REVIEW

Preimplantation genetic testing: changes in biopsy technique and clinical utility

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Abstract
The last forty years has seen vast changes in the way embryos are assessed and selected for use in Assisted reproductive technology (ART) procedures. The introduction of preimplantation genetic testing (PGT) produced a new means to do this but also caused ethical, legal and practical concerns. This review aims to identify how the type and quality of genetic testing available has changed over time, review how legislation governing PGT differs worldwide and identify the ethical issues that have emerged. It examines how the type of cell collection biopsy has evolved and discusses what this means with regard to clinical outcomes and future practice. PGT use has seen improvement to implantation rates and clinical pregnancies, reductions in miscarriage rates and quicker cumulative pregnancy rates. Issues of cost-effectiveness remain the next challenge to overcome.

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Introduction

Preimplantation genetic testing (PGT) is a procedure requiring the collection of cells from a developing human oocyte or embryo for the identification of genetic abnormalities. This information is used to inform the selection and transfer of optimal euploid embryos in an in vitro fertilization (IVF) cycle (Chang et al, 2016; Munne, 2012).

PGT was first introduced in the late 1980's as an alternative to prenatal diagnosis for couples with a risk of transmitting a known fatal or debilitating genetic condition. The intent was to avoid ethical and emotional issues regarding pregnancy termination (Chang et al, 2016; Gleicher & Orvieto, 2017). This extended to sex selection in couples with family histories of X-linked disorders (Chen et al, 2018; Verlinsky et al, 1990).

Presently, the aim of PGT is two-fold, with one arm focusing on the diagnosis of monogenic disease and chromosomal translocations and the other arm focusing on identifying and screening the ploidy state of the embryo as either euploid or aneuploid. This allows selection of embryos with the highest implantation potential and reduces the financial costs and emotional trauma of multiple transfers and miscarriages (Cimadomo et al, 2016).

This review will identify how the type and quality of genetic testing availability has changed over time, review how legislation governing PGT differs worldwide and the ethical issues that have emerged. It will show how the type of cell collection biopsy has evolved and discuss what this means with regard to clinical outcomes and future practice

Types of PGT and Changes to Platforms Used

PGT is aimed at preventing monogenic disease transference to embryos of high-risk couples, the transfer of unbalanced chromosomes to embryos where parents have balanced rearrangements and the use of aneuploid embryos in IVF procedures (Treff & Zimmerman, 2017). Currently there are three main types of PGT used to diagnose different genetic abnormalities in embryos prior to transfer. What was once called preimplantation genetic diagnosis has been split into two groups – Preimplantation genetic testing for monogenic (single gene) disorders (PGT-M) and preimplantation genetic testing for structural rearrangements (PGT-SR) including translocations, inversions and deletions. Preimplantation genetic screening is now known as preimplantation genetic testing for aneuploidy (PGT-A) (Harper & Harton, 2010; Wang et al, 2017).

Aneuploidy is the most common form of chromosomal abnormality and can be directly correlated to implantation failure, miscarriage and congenital malformation in humans (Chambers et al, 2015; Treff & Zimmerman, 2017). Aimed at improving IVF clinical outcomes, PGT-A is used to identify the most competent / euploid embryos for transfer in an IVF cycle (Wang et al, 2017). Indicators most frequently cited for use include advanced maternal age (Lee et al, 2017; Milan et al, 2010), repeated implantation failure (Harper & Harton, 2010; Rubio et al, 2013), recurrent spontaneous abortion (Munne & Wells, 2017; Harper et al, 2010) and severe male factor infertility (Harper & Harton, 2010). These indicators remain controversial as there is not sufficient evidence in the literature to show benefit of PGT-A use in all cases.

However, these patient populations are at higher risk of IVF failure because of their higher risk of aneuploidy, meaning selection of the most competent / euploid embryos would be expected to increase clinical outcomes (Penzias et al, 2018; Treff & Zimmerman, 2017l). PGT-A is also used to aid selection for single embryo transfers by identifying the embryo with the highest implantation potential. This lessens the incidence of multiple births and the health costs and associated risks (Chambers et al, 2015; Dahdouh et al, 2015; Simon et al, 2018).

