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Updates in preimplantation genetic testing (PGT)

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ABSTRACT

Preimplantation genetic testing (PGT) involves taking a biopsy of an early embryo created through in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI). Genetic testing is performed on the biopsy, in order to select which embryo to transfer. PGT began as an experimental procedure in the 1990s, but is now an integral part of assisted human reproduction (AHR). PGT allows for embryo selection which can reduce the risk of transmission of inherited disease and may reduce the chance of implantation failure and pregnancy loss. This is a rapidly evolving area, which raises important ethical issues. This review article aims to give a brief history of PGT, an overview of the current evidence in PGT along with highlighting exciting areas of research to advance this technology.

1. Introduction

Preimplantation genetic testing (PGT) involves taking a biopsy of an early embryo created through assisted reproductive technology in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) and performing genetic testing on the embryo, prior to implantation of the embryo. Following the 2017 consensus based International Glossary on Infertility and Fertility Care, the term PGT has replaced previous terminology-preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD) [1]. The impetus of PGT is to allow for selection and transfer of genetically normal embryos to reduce the risk of transmission of genetic diseases, pregnancy loss, and termination of pregnancy. Since the first baby was born following IVF in 1978, our understanding of embryo development has advanced and technologies have evolved to assist with embryo selection [2].

The first clinical application of PGT was in 1990, where it was used to select female embryos for transfer in two couples known to be at risk of transmitting X-chromosome linked disease [3]. In the subsequent decades, technologies have evolved to improve the accuracy of testing and PGT has become an important part of assisted human reproduction (AHR) for many patients. Now, over one third of IVF/ICSI cycles in the United States (US) are PGT cycles [4].

This is a rapidly evolving area in AHR and it plays an integral part in treatment for many patients with subfertility, recurrent pregnancy loss and inherited diseases. The aim of this review is to give an overview of up to date evidence in PGT and to highlight exciting areas of future research. We also aim to briefly highlight some of the ethical issues that arise with PGT, including the inequity of access to this technology globally.

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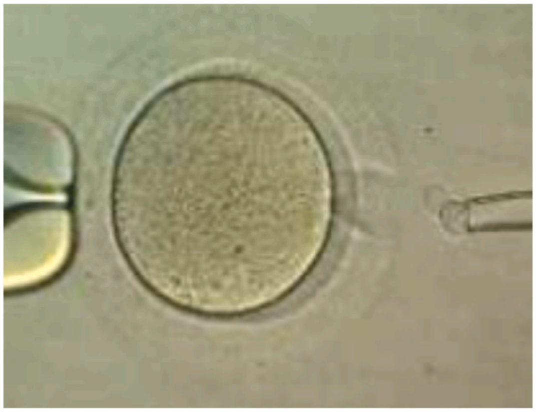

Fig. 1. Polar body biopsy. Permission to use image granted by Dr Montserrat Boada [7].

Fig. 2. Cleavage Stage Biopsy. Permission to use image granted by Dr Montserrat Boada(7).

2. Biopsy timing and technique

2.1. Polar body biopsy

During meiosis, polar bodies (PB) separate from the immature ovum resulting in a large mature oocyte and two small PBs. PBs contain a nucleus but very little cytoplasm and do not have any influence on the future development of the embryo. PBs can provide genetic information about the oocyte without destroying it [5]. One or both PBs may be biopsied by creating an opening in the zona pellucida (ZP) of the unfertilised oocyte. PB biopsy only provides genetic information pertaining to the oocyte, not the embryo. Embryo biopsy is used more frequently than PB biopsy these days, though PB biopsy may be used to investigate maternal pathogenic variants or structural rearrangements or it may be used in countries/regions where embryo biopsy is prohibited [6]. Fig. 1 shows a PB biopsy. Permission to use image was granted by Dr Montserrat Boada [7].

