Fiorentino et al, 2014a).

3.5. <u>The future</u>

3.5.1. Data from these and other studies suggests that it is technically feasible to extend NGS to allow whole genome sequencing of embryos. This coupled with the growing ease with which preconception carrier screening can be conducted (including commercially by companies such as 23andMe) may be leading us towards a future where IVF and PGS/D will be used not for the treatment of infertility or avoidance of disease, but to allow people to select to have the healthiest possible child (Hens et al, 2013). Although this would not be permitted under current HFEA regulation, it is likely technically possible already, and it seems plausible that some people might have an appetite for such innovations. It is important to be mindful of this and to make sure that the ethical and regulatory framework keeps pace with the science.

4. Conclusions and recommendations

4.1. Recent research and literature demonstrates that considerable progress has been made in the techniques available for use in PGS, but there is still considerable controversy surrounding its practice in IVF, and a continuing need for a greater number of well-designed RCTs.

4.2. The aim of the patient information is to provide a fair, balanced and accurate picture on current progress regarding PGS to assist patients who are seeking to make decisions about fertility treatment.

4.3. The Executive recommends that the current website information for patients is updated and consideration is given to whether guidance in the Code of Practice, particularly regarding information clinics should provide to patients about PGS, should be amended.

4.4. Members are asked to:

- Review the recent literature in this area and consider the safety and efficacy issues that may arise from such techniques.
- Review the HFEA website text (at Annex A) and provide comments to the Executive, relating to possible updates and changes including any studies they feel should be added to the website text as highlighted articles.
- Consider whether any points in the Code of Practice guidance regarding information to be provided to patients prior to PGS (9.1 at Annex B) should be amended and, if so, provide comments to the Executive regarding possible amendments.

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Annex C – Summary of SCAAC focus meeting: New technologies in embryo testing

Background information

Technological advances over recent years have made it possible to simultaneously screen embryos for both genetic and chromosomal abnormalities, without the need to develop disease-specific tests. In turn, it presents the ability to generate extra genetic information and to simultaneously conduct preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) on a sample retrieved through one embryo biopsy procedure.

The Human Fertilisation and Embryology Act 1990 (as amended) ('the Act') states that embryo testing can only be used to establish whether the embryo has a gene, chromosome or mitochondrial abnormality that may affect its ability to result in a live birth, in the case of PGS; or in a case where there is particular risk that the embryo may have any gene, chromosome or mitochondrion abnormality, establishing whether it has that abnormality or any other gene, chromosome or mitochondrion abnormality, in the case of PGD.

Taking the Act into consideration, the Authority is currently seeking advice on the remit for testing embryos for genetic conditions and chromosomal abnormalities using new methods of embryo testing. Fundamentally, the question it considers is:

> If one of more of the established purposes for embryo testing, as defined by the Act, is met, is it acceptable for the embryo to be tested for other genetic conditions and chromosomal abnormalities, for which no particular risk (for that particular embryo) has been identified at the same time?

In addition, the Authority's Scientific and Clinical Advances Advisory Committee (SCAAC) has highlighted that the majority of recent research investigating the efficacy of newer PGS techniques has focussed on good prognosis patients, rather than patients with typical indications for PGS, such as advanced maternal age, recurrent IVF failure or miscarriages and severe male factor infertility. The current website information for patients regarding PGS was also flagged as being outdated; it was recommended that changes should be made to reflect that fluorescent in situ hybridisation (FISH) is no longer the technique of choice for PGS, to provide information on the accuracy and efficacy of newer PGS techniques, and to include recent citations and recent data on success rates with PGS.

Having identified these areas of concern, SCAAC decided to explore the area further. It was felt that a dedicated meeting involving expert stakeholders was required to:

- Discuss advances in embryo testing and the ways in which they might impact the guidance and information that the HFEA provides to the sector and patients, prospectively.
- Discuss PGS validation and general patient information on this area.

Summary of presentations and discussions

To better understand the use of new technologies in embryo testing for PGD and PGS, SCAAC invited three experts in the area to present their thoughts and experience by addressing:

- The technologies available for embryo testing (Tony Gordon, Genesis Genetics);
- The current use of embryo testing technologies in clinical practice (Alan Thornhill, Illumina and HFEA member); and
- The latest and future research (Dagan Wells, Reprogenetics).