Initially PGT-A was performed using cleavage stage biopsies and fluorescence in situ hybridization (FISH) techniques (Munne, 2012). FISH is only able to assess 5 – 10 unique chromosomes, is prone to failure and inaccuracy in interpretation and suffered from variable reproducibility between laboratories (Chambers et al, 2015; Mastenbroek & Repping, 2014; Munne, 2012). This resulted in a failure of the technology to improve pregnancy rates leading instead to poorer clinical outcomes (Chambers et al, 2015; Gleicher & Orvieto, 2017; Mastenbroek et al, 2007).

Current technology utilizes genetic platforms and strategies that are focused on specific gene amplification or that allow whole genome amplification (WGA) (Treff & Zimmerman, 2017). In order of decreasing cost these include single-nucleotide polymorphism array (aSNP), comparative genomic hybridization array (aCGH), real-time quantitative PCR (qPCR), and next-generation sequencing (NGS) (Chen et al, 2018; Dahdouh et al, 2015; Penzias et al, 2018). While WGA has the ability to detect abnormalities across the entire genome, the role of many genes remains unknown. This means meaningful interpretation of results requires the development of complex analytical algorithms, but still clinical significance can be difficult to interpret (Chrystoja & Diamandis, 2014).

A move towards blastocyst biopsy of trophectoderm cells, coupled with the new genetic platforms, means minimizing embryonic risk compared to cleavage stage biopsy and increasing the amount of starting DNA. This improves the sensitivity and specificity of results over FISH and cleavage stage biopsies (Dahdouh et al, 2015; Lee et al, 2017).

The greatest argument against the use of PGT-A is the ability of the technology to produce false positive predictions of aneuploidy which could result in discarding reproductively competent embryos (Treff & Zimmerman, 2017). Different platforms incur different degrees of inaccuracy and because multiple cells are tested instead of single cells a diagnosis of mosaicism can result (Friedenthal et al, 2018). A mosaic embryo contains cells with different chromosomal arrangements indicating some cells are euploid and some are aneuploid (Munne & Wells, 2017). Embryonic mosaicism results from post-zygotic chromosome segregation errors during mitotic non-disjunction (Capalbo et al, 2017). The clinical significance

of the mosaicism will depend on which chromosomes are involved, the timing of the anomaly thus what proportion of the embryo is involved, and the fate of the affected cells (Capalbo et al, 2017).

The location within the embryo of any aneuploid cells can impact the detection of mosaicism. Widely spaced aneuploid cells are likely to be biopsied where as if the aneuploid cells are clustered together a biopsy may miss them and result in a euploid assessment (Scott & Galliano, 2016). Biopsies that contain cells with reciprocal anomalies can also result in misdiagnosis depending on the platform used for detection (Scott & Galliano, 2016).

Currently trophectoderm biopsy is preferential, but mosaicism is higher in trophectoderm cells than inner cell mass cells due to less efficient self-correction mechanisms which could lead to higher rates of mosaicism (Gleicher & Orvieto, 2017). Despite a reduced implantation potential and higher miscarriage rate, some mosaic embryos can result in live births (Friedenthal et al, 2018; Greco et al, 2015).

As screening techniques become more sensitive the assessment of the type of mosaicism also becomes more specific. This has led to a percentage grading system and identification of the type of aneuploidy and which chromosomes are affected. This assessment can be used to aid identification of mosaic embryos with the greater potential for implantation and live birth outcome (Capalbo et al, 2017; Munne & Wells, 2017; PGDIS, 2016). The accuracy of this assessment and its clinical application remains controversial.

While fully euploid embryos are the first choice of transfer, graded mosaic embryos can be considered for transfer in their absence, provided appropriate genetic counselling is offered, regarding potential risks to the pregnancy and child, with some expectation of success (Friedenthal et al, 2018; Munne & Wells, 2017). These issues become more important with women who are poor responders, have low follicular reserve or are of advanced age with fewer follicles and embryos to use (Munne & Wells, 2017).

The use of genetic platforms for PGT-M and PGT-SR investigation is less controversial. Many platforms are able to identify specific known potential mutant alleles and also co-amplify multiple surrounding single tandem repeat (STR) markers linked to the mutant alleles to help reduce misdiagnosis (Chen et al, 2018). STRs are found throughout the genome and are extremely polymorphic, thus aiding identification of mutations (Treff & Zimmerman, 2017). Single nucleotide polymorphism (SNP) based approaches are also utilized and are particularly useful for diagnosing specific mutations in family groups (Treff & Zimmerman, 2017).

