Preimplantation genetic screening: Changes in technology and methodology, ethical implications, and areas of future research

April Vanderwal

Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, ON Canada. Faculty supervisor: Nina Jones.
For correspondence, please email: april_vanderwal@hotmail.com

Abstract

Advances made in the field of genetics have positively contributed to the ability to screen for genetic diseases. Preimplantation genetic screening (PGS) is a type of Reproductive Genetic Screening that involves day 5 embryo biopsies and DNA testing prior to embryo implantation in the uterus. Historically, this DNA testing was performed using fluorescent *in situ* hybridization. However, this was deemed ineffective, so new procedures involving methods such as array comparative genomic hybridization and real time polymerase chain reaction have been implemented. As PGS technology develops, ethical issues arise, as well as a need for further research.

Keywords: preimplantation, genetic, screening, PGS, reproductive, University of Guelph

Introduction

With the continued discoveries and advances made in the field of genetics, and with the ongoing advancement of molecular techniques, there comes the opportunity for real life application for the purpose of improving human life. One way that genetic information and technology has been applied to everyday life is with the implementation of genetic screening. Genetic screening takes many forms and involves many different molecular techniques, but has the overall encompassing purpose of detection of a disease or risk that can be addressed through medical or dietary intervention, or through counselling to improve the outcome for the individuals and families involved (Burke et al., 2011). Genetic screening involves the evaluation of a large group of individuals without symptoms for the purpose of detecting numerous conditions, while genetic testing involves testing an individual with signs or symptoms of a specific genetic condition or with no signs or symptoms but with a family history of a specific genetic disease (American Academy of Pediatrics, 2001; Burke et al., 2011). Through the use of the knowledge gained from genetic screening, an educated professional can work with the affected individuals and families to ensure they are provided with all of the information and support needed to make an informed decision regarding further actions that may be taken.

Different Types of Genetic Screening

Genetic screening can involve newborn screening, family history assessment, or reproductive genetic screening (Burke et al., 2011). Each screening technique targets a different group of individuals with different suspected risks. While the types of genetic screening vary in which populations are targeted, all screening techniques utilize molecular procedures and share a common goal of early identification for the purpose of intervention, whether that involves medical or dietary intervention, or counselling.

Newborn Genetic Screening

Newborn genetic screening was designed for the purpose of identifying infants with conditions where early implementation of treatment is beneficial (Burke et al., 2011). Newborn screening tests for metabolic, hematologic, and endocrine abnormalities, ultimately allowing for early intervention to reduce infant mortality (American Academy of Pediatrics, 2001). Parents are informed of the procedures involved in newborn screening, as well as the associated risks and benefits, and then given the option of refusing screening of their newborn (American Academy of Pediatrics, 2001). Newborn screening originally began in the 1960s in response to the genetic disease phenylketonuria, where implementation

of a specific diet within 2 to 3 weeks of birth greatly improves the outcome for affected individuals (Burke et al., 2011). Since then, newborn screening has grown, particularly with the implementation of tandem mass spectrometry technology, allowing for testing of over 50 conditions at birth (Burke et al., 2011). If a newborn tests positive for any of the screened diseases, appropriate referrals to diagnostic testing and next steps are made for the parents of the affected newborn (American Academy of Pediatrics, 2001).

Family History Assessment Screening

Screening can also occur later in life, in an asymptomatic individual who has a family history involving a genetic condition (American Academy of Pediatrics, 2001). This screening involves analysis of family pedigrees for diseases with a common genetic element, such as cardiovascular disease, diabetes, and several cancers (Scheuner et al., 1997). This type of screening involves more theoretical analysis of risk than newborn and reproductive genetic screening, and has been criticized as being less effective in detecting individuals at risk (Burke et al., 2011). If an individual is identified as at risk for the disease in question, additional preventative measures can be implemented, such as lifestyle changes or further testing to better assess the risk (Burke et al., 2011).

Reproductive Genetic Screening

Reproductive genetic screening can involve a number of different strategies, all with the purpose of increasing reproductive success, which is defined as a healthy infant outcome (Burke et al., 2011). This could involve screening individuals to determine if they are carriers, prenatal screening for disease, or screening of gametes (Burke et al., 2011). Reproductive genetic screening is unique in that it offers parents without a specific family history or known risk the opportunity to learn their chances of conceiving a child with a specific genetic condition, whether in a current or future pregnancy (Ames et al., 2015). Whether the decision to investigate this risk comes from general curiosity, from knowledge of past family risk, or after numerous miscarriages due to an unknown cause, parents have the opportunity to request screening involving technologies such as multiplex panels, whole genome sequencing, or non-invasive prenatal testing (Ames et al., 2015).