A summary of the presentations given and discussions had on the day is outlined below.

Technologies available for embryo testing

It was established that embryo testing technologies are used to determine single gene disorders and chromosomal abnormalities as follows:

- To determine monogenic disease via PGD
  - Multiplex polymerase chain reaction (PCR) short tandem repeat (STR)-based haplotyping (in use for around ~25 years)
  - Whole genome amplification (WGA) and STR-based haplotyping (around ~8 years)
  - Single nucleotide polymorphism (SNP)-based haplotyping (involves WGA and SNP arrays, such as karyomapping; around ~2 years)
- To determine a chromosomal translocation carrier via PGD
  - Fluorescent *in-situ* hybridisation (FISH) (around ~20 years; still used in clinical practice for specific translocations)
  - Array comparative genomic hybridisation (aCGH) (around ~7 years)
  - Next generation sequencing (NGS) (around ~1 year)
- To determine chromosome aneuploidy via PGS
  - aCGH (around ~7 years)
  - NGS (around ~1 year)
- To determine monogenic disease, translocation carriers and aneuploidy via PGD and PGS
  - WGA, STR-based haplotyping and aCGH (around ~4 years)
  - SNP-based haplotyping (involves WGA and SNP arrays, such as karyomapping; around ~2 years)

It was highlighted that NGS mimics aCGH and that it is often mistaken as being a form of whole genome sequencing – rather, it sequences around 10% of the genome and phasing for haplotyping is not possible. Additionally, although FISH is not the newest of technologies, it is still used in some diagnostic laboratories/centres to determine particular chromosomal translocation carriers.

The accuracy of new technologies for PGS

It was highlighted that research over recent years has shown that PGS is beneficial to good prognosis patients and that in this patient group it may help to select an optimal embryo for transfer. It was also noted, however, that PGS may not be ideal for patients with a reduced number of embryos to transfer. In short, PGS could be used to improve the overall success of IVF/ICSI and to improve clinical decision making.

It was mentioned that there are degrees of aneuploidy within the general population. The majority of segmental aneuploidies – for which the effect on an embryo's capacity to result in a live birth is largely unknown –detected in embryos through embryo testing are mosaic and that the ability to implant is reduced (although not zero).

To conclude, it was raised that PGS is carried out in around 20% of all cycles in the US due to new technologies being more accurate and reliable. In turn it was stressed that any current assessment of the efficacy of PGS should be based on new technologies (rather than the dated, as there are significant differences.

Current use of embryo testing technologies in clinical practice

The benefits and challenges of working with embryo testing technologies

With regards to benefits of new technologies in embryo testing, it was explained that on a technical level they can be more accessible, accurate, reproducible and reliable, and can reduce waiting times for patients compared to older technologies. While new technologies for PGD can reduce the likelihood of an affected child being born, for chromosomal translocation carriers specifically, they can reduce the likelihood or an affected child being born, miscarriage rates and the time a patient may have to wait to have a live birth. In terms of PGS, it was noted that new technologies are of greater benefit to good prognosis patients and can also reduce the miscarriage rate, dropout rate, and cost and time to live birth (where multiple cycles result in miscarriages).

However, there are challenges to using these technologies which include:

- Technical difficulties;
- That they require a high level of embryology competency (for embryo biopsies and freezing);
- That there are often logistical and cost issues;
- That patients may not have access to a counsellor before and after tests are performed; and
- That the interpretation of incidental findings can be challenging.

To address these, it was suggested that embryo biopsy and freezing practitioners should demonstrate their competency on inspections; ongoing key performance indicators should be monitored that include misdiagnosis rate and inconclusive results rate; and that patients should have access to a counsellor pre- and post-testing.

Stage of embryo biopsy

Although there is a growing preference towards day five biopsy in the UK, it was noted that the stage of embryo biopsy remains equal between day three and day five (ie, 50:50). In the US, however, practice is much more in favour of day five biopsy, with around 80% of biopsies being performed at this stage.

Although day five biopsies are technically challenging, the trend towards biopsies at this stage was attributed to the notion that:

- Results are more accurate (chromosomal mosaicism is less common and can be identified in the sample)
- A smaller mass of the embryo is taken overall compared to day three biopsy even though multiple cells are taken; and
- It is the least expensive option diagnostically (although there is an increased cost to the patient regarding freezing and storage costs).