PGT-M is used to identify known familiar heritable disorders that are autosomal dominant (50% risk) or recessive (25% risk), X-linked dominant (50% in males, 25% carrier in females) or recessive (variable risk) or from mitochondrial inheritance (Chen et al, 2018 It is also possible to screen for mutations that cause a specific condition but have variable penetrance thus the extent of the clinical significance of the condition remains unknown. This type of testing can be controversial and may require permission from a regulating body before testing can be conducted (Bayefsky, 2016).

PGT-SR is used to identify unbalanced chromosome complements, especially when one or both parents carry a balanced translocation. It is estimated that 0.2 percent of the population carry a balanced translocation without an observable phenotype (Wang et al, 2017). While detecting an unbalanced translocation is possible, predicting whether an embryo is ‘normal’ or carries a balanced translocation is problematic. A small study by Treff et al, (2016) using a SNP array platform validated a methodology that utilized parental DNA and an unbalanced IVF embryo to distinguish a normal embryo from an embryo with a balanced translocation in sibling embryos. This technology would give greater power of choice to parents to avoided passing balanced translocations if euploid embryos are an option.

Biopsy Types

Preimplantation genetic testing of any type requires the collection of cellular DNA from the pre-implantation embryo (Kirkegaard et al, 2012). Along with the genetic testing procedures the biopsy process itself has an impact on the implantation potential of the embryo and subsequent pregnancy outcomes (Leigh, 2019). This is related to the type of biopsy procedure chosen and the stage of development of the embryo. The literature now reflects that earlier

stage biopsies can reduce implantation rates by up to 40% while the impact of later stage trophectoderm biopsies is under 2% (Leigh, 2019). Some novel, less invasive techniques have more recently been considered that involve the testing of spent culture medium and blastocoel fluid (Li et al, 2018; Shamonki et al, 2016). While embryonic DNA is able to be collected, these techniques currently suffer from low sensitivity and specificity as well as contamination issues from apoptotic cells and shedding from developing embryos and maternal cells (Feichtinger et al, 2017; Li et al, 2018). Further research and assessment are required before clinical application can be utilized (Leigh, 2019).

There are three, more invasive biopsy techniques used, namely, polar body collection from a mature oocyte and/or zygote, cleavage stage blastomere collection and trophectoderm collection at the blastocyst stage (Cimadomo et al, 2016; Chen et al, 2018). A fourth timed collection at the morula stage has also been investigated. The goals of these biopsies are to remove testable DNA that represents the embryos genetic constitution while maintaining embryo viability and reproductive potential (Capalbo et al, 2016).

Polar Body Biopsy

As an oocyte develops, a series of meiotic divisions occur resulting in the extrusion of two sets of haploid maternal DNA known as polar bodies (Munne et al, 1995; Verlinsky et al, 1990). As they are unused products of meiosis their removal will have no effect on embryo integrity and the process is considered less invasive than biopsy at other stages (Chen et al, 2018; Cimadomo et al, 2016; Montag et al, 2009).

Both polar bodies are required for assessment as they represent different stages of development thus two potential nondisjunction / recombination events (Harton et al, 2010). These polar bodies can be collected sequentially or simultaneously depending on the analysis process to be used. Using zona drilling techniques, the first polar body can be removed between 36 and 42 hours post hCG injection and the second +/- the first polar body between 9 and 22 hours post fertilization, thus avoiding degeneration (Cimadomo et al, 2016; Harton et al, 2010).

The major limitation of this process is that only maternal DNA can be tested with no assessment of mitotic division, post-zygotic aneuploidy or paternal DNA available (Capalbo et al. 2016; Cimadomo et al, 2016). This means the major application of polar body biopsy is to identify maternally derived translocations and X-linked heritable disorders. This is especially relevant when maternal age is a factor as aneuploidy is known to increase with increasing maternal age (Dahdouh et al, 2015; Harton et al, 2010; Montag et al, 2009). While this process is not the biopsy of choice it can be used in countries where ethical concerns around embryo biopsy have resulted in restrictive legislation as the procedure can be performed prior to fertilization (Brezina et al, 2012; Harton et al, 2010).The other disadvantage of polar body biopsy is the cost. The procedure requires two samples per oocyte which is both time consuming and expensive to process for only half the required information and poor predictive value for the ploidy of any resultant embryo (Cimadomo et al, 2016; Treff & Zimmermann, 2017).