2.2. Cleavage stage biopsy

The cleavage stage of an embryo created through IVF/ICSI is the stage of rapid mitotic division when the fertilised egg divides into numerous smaller nucleated cells called blastomeres, at day two to three following oocyte retrieval. Cleavage stage biopsy involves making an opening in the ZP and removing one or two blastomeres for genetic testing. This was the most widely practiced form of PGT for over a decade, but has now been largely replaced by trophectoderm (TE) biopsy (outlined below) due to the increased amount of genetic material available for analysis with TE biopsy (5–10 cells versus 1–2) [8]. Fig. 2 shows a cleavage stage biopsy. Permission to use image was granted by Dr Montserrat Boada [7].

2.3. Trophectoderm biopsy

TE biopsy is the most widely used technique in current PGT practice [9]. It involves taking a biopsy from the trophectoderm of a blastocyst (day five to seven following oocyte retrieval). See Fig. 3 below for a diagram of TE biopsy and Fig. 4 below for an image of a

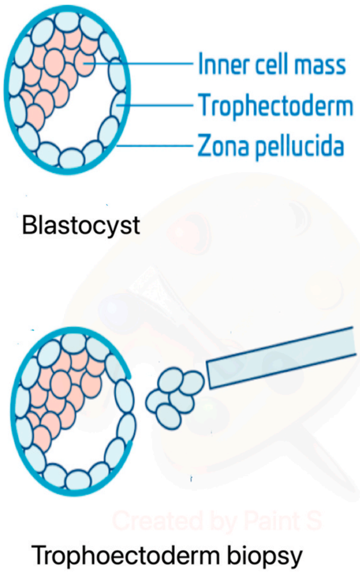

Fig. 3. Trophectoderm biopsy. Created by author SP on Paint S.

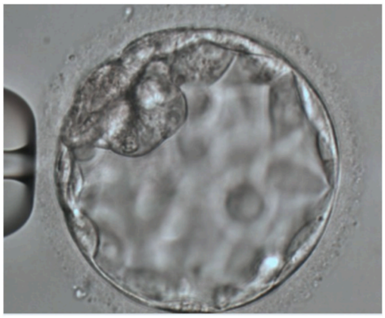

Fig. 4. Blastocyst for trophectoderm biopsy. Permission to use image granted by Dr Montserrat Boada(7).

blastocyst for TE biopsy. TE biopsy is the type of biopsy recommended by the 2020 European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium on biopsy technique [10]. Compared with cleavage stage biopsy and PB biopsy, TE biopsy provides more genetic material for analysis and is less likely to result in an inconclusive diagnosis (<5% vs 10%) [10]. TE biopsy requires a skilled and highly trained embryologist. First, an opening must be created in the ZP, most commonly using a laser, followed by aspiration of cells using a micropipette. Fig. 3 shows a diagram of a TE biopsy and Fig. 4 shows an embryoscope image of a TE biopsy. Permission to use image was granted by Dr Montserrat Boada [7].

3. Types of PGT

There are several different types of PGT which will be described below:

- PGT for aneuploidy (PGT-A)
- PGT for monogenic disorders (PGT-M)
- PGT for structural rearrangements (PGT-SR)
- PGT for human leukocyte antigen matching (PT-HLA)
- PGT for polygenic disorders (PGT-P)
- PGT for mitochondrial DNA (PGT-mtDNA)

3.1. PGT-A

PGT-A involves testing embryos for aneuploidy (an abnormal number of chromosomes). Aneuploidy has been established to be the leading cause of pregnancy loss and implantation failure [11,12]. PGT-A aims to allow selection of euploid embryos (embryos with a normal number of chromosomes) for transfer, to reduce the likelihood of implantation failure or miscarriage. The use of PGT-A is increasing, particularly in the US, but its use is debated. A 2021 randomised control trial (RCT) randomized patients to PGT-A or conventional IVF found that the cumulative live birth rate over three successive embryo transfers was non inferior in the conventional IVF group [13]. However, though this was a large study with >1200 patients randomized, an RCT may not be the best way of determining the benefit of PGT-A as the pool of embryos transferred are the same. In the same year that this RCT was published, Tiegs and colleagues carried out a multicentre, prospective, blinded, non-selection study evaluating the predictive value of an aneuploid diagnosis [14]. Their primary outcome was the ability of the analytical result of aneuploidy to predict failure to deliver. The embryos transferred were based on morpho kinetics only and results of the PGT-A biopsy were not disclosed until after the embryo transfer. No aneuploid transfers resulted in a live birth [14].