Carrier screening involves genotyping individuals to see if they carry certain recessive genes, and could therefore pass these genes onto their children and possibly conceive an individual that expresses the recessive disease (Cho et al., 2013). This screening could involve whole genome sequencing, where the entire genome of the individual being tested is sequenced through a molecular technique such as next generation sequencing or multiplex panels (Ames et al., 2015). Carrier screening can be targeted for a specific disease, such as cystic fibrosis, or more general and test for a large number of recessive diseases (Cho et al., 2013).

Prenatal screening for disease takes many forms, and often does not begin with DNA screening (Burke et al., 2011). Prenatal screening usually begins with visual examination of the fetus during an ultrasound, to screen for abnormal growth (Burke et al., 2011). If abnormal growth is observed, further testing may be recommended (Burke et al., 2011). One example of further testing is maternal blood cell testing for alpha-fetoprotein, which can indicate a risk for neural tube defects (Burke et al., 2011). Maternal blood can also be used for DNA testing, to detect aneuploidies in the developing fetus (Gil et al., 2013). Maternal blood testing has a high success rate of determining trisomies 21, 18, and 13, and when implemented in the first trimester of pregnancy, can either reassure parents early on that their child is unlikely to possess a trisomy mutation or provide parents of an affected fetus early counselling or access to earlier and safer pregnancy termination options (Gil et al., 2013).

Issues with Reproductive Genetic Screening

An issue associated with carrier screening is that although it determines the probability of conception of an affected child, it does nothing to prevent or influence whether the child inherits the disease. Although providing parents with accurate information can assist in making an informed decision, if the parents choose to conceive they are still faced with the same risks had they not known they were carriers.

The main issue associated with prenatal screening, such as maternal blood screening, is that these tests can only detect trisomies or other defects after embryo implantation has already been established. This results in unnecessary emotional stress on the parents, as they have to wait until partway through the first trimester for the results of current prenatal screening techniques, and have to then make the decision to either terminate or follow through with the pregnancy if it is determined the fetus is affected. This can cause unnecessary heartache and strain, as the parents are faced with a decision that may cause conflict between their personal ethics and what they determine to be best for their family and lifestyle. If the parents do choose to follow through with the pregnancy, they will likely be faced with a lifetime of expenses related to the disease of their affected child, such as therapy, medical expenses, and emotional counselling and support. In the case of some lethal genetic diseases, the parents are not given this choice; a positive screening result means an ultimately unsuccessful pregnancy. This also results in unnecessary heartache and stress for the affected parents.

To combat these issues, a screening technique would need to be developed that could accurately determine if the fetus would inherit the disease, while at the same time occurring early enough to limit parental emotional attachment to the fetus, preventing stress and heartbreak and allowing for the parents to make an informed decision regarding the outcome of their pregnancy. This seems like an impossible feat, but with the development of preimplantation genetic

screening, researchers have developed a reproductive genetic screening technique that meets these criteria.

Preimplantation Genetic Screening

Preimplantation genetic screening (PGS) is a type of Reproductive Genetic Screening that involves embryo biopsies and DNA testing prior to embryo implantation in the uterus (Hens et al., 2012). PGS technology is used in conjunction with in-vitro fertilization (IVF), and aims to detect genetic conditions and increase the overall chance of a successful, single embryo pregnancy for couples with low fertility (Kang et al., 2016). PGS is primarily used for aneuploidy screening, but can also be used to detect genetic conditions that accompany other chromosomal abnormalities, such as chromosomal translocations or larger gene deletions (Ginsburg et al., 2011). This differs from preimplantation genetic diagnosis (PGD), performed for single-gene defects and translocations (Ginsburg et al., 2011). PGD would typically be utilized in a situation where a couple knows that they are carriers for a specific genetic condition, or following a family history assessment where this information is discovered, while PGS would be used in a situation where multiple failed pregnancies have occurred, and it is expected this is due to aneuploidy or due to an unknown factor (Ginsburg et al., 2011). PGD and PGS can both be classified under the broader category of preimplantation genetic testing (PGT) (Ginsburg et al., 2011). When PGS technology was first implemented in the 1990s, the procedure was performed on day 3 embryos; however, it was determined that this could be detrimental to embryo survival, due to the invasive nature of the procedure, so PGS now occurs at the blastocyst stage, which is typically 5 days post fertilization (Kang et al., 2016). PGS on day 3 embryos can also be inaccurate due to mosaicism within the embryo at this stage of development (Ginsburg et al., 2011).