Cleavage stage biopsy

After fertilization the formed zygote undergoes isolecithal mitotic cleavage forming blastomeres. After three days a normally developed embryo consists of six to eight blastomeres and is considered large enough for the removal of one or two blastomeres for genetic testing (Chen et al, 2018; Munne, 2012). Blastomere removal is done by first breeching the zona pellucida by either using non-contact lasers, mechanical action or an acidified Tyrode's solution. The embryo is placed in a Ca++ / Mg++ free media to loosen the cell-to-cell adhesions between blastomeres and using a holding pipette to secure the embryo, one or two blastomeres are aspirated (Cimadomo et al, 2016; Munne, 2012). In recent times non-contact lasers are the breeching method of choice of the majority of fertility laboratories (Harton et al, 2010). Not only is the process more precise, faster and requires less time in Ca++/Mg++ solution but the procedure is also associated with an increased number of blastocysts for transfer and better clinical outcomes when compared to acid Tyrode methods (Geber et al, 2011). The procedure to create the hole in the zona pellucida can be standardised and has greater reproducibility than acid Tyrode's solution as it is

less operator dependent (Cimadomo et al, 2016).

Many studies have observed a significant delay in the timing of compaction and the commencement of blastulation leading to a decreased implantation rate of embryos that undergo cleavage stage biopsy (Bar-El et al, 2016; Cimadomo et al, 2016; Dahdouh et al, 2015; Kirkegaard et al, 2012). Time lapse videography has also shown altered blastocyst hatching with biopsied embryos bypassing the period of zona pellucida thinning and instead hatching through the hole created during biopsy (Kirkegaard et al, 2012).

Despite this trend towards less advantageous outcomes, cleavage stage has been a popular choice of biopsy method. This is in part because biopsy results can be obtained in time for a fresh embryo transfer within the collection cycle. Despite this advantage the current favoured trend has moved towards trophectoderm biopsy and cryopreservation for transfer in a later cycle (Leigh, 2019; Brezina et al, 2012).

Timing of blastomere biopsy is an important factor affecting implantation potential (Cimadomo et al, 2016; Kalma et al, 2018). While laboratory routine can dictate when biopsy procedures take place and guidelines indicate day three post insemination / ICSI when six to eight blastomeres are seen as the recommended time, further refinement is called for (Harton et al, 2010; Kalma et al, 2018). The use of time-lapse videography of embryos can pin point the exact time an embryo reaches the eight-cell stage. In human embryos a long arrest phase of up to 20 hours occurs at the eight-cell stage while embryonic genome activation and cell differentiation occurs (Cimadomo et al, 2016; Kalma et al, 2018). Blastomere biopsy at the 15 to 20 hour mark post eight cell stage has been shown to be least affected by the biopsy procedure and their implantation potential is greater than embryos biopsied between 0 to 15 hours post eight cell stage (Kalma et al, 2018).This timing is another practice point for reducing the known effects of cleavage stage biopsy and improving clinical outcomes.

Morula stage biopsy

The compaction process transforms the growing embryo into a tightly packed group of cells, firmly adhered and called a morula, at about day four after fertilization. From a structural point of view this makes blastomere collection difficult (Zakharova et al, 2014). However, the use of Ca++/Mg++ free culture media can induce decompaction and allow the collection of blastomeres using the same method as cleavage stage blastomeres. The addition of Ca++/Mg++ to the culture media reverses the decompaction within a few hours (Cimadomo et al, 2016; Zakharova et al, 2014). The difference being three to seven cells can be obtained thus providing more genetic material for testing to overcome the limitation of single cell genetic analysis seen in cleavage stage biopsies (Cimadomo et al, 2016; Zakharova et al, 2014).

While more cells are available for biopsy, they have still not completed differentiation into trophectoderm and inner cell mass cells meaning the fate of the cells removed is yet to be determined. The potential removal of cells destined to become inner cell mass cells is a contributing factor to implantation failure for both cleavage stage and morula stage biopsies (Irani et al, 2018; Zakharova et al, 2014).

Morula stage biopsy is also advantageous for women with slow-developing embryos that have not moved past the cavitating stage by day 6. This is especially relevant in younger women with diminished ovarian reserve and low blastocyst yields (Irani et al, 2018). While the rate of embryo development and morphology is correlated to higher implantation rates and positive pregnancy outcomes, a study by Irani et al (2018), showed that slow developing embryos should not be deselected and discarded as their potential for euploid status remained.