Using PGT-A to avoid transferring an aneuploid embryo may avoid the cost and psychological harm associated with an unsuccessful embryo transfer or pregnancy loss. Whilst PGT-A does not seem to be of benefit in an unselected population of fertility patients, it significantly improves outcomes in women of advanced maternal age (AMA) [15,16]. In particular, PGT-A seems to reduce the likelihood of miscarriage in women of AMA. A prospective cohort study of 2538 couples where the woman was aged between 38 and 44 years compared PGT-A cycles (n = 370) to IVF cycles without embryo testing (n = 2168) and found an 80% relative reduction in the miscarriage risk following the transfer of euploid embryos (OR = 0.19, 95% CI 0.09–0.41) [16].

Some have called into question the ‘diagnosis’ of recurrent implantation failure, stating that failed implantation is usually due to embryonic factors and that repeated transfer of euploid embryos results in a high likelihood of a live birth. A recently published multicentre retrospective study of over 64,000 euploid embryo transfers found that the cumulative LBR with five consecutive euploid transfers was 98.1% (95% CI = 96.5–99.6%) [17]. Whilst this statistic is encouraging, it could take multiple cycles for a couple to create five euploid embryos, particularly for women of advanced age or with low ovarian reserve.

PGT-A may be considered an ‘add on’ to IVF treatment. The Human Fertilisation & Embryology Authority (HFEA) is the regulatory body in the United Kingdom (UK). They provide a rating system providing evidence-based recommendations on ‘add on’ treatments, including PGT-A. The HFEA have assigned a ‘red’ rating (potential concerns/may reduce effectiveness) to PGT-A for most fertility patients as PGT-A reduces the number of embryos available to transfer and increases the time to conception and live birth [18]. However, they assign a ‘green’ rating (effective at improving the treatment outcome) for the reduction in miscarriage risk for most fertility patients [18].

Counselling of patients prior to electing for PGT-A is of great importance. The potential results that may be obtained from a PGT-A biopsy:

- Euploid (normal number of chromosomes)
- Aneuploid (abnormal number of chromosomes)
- Mosaic (euploid and aneuploid mixture)
- No result

3.1.1. Euploid/aneuploid result

The likelihood of a euploid result depends largely on the woman’s age and her ovarian reserve. The prevalence of aneuploidy ranges between 25 and 90% depending on maternal age [19]. Older oocytes are more likely to result in aneuploid embryos, both in vivo and in vitro. The aneuploidy rate of embryos at blastocyst stage in women of advanced maternal age (mean 39-year-old) is 55% [20]. A retrospective analysis of >7000 embryos from 990 patients found that the odds of having at least one euploid embryo was significantly decreased by increasing female age ($P < 0.01$ for both day three and day five embryos) and was significantly increased by every additional embryo available for analysis ($P < 0.001$ for both day three and day five embryos) [21].

The **P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**DO**ocyte **N**umber (POSEIDON) group came up with a validated predictive tool to calculate the number of mature ooctyes a patient would need to have a euploid embryo for transfer following a PGT-a cycle [22–24]. The ART Calculator can be used as a predictive tool to calculate the number of mature oocytes needed in order to have one euploid embryo [23]. This may serve as a useful counselling tool for couples considering PGT-A.

3.1.2. Mosaic result

Mosaicism is the presence of more than one genotypically distinct cell population within a single zygote [25]. In a blastocyst the inner cell mass (ICM) develops into the fetus and the trophectoderm (TE) will develop into the placenta, which contains both maternal and fetal DNA. The prevalence of true biological mosaicism is estimated to be <1% [26]. For PGT-A result reporting, the Preimplantation Genetic Diagnosis International Society (PGDIS) recommend defining mosaicism as a mixture of 20–80% aneuploid and euploid DNA content [27,28]. Embryos are considered non-mosaic euploid if there is <20% non-model DNA, and non-mosaic aneuploid if there is > 80% non-model DNA(28).