After the embryos have been biopsied and screened for aneuploidy, the euploid embryos are selected and prepared for transfer, while the aneuploid embryos are analysed to confirm diagnosis and then discarded (Harper et al., 2010). It has been reported that the most common cause for failed implantation during IVF is embryo aneuploidy; by using PGS to detect and then discard the aneuploid embryos prior to embryo transfer, the number of failed implantations greatly decreases (Kang et al., 2016). PGS has also been reported to decrease miscarriage rates in couples undergoing IVF (Kushnir et al., 2016). What PGS aims to avoid is establishing a pregnancy where heart motion and fetal growth is observed by the couple and fetal death occurs, a situation that can lead to significant emotional distress and a financial loss (Meldrum, 2013). PGS was implemented to address the need for a high accuracy screening technique that can be implemented very early in embryo development.

Preimplantation Genetic Screening Techniques and Molecular and Cellular Genetics

PGS involves embryo biopsy followed by genetic screening, which can be done using a number of molecular techniques (Hens et al., 2012). PGS can begin when blastocysts have been obtained through standard IVF procedures; this involves controlled ovarian hyperstimulation, oocyte retrieval, *in vitro* fertilization, and incubation to allow for aging to the blastocyst stage (Kang et al., 2016). Before embryo biopsy, embryos are evaluated for viability based on the trophoblast—the outer cells of the blastocyst that later develop into part of the placenta (Gardner et al., 2000). The trophoblast is assessed on the number of cells forming a cohesive epithelium, the number of cells forming a loose epithelium, and the number of large cells, and then given a score based on these qualities (Gardner et al., 2000). Embryos must receive a certain trophectoderm score to then be biopsied, ensuring that only high quality embryos that are more likely to survive are utilized (Kang et al., 2016). Embryo biopsy first involves immobilization of the embryo with a holding pipette, followed by laser pulses to penetrate the zona pellucida (Kang et al., 2016). A biopsy pipette is then used to remove anywhere between one and five cells from the trophoblast in a trophectoderm biopsy (Kang et al., 2016). Previously, one cell was removed from the part of the embryo destined to become the fetus; however, it was found that this could be detrimental to embryo development (Ginsburg et al., 2011). It has now become more common to use a trophectoderm biopsy, which involves biopsy of the trophoblast, as this leaves the cells destined to become the fetus undisturbed and still provides all of the required genetic information (Ginsburg et al., 2011). These cells are removed from the embryo through gentle traction and laser pulsation (Kang et al., 2016). The collected cells must then be analyzed for genetic defects, which can be done through a number of different molecular techniques (Hens et al., 2012).

Fluorescent in Situ Hybridization (FISH)

Historically, PGS for aneuploidy was performed using fluorescent *in situ* hybridization (FISH) (Hens et al., 2012). FISH is based on the concept that single stranded DNA will anneal to complementary DNA (Gole, 2005). After the cells have been collected from the blastocyst, FISH is used to selectively stain specific DNA sequences with fluorescent markers (Zucker, 2006). This is done through constructing a DNA probe complementary to the DNA sequence of interest, and labelling this probe with the fluorescent markers (Gole, 2005). In PGS, the DNA probes constructed are associated with aneuploidy or other genetic defects, such as chromosomes 13, 15, 16, 17, 18, 21, 22, X, and Y (Rubio et al., 2013). Which probes are constructed varies depending on what areas of the genome are being examined (Rubio et al.,

2013). The target DNA collected from the embryo is fixed to a glass microscope slide, and then hybridized to the labelled DNA probe (Gole, 2005). After hybridization, the DNA is visualized using an epifluorescence microscope (Gole, 2005). This procedure allows for detection, analysis, and quantification of genetic abnormalities within the cell, therefore indicating the abnormalities present in the embryo from which the cell was removed (Zucker, 2006).