It was highlighted that research is underway outside of the UK regarding morula stage biopsy; however, there was concern that part of the inner cell mass could be biopsied and that the embryo could fall apart. It was also flagged that embryology competency at the biopsy stage is crucial, and go hand-in-hand with a centres ability to freeze embryos. As such, diagnostic laboratories create reports for each centre that they work with to show how it compares with others they work with, and it was suggested that embryo biopsy practitioners should demonstrate their competency on HFEA inspections.

Issues around data interpretation

To interpret data accurately, tests and software should be appropriately validated, embryo testing technologies should be used in accredited laboratories, and results should be reviewed and signed-off by appropriately qualified staff. It was suggested that a key performance indicator for PGS could be introduced that flags misdiagnosis and inconclusive results rates.

Incidental findings have been a concern regarding new technologies in embryo testing. It was clarified that in order to carry out PGD for single gene disorders using karyomapping or SNP arrays, a reference sample is currently required in order to follow haplotypes and determine whether an embryo will carry a specific disorder. However, given the technologies, they may reveal chromosome aneuploidies. In addition, if completing PGS to determine chromosome aneuploidy using the same technologies, tests may find that an embryo is a chromosomal translocation carrier by looking at recurrent break points, and vice versa.

As the clinical relevance of incidental findings cannot always be determined, current practice – from a diagnostic laboratories perspective – is to flag any anomalies to the requesting centre and highlight that its clinical significance cannot be determined. It is then the decision of the treating clinician, genetic counsellor or other qualified professional to determine whether the embryo should be transferred in accordance with the law/with the aid of a local ethics board. It was noted that around 3-5% of PGS cycles produce inconclusive test results.

The latest research and looking ahead

Summary of the latest research

With regards to PGS, it was highlighted that recent studies[16] have shown that:

- PGS helps to reduce the effect of maternal age on miscarriage rates
- PGS helps to reduce the negative effect of maternal age on implantation

[16] Harton et al, 2013; Forman et al, 2013; Yang et al, 2012; Schoolcraft et al, 2012; Scott et al, 2013; Rubio et al 2014.

- Randomised controlled trials demonstrate that PGS has clinical value
- PGS can achieve high efficiency eSET and a faster time to pregnancy
- PGS can avoid unnecessary embryo transfers and cryopreservation of non-viable embryos
- PGS can reduce miscarriage rate and the risk of Down's syndrome

Given these results, it was noted that in the US there is a growing acceptance that PGS should be widely adapted.

With regards to the work being carried out by the Authority, it was flagged that even if only two conditions were tested for, the number of viable embryos would be too few so to screen for any more conditions (eg, on an array) would not be realistic.

The increased awareness of preconception screening was also raised. While it was explained that preconception tests are possible based on the increased amount of DNA available in blood, to conduct such screening in embryos is not currently possible as the amount of DNA is too small and amplification methods are not well adapted. It was flagged however, that embryo testing is a rapidly evolving field and should there be significant advances in genome amplification, this may be possible in around 5-7 years although it would only be useful for research purposes as not all data could be interpreted.

The Authority's work

As it stands, however, and in the context of the question posed by the Authority above, PGD can only be carried out for the focussed finding of single gene disorders for which a reference sample can be provided. In short, an embryo could not be screened for a list of conditions if there was no affected family member. If a couple were to go through WGA and were found to be carriers for a recessive condition or late onset condition, they would undergo genetic counselling, and as long as a condition is on the list of PGD authorised conditions, an embryo could be tested for the condition without there being an affected child or relative (as there would still be a sample to reference). As a result, this parental screening may lead to an increase in the number of PGD cycles, although only in cases where an individual/couple are carriers or where they/a relative suffer from a condition.

Looking ahead, with the notion of future proofing any guidance the Authority may provide, it was suggested that the area should be reviewed in five years given the advances that may be made – including that arrays could be developed for specific demographics/ethnic backgrounds. In the interim, the Authority should inform patients that this is a rapidly evolving field, that there is data available although the results are inconclusive, and which groups would benefit from testing. The Authority should also encourage patients to ask questions when offered treatment so that it is given to those most suitable. These recommendations regarding information provisions around PGS will be taken to SCAAC for consideration and advice on possible changes to the Code of Practice guidance and patient information.