The definition of mosaicism used in practice varies greatly, and some laboratories do not report mosaicism at all which can lead to confusion and incorrect interpretation of results. Mosaic results are less likely to occur in TE biopsy (5–15%) [29,30] compared to cleavage stage biopsy (30–70%) [31]. The frequency of mosaic results may be greater with newer molecular technologies such as next generation sequencing (NGS) [32]. False positive mosaicism is a major concern and can pose difficult decisions for physicians and patients, particularly if the results from a PGT-A cycle yield only aneuploid and mosaic results. A systematic review of 26 studies found that, on re-biopsy 57% of previously labelled mosaic embryos, were unlikely to have been mosaic [33]. Grati and colleagues devised an evidence-based scoring system for prioritizing mosaic embryos for transfer based on a retrospective analysis of cytogenetic and molecular results on chorionic villi samples (n = 72,472) and products of conception (n = 3806) analysed which may be helpful to embryologist and clinicians [34].

The PGDIS published a position paper in 2021 on transferring mosaic embryos, they recommend proper counselling of patients and selecting embryos with lower levels of mosaicism for transfer first [28]. Embryos that show less than 50% aneuploidy have yielded a favourable live birth rates [35,36]. If there are more than one embryo with similar levels of mosaicism the PGDIS recommends transferring the embryo with a higher morphological grade is these are associated with better outcomes [28,37]. The PGDIS also recommends considering prenatal screening following mosaic embryo transfer. Non-invasive prenatal testing (NIPT) which involves testing of cell free DNA (cfDNA) in maternal serum and can be performed in the first trimester, has the advantage of being non-invasive however, it is a screening test and placental mosaicism may still be detected. Amniocentesis, a diagnostic test which involves testing a sample of amniotic fluid (from 14 weeks' gestation) gives a more accurate representation of the fetal DNA but carries a small risk of pregnancy loss [28]. Reassuringly, reviews of live births following mosaic embryo transfer have not demonstrated abnormal phenotypes in the children born [38,39]. Mosaic embryo transfers are certainly possible, provided the patients have been adequately counselled.

3.1.3. *No result*

Occasionally, an embryo biopsy for the purpose of PGT-A fails to yield a diagnostic result. The frequency of a 'no result' biopsy is approximately 1–3% [40–42]. There can be many reasons for this, including poor embryo quality, biopsy technique, shipping conditions and technical failures [42]. When patients have embryos with a euploid result, these should be given preference for transfer. However, some patients may be in a position of having no euploid embryos and only embryos with 'no result'. Re-biopsy is possible and results from studies of re-biopsied embryos show that a result is obtained in >95% of cases and the majority (53–55%) were euploid with favourable pregnancy outcomes [43,44].

3.2. *PGT-M*

PGT for monogenic disorders (PGT-M) involves testing for nuclear DNA pathogenic or likely pathogenic variant(s) causing a monogenic disorder. Pathogenic or likely pathogenic variants with autosomal dominant, autosomal recessive and X-linked inheritance patterns may be tested. This test may be offered to couples who are at risk of transmitting a particular genetic condition testing embryos and selecting only unaffected embryos for transfer. PGT-M can be offered for all monogenic disorders for which the disease-causing loci have been identified [45]. Countries have different regulations when it comes to PGT-M indications. The HFEA in the UK authorises PGT-M for over 600 genetic conditions with strict criteria including that the genetic condition must be clinically significant [46]. By contrast, in the US, PGT-M is not regulated [45]. It is now possible to combine PGT-M with PGT-A and PGT-SR, however, this may present challenges for genetic counselling.

3.3. *PGT- SR*

PGT for structural rearrangements (PGT-SR) forms a major indication category for PGT. Structural chromosomal rearrangements may include reciprocal translocations (where two different chromosomes exchange segments with each other), Robertsonian translocations (an entire chromosome attaches to another chromosome at the centromere), deletions, duplications, and inversions.