FISH has improved speed and spatial resolution when compared to other *in situ* hybridization methods (Gole, 2005). Another advantage of FISH is that the results are easier to interpret than a karyotype (Zucker, 2006). Although FISH has been the primary method behind PGS, recent studies have demonstrated that FISH is ineffective at detecting aneuploidies, and the use of FISH for PGS has since been discouraged clinically (Hens et al., 2012). Through comparing FISH results to the results of newer and more accurate technologies, such as array comparative genomic hybridization (aCGH), it was shown that FISH results would often label normal embryos as aneuploidy, leading to discarding of normal, viable embryos (Balmir et al., 2013). FISH can also lead to mislabelling aneuploidy embryos as normal, as typically not all of the chromosomes are tested during FISH, meaning that an abnormality on a chromosome that was not tested could be missed (Rubio et al., 2013). FISH technology in PGS has since been eliminated, and has been replaced by newer genetic screening methods that have the ability to accurately screen all 24 chromosomes for defects (Kang et al., 2016). These newer screening methods include array comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) array, next generation sequencing (NGS), and real time polymerase chain reaction (qPCR) (Yang et al., 2015). It is believed that these array based techniques for aneuploidy testing not only provide more accurate information, but also improve the overall IVF outcome in terms of live birth rates when compared to FISH (Hens et al., 2012).

Array Comparative Genomic Hybridization (aCGH)

aCGH is currently the most common and most widely used aneuploidy screening technique in PGS (Fiorentino et al., 2014). aCGH utilizes similar methodology and procedures to FISH; however, it involves whole genome analysis (Vestergaard et al., 2013). The target DNA from the cell taken from the blastocyst is isolated and fluorescently labeled (Maloy et al., 2013). Next, DNA is taken from a genetically normal reference source and fluorescently labeled a different colour from the target DNA (Maloy et al., 2013). The two samples are hybridized together in a 1:1 ratio, and the two coloured fluorescent DNA samples, typically red and green, are visually compared to determine similarities and differences in DNA content (Maloy et al., 2013). The purpose of aCGH is to detect DNA copy number changes, as well as to determine where deletions or additions to the genome have

occurred (Maloy et al., 2013). In PGS, aCGH can be used to detect common aneuploidies such as trisomies 21, 18, and 13, and monosomy X (Vestergaard et al., 2013). aCGH has significant advantages over karyotyping and FISH, such as improved sensitivity for detection of genome abnormalities, decreased time required for the test to be performed, and higher resolution (Vestergaard et al., 2013).

Single Nucleotide Polymorphism (SNP) Array

SNP arrays are the highest resolution array based genomic test currently available (Maloy et al., 2013). Unlike aCGH, hybridization of both target DNA and reference DNA is not required; instead, fluorescently labeled target DNA is hybridized directly to the SNP array, to detect single nucleotide polymorphisms in the DNA sample (Maloy et al., 2013). SNP arrays have increased sensitivity when compared to other array based methods as they detect amplifications, deletions, mosaicism, loss of heterozygosity, and uniparental disomy, as well as provide a highly accurate copy number assessment (Maloy et al., 2013). When comparing SNP arrays to aCGH, SNP arrays have some disadvantages in terms of copy number variation detection; this is due to the high level of sensitivity and resolution involved with SNP arrays (Maloy et al., 2013). Analysis of copy number variation involves a more broad visualization of the cell genome, compared to the specific in depth visualization SNP array provides (Maloy et al., 2013).

Next Generation Sequencing (NGS)

The use of NGS for identifying aneuploidy embryos in PGS is still relatively new, and is not yet common in a clinical setting (Hens et al., 2012). NGS involves parallel sequencing of millions of DNA fragments that make up the entire genome, followed by alignment of these sequences, resulting in an ordered readout of the genome at the base pair level (Maloy et al., 2013). This technique involves the use of bioinformatics and computer analysis, and is not readily available in all laboratory settings (Maloy et al., 2013). It has been determined, however, that NGS is a reliable method for determining chromosomal copy number variations in PGS, and when used in tandem with aCGH, the two techniques showed the same results (Fiorentino et al., 2014). NGS offers several advantages when compared to aCGH, such as reduced cost, enhanced detection of partial aneuploidies due to increased resolution, ability to detect mosaicism, and potential implementation of sequencing libraries to reduce human error (Fiorentino et al., 2014). NGS is an emerging technology in the field of PGS, and its use in a clinical setting may increase as more research is done.