Blastocyst stage biopsy

By day five or six the average embryo has passed the compaction and cavitation phases and formed an expanded blastocyst. This clearly differentiates the inner cell mass cells that will form the embryo from the trophectoderm cells that will form the placenta and related structures (Cimadomo et al, 2016; Munne, 2012). The collection of cells for genetic testing is technically similar to the collection of cleavage stage cell biopsies. The difference being that as collection occurs on day five or six, only trophectoderm cells are removed so no embryonic cells are compromised, and a greater number of cells can be removed without compromising total blastocyst cell numbers (Capalbo et al, 2016; Chen et al, 2018;

Cimadomo et al, 2016). Collection at this late stage of development necessitates the cryopreservation of the embryo as the current turnaround time for complete results is not fast enough for transfer within this cycle.

Blastocyst stage biopsy has no deleterious effects on embryo development, viability or reproductive potential (Cimadomo et al, 2016; Dahdouh et al, 2015; Scott, Upham, Forman, Zhao & Treff, 2013). The major issues revolve around the ability of embryos to survive in vitro culture until day five. If embryos are not viable on day five, not only is there nothing to biopsy, there is also nothing to transfer when if transferred on day three some embryos may have been able to establish a viable pregnancy (Gleicher et al, 2014). This can be of particular importance to older women and those with prematurely diminished ovarian reserves who have fewer follicles to start with and incur a higher rate of aneuploidy (Lee et al, 2015). This highlights the need for quality embryo culture conditions, high grade biopsy media and focused biopsy timing and technique to achieve day 5 blastocyst growth (Rubio et al, 2013). Vitrification techniques also need to be of a high standard to reduce the potential of rethawing issues.

It has also been demonstrated that lower levels of mosaicism are seen in blastocyst stage biopsies compared to cleavage stage biopsies (Capalbo et al, 2017). Current research is focused on developing an improved mosaicism classification scheme to aid genetic interpretation and transfer decisions (Capalbo et al, 2017). Research studies are also examining aneuploidy concordance between trophectoderm biopsy and inner cell mass biopsy. A small study by Victor et al (2019) found that utilizing NGS, a multicell trophectoderm biopsy classified as aneuploid is predictive of an aneuploid inner cell mass. Alternatively, segmental aneuploidies are rarely concordant and hold the potential for implantation and self-correction (Victor et al, 2019). The interpretation of segmental aneuploidies is a source of conflict and controversy that requires validation of the accuracy of results (Treff & Zimmerman, 2017). Notably with high quality biopsy, vitrification and chromosomal procedures inconclusive results can be retested without compromising euploid blastocysts (Cimadomo et al, 2016).

Random control trials have demonstrated that when PGT-A is undertaken using comprehensive genetic screening platforms, implantation rates per transfer have increased when compared to morphologically assessed only groups (Scott et al, 2013; Yang et al 2012). Higher implantation and clinical pregnancy rates have led to higher live birth rates (Scott et al, 2013) and similar pregnancy rates are found between one PGT embryo transfer and two morphologically assessed embryo transfers (Forman et al 2013). These results also encourage the use of single embryo transfers (Keltz et al, 2013).The limiting factor with these studies is the age of the women being below 35 years, meaning it is unclear if the data can be extrapolated to older women above 35 years where the use of PGT-A is encouraged (Chambers et al, 2015; Irani et al, 2018). A study by Rubio et al, (2013) found an increase in live-birth rates per patient between a PGT-A group compared with a blastocyst only group despite there being a lower transfer rate.

It can be concluded that the introduction of trophectoderm biopsy and whole genomic screening platforms have improved the ability of PGT, particularly PGT-A, to select euploid embryos for transfer in IVF cycles when compared to morphological assessment only (Irani et al, 2018; Lee et al, 2015).

Legal Aspects of PGT

Legislation and policy regarding the use of the various PGT techniques varies considerably worldwide from severely restrictive to minimal regulation. This is in part due to the impact of religious belief, medical rebate policy and public opinion in different countries around the world (Bayefsky, 2016). These different regulations have created a phenomena called ‘reproductive tourism’ where couples are willing to travel to other countries to circumvent restrictions in their own home country. This can lead to exploitation of couples desperate for a child as well as legal, health, financial and ethical complications (Chen et al, 2018; Ferraretti et al, 2010).