3.4. *PGT- HLA*

Preimplantation genetic testing-human leukocyte antigen (PGT-HLA) aims to allow for embryo selection to create an HLA matched sibling for an existing child who may require a hematopoietic stem cell transplantation (HSCT). Conditions where PGT-HLA may be of use include beta-thalassaemia, severe congenital immunodeficiency syndrome (SCID), Fanconi anaemia and haematological malignancies [19]. PGT-HLA may be combined with PGT-M and select an embryo HLA matched to the existing child and unaffected by the disease. A 2020 cohort study reported successful HSCT in 25 cases following PGT-(M-) HLA [47]. The largest study of PGT-(M-) HLA in 197 cycles for families affected by beta-thalassaemia, resulting in the birth of 45 thalassaemia-free children who were also HLA identical to their affected sibling, with successful stem cell transplantation in one case [48]. This may be a very valuable procedure for families who need it.

3.5. *PGT- P*

Preimplantation genetic testing for polygenic disorders (PGT-P) is a way of using PGT technology for embryo selection with a view to reducing the risk of polygenic disorders such as Type 1 diabetes, heart disease and cancer. This may be of interest to patients who have a strong family history of a particular polygenic disorder, and some argue it is of benefit even in cases where there is no known family history [49]. This use of PGT remains controversial and is only offered in a small number of clinics worldwide.

3.6. PGT-mt DNA

All the above types of PGT focus on nuclear DNA. Preimplantation genetic testing for mitochondrial DNA (PGT-mtDNA) specifically targets the mitochondrial DNA (mtDNA) within embryos. Mitochondria are organelles found within cells that play a crucial role in energy production and various cellular processes. They contain their own small circular DNA, separate from the nuclear DNA, which is maternally inherited [50]. PGT-mtDNA is typically utilized in cases where there is a risk of transmitting mitochondrial DNA diseases from the mother to the offspring [51]. These diseases can result from mutations or abnormalities in the mitochondrial DNA and can lead to a wide range of clinical manifestations, including neurological disorders, muscle weakness, and organ dysfunction.

4. Molecular techniques

Since PGT began over three decades ago, molecular technology has evolved hugely, and with its evolution the accuracy of PGT has improved. ESHRE published a series of Good Practice Recommendations on PGT in 2020, including the molecular techniques recommended for PGT-A, PGT-SR and PGT M [52,53]. Below some of the different molecular technologies are described in brief.

4.1. Fluorescence in situ hybridization (FISH)

FISH is a cytogenetic technique that uses a fluorescent DNA probe to target specific sequences. This technology was originally described in 1969 and was used for much of the early PGT in the 1990's [19,54]. It is still possible to perform FISH for PGT-SR [53], however since the introduction of newer technologies, described below, FISH is no longer widely used.

4.2. Array based comparative genetic hybridization (aCGH)

aCGH is a cytogenetic technique, developed in the early 1990's, used for the detection of chromosomal copy number changes on a genome wide and high-resolution scale [55]. Arrays are more reliable than FISH as they provide multiple points of measure for each translocation segment [53]. This technique may be used for PGT-A, by analysing copy number variants relative to ploidy in DNA compared to a reference sample, and it may also be used for PGT-SR(53).

4.3. Next generation sequencing (NGS)

NGS involves the amplification of DNA with priming, generating millions of genome wide views of a genetic background, allowing for genome wide sequencing. NGS can complement aCGH by providing additional information on single nucleotide level sequence variations, and chromosomal abnormalities such as balanced translocations. NGS is the present gold standard for PGT(19).

5. Accuracy of testing in PGT

The diagnostic accuracy of PGT is high and has improved with the development of newer technologies.

Reports from the 1990s and early 2000s from centres with experience of many PGT-M cycles reported a diagnostic accuracy of above 99% with polar body biopsy and blastomere biopsy [56,57]. Since then, the diagnostic accuracy has continued to improve with advancements in biopsy and molecular techniques. The most recent ESHRE consortium on PGT-M reported that misdiagnosis occurs in <0.1% of cases [58].

Since the development of TE biopsy, aCGH and NGS the diagnostic accuracy of PGT-A has also increased. PGT-A is highly accurate for the detection of uniform aneuploidy but it's accuracy for euploid and mosaic embryos is debated. The previously mentioned non-selection study by Tiegs et al. demonstrated no live births following the transfer of 102 aneuploid embryos [14].