Real Time Polymerase Chain Reaction (qPCR)

qPCR also provides the ability to accurately screen the entire genome for chromosomal abnormalities, and is another prominent technology associated with PGS (Kang et al., 2016). qPCR is used to quantify the amount of DNA

expression in a cell (França et al., 2012). To perform qPCR, RNA must first be extracted from the cell removed from the blastocyst, followed by synthesis of cDNA from the RNA using reverse transcriptase (França et al., 2012). Once the cDNA has been synthesized, gene expression can now be quantified using PCR, which involves denaturing the double stranded DNA into single stranded DNA, annealing of specific primers, and elongation via Taq polymerase (França et al., 2012). qPCR is used to measure the amplification of the target DNA after each round of PCR, through the use of fluorescent labeling, allowing for quantification of DNA expression (França et al., 2012). When applied to PGS, qPCR can be used to examine expression levels of molecular markers on each chromosome, and if expression levels are higher or lower than expected, this indicates aneuploidy (Yang et al., 2015). When comparing aCGH and qPCR, aCGH has a better resolution; however, qPCR results in fewer false positives for aneuploidy screening (Yang et al., 2015). Some problems associated with using qPCR for PGS include designing primers to accurately amplify the correct molecular markers and optimizing melting temperatures and time intervals (Yang et al., 2015). Another issue associated with qPCR for PGS is that for rapid overnight diagnosis, to allow for fresh embryo transfer, a qPCR machine with 384 wells would be required to detect each chromosome's expression level for one embryo, which is not feasible in most genetics laboratories (Yang et al., 2015).

Preimplantation Genetic Screening and Genetic Counselling

With the continued advancements in the field of medical genetics, and the increasing availability of personal genetic information, there comes a need for accurate communication of this complex information and what it means on a broader scope to impacted individuals (Bhat, 2015). Genetic counselling aims to provide patients with accurate genetic information as well as support in regards to decision making, such as future testing and available procedures (Zaccaro and Freda, 2014). The underlying goal of genetic counsellors is communication, for the purpose of giving affected families or individuals a regained sense of control (Albada et al., 2014). It is important that the affected individuals make their own decisions and the genetic counsellor acts as a neutral party; while genetic counsellors work to help individuals and families determine their preferred course of action, the genetic counsellor must keep their own opinions and feelings separate (Sahin et al., 2014). The correct course of action varies with the patients' personal morals and standards, and therefore must be determined by the patient themselves, while the genetic counsellor provides support and information regarding available resources (Albada et al., 2014).

In terms of reproductive genetic counselling, preimplantation genetic screening may be discussed as an option for couples when multiple miscarriages have occurred,

whether due to a known aneuploidy or unknown factors, or when several failed rounds of IVF have occurred (Ginsburg et al., 2011; Meldrum, 2013). PGS may also be advised for women over 37, as PGS has been shown to improve clinical pregnancy and live birth rates in this age group, and as the chance of aneuploidy increases with maternal age (Kang et al., 2016). In general, in any situation where aneuploidy is a possibility, PGS may be discussed as an option, as this is what PGS primarily screens for (Ginsburg et al., 2011). PGS is one of many screening options available to parents, and although this procedure may be the right fit for one couple, not every patient will find this procedure to fit with their personal morals and principles. Genetic counsellors must communicate how PGS works as well as the advantages and disadvantages of this procedure to parents in an effective way that does not influence the parents to make a certain decision.

Genetic counselling has historically followed a non-directive approach—where genetic counsellors provide information and support without engaging in value clarification—to avoid influencing the patient to choose a certain option (Vortel et al., 2016). Recent studies, however, have illustrated that this approach may not always be possible or the most valuable, and can result in patients feeling frustrated or abandoned, particularly when difficult decisions such as those involved with prenatal genetics are being discussed (Vortel et al., 2016). This is applicable to PGS as the decision to utilize this screening technique can cause conflict between personal morals and needs, and can force patients to critically evaluate their own opinions and worldviews. Some patients may feel conflicted on the ethics behind PGS, or whether PGS is worth the financial and emotional cost. In situations such as these, shared decision making may be more appropriate, where the patient and genetic counsellor collaborate to make the best decision based on available information and the patients' preferences (Vortel et al., 2016). Using this method in a genetic counselling setting could provide patients with the needed support to decide whether PGS is right for them.