Italian legislation passed in 2004, saw some of the most restrictive assisted reproductive technology (ART) regulations created (Biondi, 2013). Specifically, the number of oocytes fertilized was limited to three at one time within an IVF cycle and all viable embryos were required to be transferred, not stored or

destroyed. Cryopreservation of any remaining oocytes was allowed but embryo cryostorage was banned as was any form of PGT (Fineschi, Neri & Turillazzi, 2004; Ragni et al, 2005). Access to ART was restricted to those with a diagnosis of 'infertility'. This meant no access to fertile couples with known heritable genetic conditions forcing couples to seek treatment out of country and risk legal prosecution (Ferraretti et al, 2010; Gianaroli et al, 2014). Public outcry and scientific concern regarding the rigid dictation of the number of embryos transferred, the inability to cryopreserve embryos, the risks to women's health relating to increases in hyperstimulation cycles needed and the increased chance of multiple pregnancy eventually forced changes to the regulations and new legislation was introduced (Fineschi, Neri & Turillazzi, 2004; Ragni et al, 2005). Current laws allow the use of PGT when the purpose is for the protection and health of the developing embryo provided no form of eugenic selection is undertaken (Bayefsky, 2016).

After initial bans placed on PGT use in both Switzerland and France, laws were passed between 2013 and 2015 to allow PGD for recognised serious heritable conditions and PGS for aneuploidy screening. Specific criteria still need to be met and approval from regulatory bodies obtained prior to the testing (Bayefsky, 2016).

In the United Kingdom ART procedures are regulated by the Human Fertilization and Embryology Authority (HFEA) which obtains its authority from the Human Fertilization and Embryology Acts passed in 1990 and revised in 2008. These acts allow PGT for medical purposes, including human leukocyte antigen (HLA) matching, and provide a list of heritable conditions that, with permission, PGD can be used for (Bayefsky, 2016; Human Fertilization and Embryology Act, 1990).

In Australia relevant legislation exists regarding the use of PGT in Victoria, South Australia and Western Australia. PGD is permitted in Victoria for use to prevent a genetic abnormality or disease (Assisted Reproductive Treatment Act of 2008 (VIC) s 10(2)(a)(iii)). What is considered a genetic abnormality or disease is not defined by the act and permission is not required prior to testing. South Australia allows PGD if the risk of a serious genetic defect, disease or illness could be inherited if natural conception occurred (Assisted Reproductive Treatment Act 1988 (SA) s 9(1)(c)(iii)). Western Australian law allows diagnostic procedures on embryos when there is a significant risk of serious genetic disease or abnormality (Human Reproductive Technology Act 1991 (WA) s 14(2b)(ii)). There is a proviso that the embryo must most likely remain fit for transfer and not be harmed by the procedure (Human Reproductive Technology Act 1991 (WA) s 14(2b)(i)). For the other states and territories, and to supplement the above Acts, the National Health and Medical Research Council (NHMRC) provides fertility clinics with ethical guidelines for use in utilising PGT technologies for genetic diagnosis, aneuploidy screening, HLA testing and sex selection. The use of PGT for non-medical reasons in Australia is prohibited (NHRMC, 2017).

In the United States of America (USA), apart from New York States Civil Rights Law §79-1 which is directed at clinical genetic testing, PGT is not regulated. There is no overriding regulatory body or state-based legislation outlining the acceptable or unacceptable use of PGT (Bayefsky, 2016). This means that legally PGT can be used to detect and select for any condition or trait that genetic testing is available for, including conditions like deafness and achondroplasia (Baruch et al, 2008). A 2018 study found that 72.7% of US fertility clinics offer embryo sex selection with 83.5% of those clinics offering sex selection without an infertility diagnosis (Capelouto et al, 2018). This lack of regulation enables reproductive tourism to flourish in the US (Bayefsky, 2016).

While accredited bodies like the American College of Medical Genetics (ACMG) and the American Society for Reproductive Medicine (ASRM) do put forward some guidelines they are not bound by legislation and the decision to use PGT is at the discretion of the individual fertility clinics and clinicians (Bayefsky, 2018).

While countries have developed legislation to reflect the moral and ethical beliefs of the majority of their populace there remains room for legal and financial exploitation of many. With geographical barriers easier to navigate than legal ones, for those with financial means cross border reproductive care can be obtained in the pursuit of a baby (Bayefsky, 2018).

Ethical Issues

The use of PGT raises a number of ethical concerns and moral dilemmas. Its use for sex

selection for personal preference, unrelated to disease, is prohibited by law in many countries (Australia, India, China) but in others (USA) is freely advertised on fertility clinic web pages (Brezinza et at, 2012). Objections revolve around issues of discrimination, oppression of females and expanding control over creating 'designer babies' with non-medical phenotype traits (Bayefsky, 2018; Brezinza et at, 2012).