6. Insemination technique prior to PGT (IVF vs ICSI)

IVF and ICSI differ in the way oocytes are fertilised in vitro. ICSI is the insemination method of choice when there is male factor infertility. It's use for non-male factor indications has been the topic of much debate recently.

In conventional IVF (cIVF), sperm surround an oocyte in a Petri dish and one sperm fertilises the egg. Surplus sperm may remain attached to the ZP. In ICSI, an embryologist selects out an individual sperm and injects it into the oocyte. Prior to injection, oocytes are denuded (cumulus cells are removed) and oocytes are rinsed. It was therefore thought that ICSI would be the preferred insemination method prior to PGT as it would eliminate the chance of maternal contamination by excess cumulus cells and paternal contamination by excess sperm which could occur in IVF. The 2020 ESHRE PGT Consortium recommended ICSI as the insemination method of choice in PGT cycles [10]. Subsequently, however, the advice has changed. Zhang and colleagues performed a retrospective analysis of 641 couples with non-male factor subfertility undergoing PGT-A cycles, they found low rates of parental contamination with cIVF for PGT cycles and concluded that cIVF is feasible for PGT-A cycles [59].

The American Society of Reproductive Medicine (ASRM) published a committee opinion in 2020 on the use of ICSI for non-male factor indications, state that ''ICSI for PGT in the absence of male factor infertility should be limited to cases where contamination of extraneous sperm could affect the accuracy of test results'' [60]. A retrospective review of 302 PGT-A cycles with NGS (75 IVF, 227 ICSI) found similar rates of euploidy, aneuploidy and no result biopsies. There was a trend towards higher rates of mosaicism with IVF cycles (25.9%) vs ICSI cycles (20.9%) but this was not statistically significant [61]. A more recently published retrospective cohort study of the Society for Assisted Reproductive Technology (SART CORs) database in the USA compared over 30,000 PGT-A cycles for non-male factor indications (n = 4867 cIVF cycles; n = 25,579 ICSI) [62]. Their primary outcomes were number of embryos suitable to transfer and LBR following frozen embryo transfer (FET) of a PGT-A embryo. They found no significant differences in rate of embryos suitable for transfer between cIVF vs ICSI (41.6% vs 42.5%, p = 0.12). Analysis of single FET (n = 3412 IVF; n = 16,358 ICSI) found no significant differences for LBRs (50.1% vs 50.8% respectively, p = 0.51) and pregnancy loss rates (16.6% vs 15.5, p = 0.11); and sub-analysis of first FET transfers only in cIVF vs ICSI revealed a similar trend for LBRs (53.4% vs 53%, p = 0.78) and pregnancy loss rates (15.7% vs 15.5%, p = 0.84).

In conclusion, PGT alone does not appear to be an indication for ICSI over cIVF.

7. Obstetric and neonatal outcomes following PGT

As previously stated, the accuracy of PGT is high, however there is still the possibility of misdiagnosis. The ESHRE consortium on PGT suggests that when pregnancies occur following PGT that parents should be made aware of the available pre-natal screening tests [63]. The PGDIS Position Paper highly recommends prenatal testing in pregnancies following PGT, particularly after transfer of a mosaic embryo [28]. In brief, prenatal screening options include non-invasive prenatal testing (NIPT), chorionic villus sampling (CVS) and amniocentesis. NIPT involves testing for fetal cfDNA in maternal serum [64]. NIPT has the advantage that it can be performed early in pregnancy, though it is a screening test for aneuploidy and not a diagnostic test. The positive predictive value (PPV) of any test is dependent on the prevalence of the condition that is being screened for. The PPV of NIPT in pregnancies following PGT-A compared to the general obstetric population, is reduced. One study of over 1100 patients following a euploid embryo transfer found that the PPV of NIPT was 12.5%, compared to 69% in the general obstetric population [65]. CVS involves sampling the early placenta, but like PGT, CVS also carries a risk of mosaicism [66]. Amniocentesis gives the most accurate representation of fetal genetics as it involves testing fetal DNA in amniotic fluid [28]. Both CVS and amniocentesis are invasive tests which carry a small but not insignificant risk of fetal loss (0.5–1%) [66]. Practices in prenatal screening vary widely internationally and access to these tests may be limited for some patients [67].