The Ethics of Preimplantation Genetic Screening

As previously discussed, PGS is not the correct choice for everyone, and the decision to use this screening method must be based on the patients' personal ethics and moral code. Some patients may believe that life begins when sperm and ova unite, and therefore believe that it is unethical to use PGS to screen embryos and select the best candidate for a successful pregnancy. Other individuals who do not hold this belief may not believe screening embryos to be unethical, and may choose to utilize PGS. As PGS becomes more widely known and implemented as a common reproductive screening technique, the personal ethics of the prospective parents are not the only ethics that must be considered; what society

considers to be ethical and morally right and wrong must also be evaluated.

One question that has arisen is when PGS may be used and what legal regulations should be put in place. Advances have been made with this technology such that screening for susceptibility to cancer and late onset diseases is now possible (Robertson, 2003). PGS has also been used to screen for a human leukocyte antigen (HLA) match for an existing child, to obtain a bone marrow or core blood transplant (Robertson, 2003). If the child must grow up knowing that they were conceived to save a sibling, this raises the moral question of a child's "right to an open future," and the question of how this will impact their view of self and the world (Hens et al., 2012). The use of PGS for non-medical sex selection has also become prevalent (Hens et al., 2012). The United States has seen an increase in sex selection through PGS for the purpose of family balancing in recent years (Ginsburg et al., 2011). The American Society for Reproductive Medicine now discourages the use of PGS for sex selection, but allows application of this method after evaluation of individual situations (Ginsburg et al., 2011). Sex selection also occurs for cultural reasons in countries where male children are valued over female children, such as China and India (Robertson, 2003). This is considered unethical by western standards; however, it can be argued that screening for female embryos prior to implantation prevents couples from resorting to abortion of a female fetus should they choose not to use PGS (Robertson, 2003).

Some people believe that PGS technology could lead to "designer babies," where preimplantation genetic selection occurs for more favourable traits that are unrelated to health, such as eye colour or height (Hens et al., 2012). Others argue that it is a moral obligation to take into account the welfare of the future child, and to select embryos whose lives will likely be better (Hens et al., 2012). While PGS clearly benefits those who are screening for lethal aneuploidies, as they would not produce a viable fetus otherwise, it is more difficult to determine where else it is ethical to apply this technology. One example of a present application of PGS for a non-lethal aneuploidy is screening for trisomy 21, which results in Down syndrome (Twisk et al., 2007). PGS for Down syndrome prevents the establishment of a trisomy 21 embryo, and therefore prevents parents from having to make the difficult decision of whether or not to abort a Down syndrome pregnancy; however, a moral question arises as to whether Down syndrome screening should be deemed ethically acceptable (Twisk et al., 2007). Down syndrome leads to numerous developmental abnormalities, such as cognitive impairment, but is not a lethal condition; individuals with Down syndrome often live until adulthood (Michael et al., 2012). While there are many health risks associated with Down syndrome, it becomes less clear as to whether application of PGS technology to screen for Down syndrome is morally acceptable when social factors and quality of life are taken into consideration (Michael et al., 2012).

Another question regarding the ethics of PGS is how to make this technology available to everyone. Financially, it has been questioned whether the government should cover PGS, whether partially or in full. The Ontario government currently covers one round of IVF for qualifying individuals, and has set aside 70 million dollars annually to be used for reproductive services (Motluck, 2016). There has been some debate as to how this service will be allocated in a fair and consistent manner—specifically, how should it be decided who qualifies for this service (Motluck, 2016). If one round of IVF is covered by the Ontario government, it is conceivable that in the future PGS may be covered as well. IVF with PGS is more expensive than IVF on its own, and PGS only improves fertility rates in a specific subset of the population—specifically, older women who have viable euploid embryos (Thyer et al., 2015). Presently, PGS is not used by a large portion of the population, partially due to cost and partially due to a lack of necessity (Robertson, 2003). If the government chooses to financially cover PGS, would the taxpayers who choose not to use this service be expected to contribute? And if the government chooses not to cover PGS, does this prevent the whole population from utilizing this technology, and restrict availability to the wealthy? PGS is currently not covered by most insurance agencies, so financial accessibility of this technology is an issue (Twisk et al., 2007). Availability of a clinic that performs PGS can also be an issue, as this procedure is usually carried out in clinics located in large cities (Robertson, 2003).