Human Leukocyte antigen (HLA) matching allows the creation of a child as a potential stem cell or bone marrow donor to an affected sibling with a known recessive disease or malignancy (Chen et al, 2018; Kalma et al, 2017). While many countries permit PGT for this purpose public opinion raises moral concerns regarding where the technology will draw the line and what the rights of the 'rescue child' are (Samuel et al, 2009; Thomas, 2004).

Screening for late onset monogenic inherited disorders, like Huntington's disease, or susceptibility to a disease that may not develop until later life are controversial subjects (Chen et al, 2018). These children can enjoy many years of 'normal' health. By the time they are symptomatic, advances in medical science may offer treatments or cures for their condition (Thomas, 2004). The importance of a legal framework to help overcome all these concerns is important to reflect public opinion and in the ongoing use of PGT in IVF treatments.

Cost-effectiveness and Clinical Utility

PGT-A and PGT-SR have the potential to be cost effective if it can reduce the number of ART cycles needed to create a live birth by increasing implantation rates and reducing miscarriage rates (Chambers et al, 2015). This cost can also be reduced with improved vitrification procedures that can store embryos for later use without further oocyte collection cycles needed and allow single embryo transfers which also reduces costs related to multiple births and prematurity (Chambers et al, 2015).

A recent study by Lee et al, (2019) reviewed the cost-effectiveness of repeated ART cycles in Older women within the Australian health care system. It found that the PGT-A group required fewer ART cycles to reach a live birth, but it also required more stimulation cycles as fewer euploid embryos were collected. So fewer transfer cycles is a saving but an increased number of collection cycles increases costs. This shows potential for increasing the cost effectiveness of PGT-A cycles in Australia by aiming to reduce collection protocol costs with improved vitrification techniques also playing a role. Selection of euploid embryos is key to cost effectiveness. Not only do implantation rates rise but the costs of storing aneuploid embryos is removed (Chambers et al, 2015).

In Denmark, Bay et al (2016) conducted a multicenter retrospective study (results between 1999-2013) investigating the risk of a range of adverse obstetric and neonatal outcomes among three groups – PGT group, IVF/ICSI group and a spontaneously conceived group. It surmised that while the risk increased in the PGT group compared to the spontaneous group, the risk was the same among the PGT and IVF/ICSI group. This suggests that the embryo biopsy itself adds no additional risk over other well-known IVF treatment outcomes. This could be interpreted to indicate that the addition of PGT will not increase the likelihood of a complicated pregnancy or preterm delivery, thus PGT adds no additional costs above what can be expected with a routine IVF procedure.

Another application of genetic testing which can be utilized to reduce overall costs and assess the need for PGT has been introduced in Victoria, Australia. Archibald et al, (2018) assessed the use of a multi-disorder genetic carrier screening test that screened for cystic fibrosis, fragile X syndrome and spinal muscular atrophy in 12000 individuals. Notably 5.08% (1:20) were diagnosed as carriers with 88% having no family history of the conditions. Thus, the chance of having a pregnancy affected with one of these three conditions is similar to levels of Down Syndrome in the community which is routinely screened for (Archibald et al, 2018). This type of screening allows couples to be informed re calculating their risk of having an affected child which also allows a more informed choice regarding the need for PGT during ART procedures. The associate financial and emotional costs relating to frequent miscarriage, poor fertilization rates and failed transfers as a result of these unknown genetic issues can be reduced when individuals are aware of the potential issues early.

Summary

Overall the assessment of PGT technology is complex and relies on multiple factors. Vast improvements in biopsy procedure, genetic screening platforms and legal regulation have been made over the last 30 years but just as many questions and challenges have been raised. Further cost-effectiveness studies utilizing current technologies are needed to truly assess the current expenses incurred by couples seeking a baby. Theoretically PGD-A has the potential to be cost-effective if the process can reduce the number of ART cycles needed and encourage the use of less expensive thaw cycles and single embryo transfers. Emotional cost should also be considered as PGT-A can ensure only euploid embryos are stored, thus preventing the storage and subsequent transfer of aneuploid embryos which are not viable so cumulative time to pregnancy can be lessened. It remains to be seen were future technology will take ART practise and how large a role genetic assessment will have.

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