The data on the safety of PGT conceived pregnancies and children appears reassuring so far. There was a concern that by taking a biopsy of the trophectoderm, which becomes the placenta following implantation, that there may be an increased risk of placental dysfunction on PGT pregnancies. There may be a higher risk of hypertensive disorders of pregnancy (HDP) in PGT cycles. A 2021 retrospective cohort study of 756 patients who underwent a frozen embryo transfer (FET) cycle compared outcomes in those who had a FET with an embryo following a TE biopsy (n = 241) to unbiopsied embryos (n = 515), found that the probability of developing HDP was higher in the PGT group adjusted odds ratio (aOR) 1.943, 95% CI 1.072–3.521; P = 0.029) [68]. A retrospective analysis of data from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System compared placental outcomes in singleton pregnancies in FET cycles after embryo biopsy (n = 585) with unbiopsied embyros (n = 2191) and found no difference between the groups with respect to pre-eclampsia or pregnancy induced hypertension [69]. Two meta-analyses of 15 studies and 19 studies respectively, published in 2021, found an increased risk of HDP with PGT compared to IVF/ICSI (RR 1.5 and RR 1.3) but, overall obstetric and neonatal outcomes were favourable in PGT cycles compared with IVF/ICSI cycles [70,71]. Notably, only two randomized control trials were included in these meta-analyses. A more recently published (2023) scoping review of 31 articles, report a possible association between embryo biopsy and an increased risk of small for gestational ages fetus and low birth weight babies for blastomere biopsy and an increase in preterm delivery and birth defects in the case of TE biopsy [72]. For both biopsy methods, there appears to be increased risk of HDP. However, the authors point out some of the confounding factors and heterogeneity in the data that limit the reliability of studies in this area. One retrospective study from China compared natural cycle (NC) FET with hormone replacement therapy (HRT) FET following PGT and found a significantly higher rate of pregnancy complications, including pregnancy induced hypertension, in the HRT FET group (2.5% versus 0.7%, $P = 0.022$) [73]. It may be possible to mitigate some of the risk of HDP following PGT by performing a natural cycle FET but more study in this area is needed.

There are a limited number of paediatric studies on the developmental outcomes of children conceived after PGT-A cycles. A Swedish register-based study compared children born following PGT cycles (n = 390) with children born following IVF/ICSI (n = 61,060) and children born following spontaneous conception (n = 42,034) [74]. The children conceived with PGT-A cycles were followed up for a mean of 4.6 years. The results are reassuring, indicating that PGT does not seem to have any adverse effect on perinatal or early childhood outcomes compared with IVF/ICSI.

8. Ethical issues with PGT

Like any genetic test, PGT raises important social, legal, and ethical issues. PGT was developed as a test to prevent transmission of clinically significant genetic diseases. There is a concern that PGT could be used for unethical embryo selection, with some arguing that there is the potential for eugenics [75]. In addition, clustered regularly interspaced short palindromic repeats (CRISPR) technology exists which can allow 'gene editing' of embryos, a technology which has been condemned by many as unethical [76]. Others discuss the inherent nature of uncertainty carried with PGT, including mosaic results, which raises ethical and moral issues [77].

Legislation and regulation of PGT varies hugely around the world, and in some countries there is no regulation and in other countries like Malta, PGT is illegal [78]. In the UK the HFEA, a statutory body, regulates PGT. PGT-M and PGT-SR are available for approved conditions, funded by the National Health Service (NHS). By contrast, the USA has guidelines on best practice for PGT but no legislation or regulatory body and almost all PGT is privately funded [78]. Some argue that strict regulation of PGT reduces choices for

Practice Points

It is beneficial for patients who are going for PGT-M or PGT-SR to be seen by a clinical geneticist for counselling.
Female age is the single biggest predictor of euploidy and patients should be counselled appropriately, especially if the woman has low ovarian reserve.
Calculators may be useful when counselling patients prior to a PGT cycle (eg ART Calc).