Future Research

As PGS technology and methods continue to improve, the application of PGS in a clinical setting is becoming much more common. One current established issue with PGS is that the embryo biopsy stage is very invasive, and can result in damaged or non viable embryos (Kushnir et al., 2016). It was observed that among women under the age of 37, PGS did not improve pregnancy and live birth rates, nor reduce miscarriage rates (Kang et al., 2016). This demonstrates that although implementation of this technology to address infertility due to aneuploidy was logical, technological and methodology improvements can be made (Kang et al., 2016). Another problem with PGS is that women with low quality oocytes, or oocytes that cannot reach blastocyst stage, are advised not to participate in PGS due to the highly invasive nature of the procedure; this represents a whole cohort of women with fertility issues who are not benefiting from PGS (Kushnir et al., 2016).

One way these issues could be addressed is through biopsy of an older embryo. PGS used to be performed solely on day 3 embryos; however, it was determined that this could be detrimental to IVF outcomes (Kang et al., 2016). Researchers then attempted embryo biopsy at the blastocyst stage, 5 days post-fertilization, and found this to be less detrimental to embryo development (Kang et al., 2016). In theory, performing a biopsy on an embryo older than 5 days

would be even less detrimental to embryo development; the older an embryo, the more cells it possesses, meaning that removing a few cells will have less of an impact on the overall quality of the embryo. This would have to occur before the cells of the embryo become too differentiated; if cell lineage is restricted, the embryo cannot make up for the cells that were removed and the embryo will be damaged. The drawback to this approach is the ethical implications; an older embryo may result in more of a moral dilemma for the potential parents and for society at large. The introduction of later stage embryo biopsy may benefit the women who possess low quality oocytes, as by allowing the embryos to reach a later stage before biopsy PGS may be less detrimental to embryo survival; however, women whose oocytes cannot reach the blastocyst stage *in vitro* would not benefit.

Another emerging technique that results in a less invasive procedure is a trophectoderm biopsy (Ginsburg et al., 2011). A trophectoderm biopsy involves removal of several cells, usually between one and five, from the trophoblast of the embryo, which is the part of the embryo destined to become the placenta or extra embryonic membranes (Kang et al., 2016). The trophectoderm biopsy collects cells with the required genetic information and would leave the cells destined to become the fetus untouched, which would in theory be less damaging to the embryo (Kang et al., 2016). The issue with this technology is that a trophectoderm biopsy still requires penetration of the zona pellucida and still reduces the overall quality of the embryo (Kang et al., 2016). The introduction of trophectoderm biopsy in place of a standard embryo biopsy—where one cell is removed from the portion of the embryo destined to become the fetus—could benefit women who possess low quality oocytes, as the cells destined to become the fetus would be undisturbed by trophectoderm biopsy. Women with oocytes that cannot reach the blastocyst stage, however, would still not benefit. It has not been determined experimentally whether PGS using trophectoderm biopsy improves live birth rates for patients undergoing IVF compared to patients who use PGS with the standard embryo biopsy; however, the benefits of a worldwide procedural shift to trophectoderm biopsy in PGS requires further investigation (Ginsburg et al., 2011). Presently, trophectoderm biopsy is becoming more common in PGS; however, standard embryo biopsy is often still utilized.

Although both of the above areas of research could result in a less invasive procedure, therefore resulting in less overall damage to the embryo, both rely on the embryo reaching the blastocyst stage *in vitro*. As mentioned above, women who possess embryos that cannot reach the blastocyst stage during *in vitro* fertilization cannot attempt PGS, and therefore cannot benefit from this technology (Kushnir et al., 2016). When IVF is used without PGS, embryo transfer at both the cleavage stage (day 3) and the blastocyst stage (day 5) are common (Papanikolaou et al., 2006). This is because embryo transfer at both stages has associated strengths and weaknesses; while blastocyst stage transfer allows for clearer