Annex D – Summary of current PGS techniques

1. Embryo biopsy

1.1. Currently all techniques for carrying out PGS require the removal of cellular material from an embryo, in a process known as embryo biopsy. There are three stages at which embryo biopsy can be carried out. One or two cells can be removed from a cleavage stage[3] embryo (blastomere biopsy) or a small number of cells from the trophectoderm of a blastocyst stage embryo (trophectoderm biopsy (TE biopsy)). Alternatively a biopsy can be carried out on metaphase II oocytes by removing the 1st and 2nd polar bodies.

1.2. Biopsies at these different stages have different advantages and disadvantages. Polar body biopsy is considered to be the least invasive technique, as no material is removed from the embryo. However, it also provides the least complete information, only accounting for errors that occur during meiosis of the egg, but not errors that occur in the sperm or errors that occur after fertilisation (post-zygotically).

1.3. Cleavage stage biopsy is technically relatively straightforward and provides information on maternally and paternally derived errors. However, biopsy at this stage is considered to have the greatest detrimental effect on implantation potential (Scott, Upham, Forman, Zhao, et al. 2013; Magli et al. 1999), in particular when two rather than one cell is removed (De Vos et al. 2009). In addition to this, as previously mentioned, at this stage of embryo development mosaicism is common.

1.4. Finally, trophectoderm (TE) biopsy, similar to cleavage stage, provides information on maternal and paternal errors. It is technically more challenging than cleavage stage biopsy and depending on the methodology used for PGS may require embryo cryopreservation prior to embryo transfer. However, it is less damaging to the embryo (Scott, Upham, Forman, Zhao, et al. 2013), there is less chromosomal mosaicism and it can be identified in the sample. In addition to this, due to the larger amount of genetic material that can be obtained, TE biopsies tend to produce more accurate and reliable results. Perhaps for these reasons it is becoming the standard stage for embryo biopsy in the USA.

1.5. From this data it might seem from this data that TE biopsy will always represent the best choice of technique, however, some patients will not produce embryos that develop to the blastocyst stage. In this case a cleavage stage biopsy is clearly preferable.

2. Array comparative genomic hybridisation (aCGH)

2.1. aCGH is currently the most commonly used technique for carrying out CCS for PGS. Using aCGH it is possible to screen the entire human genome for variations in DNA copy number, also referred to as copy number variations (CNVs). The method works by isolating total genomic DNA, amplifying it using whole genome amplification (WGA), and fluorescently labelling in one colour. This DNA is hybridised to reference DNA, labelled in a different colour that has been bound to a microarray chip. The

relative intensity of the test colour versus the reference colour signals at a given location is proportional to any CNVs (Pinkel & Albertson 2005).

2.2. The methodology is capable of interrogating chromosomes at high resolutions, up to 6Mb in single cells (Munné 2012), meaning that duplications and deletions of small parts of chromosomes (known as segmental aneuploidies) and chromosomes rearrangements, in which pieces of chromosomes break off and reattached to other chromosomes, can be identified in addition to gross chromosomal abnormalities. The reported values for the accuracy rate for aCGH are 98 and 95% for blastomeres and TEs, respectively (Fragouli et al. 2010; Gutiérrez-Mateo et al. 2011).

3. Single nucleotide polymorphism array (SNP-array)

3.1. SNP-arrays work in a very similar manner to aCGH, but the microarray chip contains thousands of immobilized short DNA sequences that are site (allele) specific to particular single nucleotide polymorphisms (SNPs)[17]. As with aCGH, the correct software, can distinguish how many of each chromosome was inherited by an embryo using both quantitative information (hybridization intensity), but unlike aCGH SNP-arrays can analyse the expected parental combinations of inherited chromosomes and comparing them with the outcome in the embryo. By using the later method SNP-array can carry out haplotyping (tracing the inheritance of chromosomes from different parents) a method that can test for specific genetic diseases (Harper & Harton 2010).

3.2. This technique has been validated by comparing SNP-array results from multiple euploid and aneuploid cell lines of known karyotype obtained from a public repository. Analysis of 72 single cells from 9 cell lines demonstrated 99.2% accuracy of copy number assignment of more than 18 million SNPs. The authors further validated the technique by randomizing and coding 27 single cells from cell lines with known abnormalities. Twenty-five of the single cells were greater than 94% concurrent, and after decoding, were determined to give 100% accuracy. Finally the authors analysed blastomeres obtained after biopsy of cleavage stage embryos from 78 patients undergoing IVF, finding that the concurrence for more than 80 million SNPs in 335 single blastomeres was 96.5% (Treff et al. 2010).