Research Agenda

NiPGT may provide a way to obtain genetic material for testing without risking 'damaging' the embryo. Results of on-going studies in this area are awaited.
Obstetric outcomes following PGT are favourable, though there appears to be an increased risk of hypertensive disorders. Further research into whether natural cycle frozen embryo transfer mitigates this risk is needed.
Whilst studies of neonatal and early childhood outcomes following PGT are reassuring, further longitudinal studies would add to the literature in this area.

patients and results in patients travelling abroad to access care [19].

Like many aspects of AHR, there is huge inequity in access to PGT globally and in many countries where PGT is available it is only available privately at a cost that is prohibitive to many patients [78].

9. New technologies in PGT

9.1. Non-invasive PGT(NiPGT)

NiPGT involves testing of cfDNA either by blastocentesis or in spent culture medium (SCM). Blastocentesis involves aspiration of blastocoel fluid (BF) containing cfDNA from the blastocoel cavity with a fine needle, and is described as being less invasive than TE biopsy [79]. Testing of cfDNA in SCM is proposed to provide PGT results without the risk of embryo damage, carried by TE biopsy. Results from a preliminary study of NiPGT-A show that DNA can be isolated from SCM with a diagnostic accuracy of 65.38% [80]. One small study involving 47 embryos, found high concordance between a combination of cfDNA from BF and SCM and TE biopsy, the concordance rate for whole chromosome copy number per sample taken from the same embryo was 100%, and the rate of concordance per single chromosome was 98.2%, however this was not statistically significant($P > 0.05$) [81]. Further research into these techniques is needed before they can be recommended for clinical use, however. Recruitment for a randomised double-blind control trial of NiPGT in IVF is on-going [82].

10. Conclusion

PGT has evolved hugely in the last three decades and has become an important part of AHR and the technology continues to improve. For many patients PGT has improved the likelihood of conception and has allowed embryo selection to prevent the transmission of significant pathogenic mutations. Obstetric and neonatal outcomes with PGT are favourable, although it may be associated with an increased risk of maternal hypertensive disorders of pregnancy. Like any genetic technology, PGT raises important ethical questions and so regulation, and adequate expert counselling are of great importance.

Preimplantation genetic testing MCQ's

1. The following statements about PGT laboratory techniques are true:
   a. Polar body biopsy provides information pertaining to the embryo (Answer: False)
   b. Blastomere biopsy involves the removal of cells from the inner cell mass of the embryo. (Answer: False)
   c. Trophectoderm biopsy involves making a small hole in the zona pellucida and collecting 5–10 cells to analyse (Answer: True)
   d. Fluorescence in situ hybridization (FISH) is currently the most widely used molecular cytology technique for PGT. (Answer: False)
   e. Next-generation sequencing (NGS) allows for the simultaneous analysis of multiple genetic markers. (Answer: True)

2. When interpreting results of PGT it is important to counsel patients about the accuracy of testing.
    a. The likelihood of a ‘no result’ following embryo biopsy is approximately 10%. (Answer: False)
    b. It is not possible to perform a re-biopsy after a ‘no result’. (Answer: False)
    c. Mosaicism is the presence of more than one genotypically distinct cell population within a single zygote (Answer: True)
    d. Mosaicism in embryos is always indicative of a genetic disorder. (Answer: False)
    e. Mosaicism in embryos increases the likelihood of a healthy pregnancy. (Answer: False)
3. Preimplantation genetic testing for aneuploidy (PGT-A) is the most widely used form of PGT. Which of the following statements are true:
    a. Aneuploidy is the cause for the majority of first trimester pregnancy losses. (Answer: True)
    b. PGT-A can identify embryos with an abnormal number of chromosomes. (Answer: True)
    c. Intracytoplasmic sperm injection (ICSI) must be used as the fertilisation technique for PGT-A cycles. (Answer: False)
    d. PGT-A involves analysing mitochondrial DNA in embryos. (Answer: False)
    e. PGT-A results are not affected by maternal age or embryo quality. (Answer: False)

CRediT authorship contribution statement

Sarah Petch: Writing – review & editing, Writing – original draft. **David Crosby:** Writing – review & editing, Supervision.

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