selection of a morphologically normal embryo, cleavage stage transfer is a better option when embryos cannot reach the blastocyst stage in culture (Papanikolaou et al., 2006). While IVF is available to women whose oocytes cannot reach the blastocyst stage *in vitro*, PGS must be performed at the blastocyst stage, as PGS on cleavage stage embryos has been shown to be too inaccurate and detrimental to embryo survival (Ginsburg et al., 2011; Kang et al., 2016). To accommodate these women, PGS must be implemented before the blastocyst stage, so that the embryos can be screened and transferred into the uterus earlier in development, allowing for the blastocyst stage to be reached *in utero*. For this to occur, more precise procedural tools could be used; for example, PGS may be less detrimental to day 3 embryos if a thinner biopsy pipette or lower intensity laser is used (Kang et al., 2016). These improved procedural tools may become available over time, as technological advances are made in this area. Removal of one cell from the trophoblast—rather than up to five cells—may also be less detrimental to these younger embryos (Kang et al., 2016). Another possible area of research to address this issue could be the conditions under which the embryo biopsy is performed, as different chemicals in the embryos' environment may be more or less detrimental to embryo survival. For example, osmolarity of culture media—specifically NaCl concentration—is a major factor that impacts embryo development *in vitro* (Liu & Foote, 1996). Higher concentrations of NaCl have been shown to depress embryo development, so evaluating the culture media used for these highly sensitive oocytes may be worthwhile (Liu & Foote, 1996). The environment the embryos are exposed to, such as available nutrients and oxygen content, can also result in epigenetic changes in the embryo DNA, which is worth evaluating when trying to create the best media culture for embryo growth and survival (Liu & Foote, 1996).

When comparing IVF outcomes for patients who utilized PGS and those who did not, the use of PGS for women older than 37 resulted in higher live birth rates and lower miscarriage rates (Kushnir et al., 2016). It would be easy to conclude that PGS used in conjunction with IVF benefits women over the age of 37. However, patient selection bias must be taken into consideration; the women who are approved for PGS generally have higher quality oocytes and a relatively good prognosis for IVF outcome (Kushnir et al., 2016). Women with poor quality oocytes—and therefore poor IVF prognosis—or oocytes that cannot reach the blastocyst stage are usually advised against using PGS, as the cost is often not worth the benefit (Kushnir et al., 2016). It is therefore inaccurate to conclude that PGS improves fertility outcomes for women over 37, as biased patient selection plays a role in the improved fertility rates observed. To conclude with certainty that PGS is a benefit to women over 37, the issues outlined for women with poor IVF prognoses must be addressed through improving PGS and making this screening method more accessible to these women. In a randomized study independent of age, PGS resulted in lower live birth

rates and successful embryo transfers than non-PGS cases (Kushnir et al., 2016). This provides strong correlational evidence that PGS results in damage to the embryo. While implementation of PGS to detect genetic conditions prior to implantation is logical, there is a lot of room for improvement before it can be concluded that this screening method is beneficial and improves fertility outcomes (Kang et al., 2016).

Conclusion

PGS has come a long way since it was first implemented in reproductive genetic screening. Altering embryo biopsy to day 5 instead of day 3, shifting away from a standard embryo biopsy towards trophectoderm biopsy, and implementation of new technology such as aCGH, SNP array, NGS, and qPCR to replace FISH are examples of a few changes in PGS that have occurred to improve the accuracy and efficiency of this screening method (Ginsburg et al., 2011; Kang et al., 2016; Yang et al., 2015). These changes result in accurate information being provided to prospective parents more quickly. PGS was implemented to address the need for a high accuracy screening technique that can be implemented very early in embryo development. If PGS is effective, it avoids the significant emotional distress and financial loss associated with the establishment of a pregnancy where heart motion and fetal growth is observed followed by fetal death (Meldrum, 2013). Providing parents with accurate information earlier also allows for access to earlier and safer pregnancy termination options. As PGS becomes more common and more socially acceptable, there is a need for effective explanation of this technology and the information it provides through genetic counselling, as well as several ethical concerns that must be addressed on the individual and societal levels. As this technology is still relatively new, there is not much known about the long-term impacts of PGS on the resultant individuals, particularly in terms of health. As the most common cause of failed implantation during IVF is embryo aneuploidy, using PGS to detect and then discard the aneuploid embryos prior to embryo transfer is logical to address this issue, or to address other genetic conditions that may arise (Kang et al., 2016). Although this technology has greatly improved since it was first introduced, there are still many improvements that can be made to ensure this technology is accessible and beneficial to all.

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