4. Quantitative polymerase chain reaction (qPCR)

4.1. Quantitative polymerase chain reaction (qPCR) (sometimes referred to as real-time quantitative PCR, RT-qPCR) is molecular biological technique that is used to determine (quantify) the amount of starting DNA material that is used in a PCR reaction[18]. The techniques has been modified so that is can quantify the starting amount of DNA extracted from the embryo biopsy, at particular loci (chromosomal

[17] Single nucleotide polymorphisms are specific locations within the genome at which a single base pair varies within the population.

[18] Polymerase chain reaction (PCR) is a one of the most commonly used and powerful techniques used in molecular biology. Using PCR a specific sequence of DNA can be copied or ‘amplified’ many thousand- to a million-fold.

locations) spread across all 24 chromosomes, and thereby extrapolate the copy number at those loci and therefore the levels of aneuploidy also.

4.2. More specifically the technique measures changes in copy number (CNVs) at 96 chromosomal locations, four per chromosome. The technique therefore does not generate the type of high resolution data that array and next-generation sequencing (NGS) techniques are capable of. However, a particular advantage of the technique is that it can be carried out in approximately 4 hours. This is unlike the other CCS methodologies described, which typically have protocols exceeding 12 hours, and therefore are likely to require cryopreservation of embryos while the tests are carried out, if a trophectoderm biopsy is used.

4.3. This technique has been validated by comparing the results of either conventional karyotyping of 9 cell lines or microarray-based diagnoses of 71 human blastocysts with qPCR, to determine whether they give consistent results. qPCR gave 97.6% (41/42) consistency with conventional karyotyping results, and after applying a minimum threshold for concurrence, 100% consistency was achieved. An identical 24 chromosome diagnoses by qPCR compared with SNP-array was achieved in 98.6% of cases, with overall euploidy and aneuploidy assigned with 100% consistency (Treff et al. 2012).

5. Next-generation sequencing (NGS)

5.1. Advances in next generation sequencing (NGS) technology have provided new tools for detecting DNA mutations and/or chromosome aberrations for research and diagnosis purposes. Combining this technology with whole genome amplification (WGA), a technique whereby the entire genome is copied many times, has enabled the detection of CNVs in single cells (Navin et al. 2011). This has paved the way for the development of WGA and NGS protocols for use in PGS.

5.2. The technique works by lysing the cell(s) obtained by embryo biopsy, increasing the amount of DNA by whole genome amplification (WGA) and fragmenting and purifying the material to assemble a barcoded library. These are then sequencing by a NGS technology (such as paired-end sequencing), generating very short sequence reads. These can be aligned with a reference sequence of the human genome and the chromosomal origin of each fragment is identified. This allows the proportion of DNA fragments from each chromosome to be determined, as the number of reads of a particular sequence in proportional to the number of chromosomes (or chromatids) present. By adjusting the read-depth the resolution of the chromosomal analysis can be adjusted.

5.3. A number of recent studies have validated the use of NGS for PGS (See Annex ?). In 2014, Fiorentino et al. carried out a study comparing the results of NGS to conventional karyotyping and aCGH, demonstrating 100% consistency and 99.8% chromosome copy number assignment consistency with these two techniques respectively (Fiorentino, Biricik, et al. 2014). In a similar study, Wells et al. (2014) assess the accuracy of a rapid low-pass whole genome sequencing technique. The authors compared the results obtained by aCGH with those generated through their

NGS technique, demonstrating that the concordance rate per chromosome between the two techniques was 99.7% (Wells et al. 2014).

5.4. One aspect of these techniques that is particularly noteworthy is the exceptionally high resolution that they can achieve. Fiorentino et al. noted that although their study was designed to validate the performance of NGS in the detection of whole-chromosome aneuploidies, their protocol demonstrated accurate detection of segmental changes as small as 14 Mb in size (Fiorentino, Biricik, et al. 2014). In addition, Wells et al. note in their paper that their data indicates that NGS has the potential to deliver accurate mutation detection as well as aneuploidy screening, effectively enabling PGD to be carried out by a methodology that has been designed primarily for PGS.

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