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Deconstructing Preimplantation Genetic Diagnosis

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ABSTRACT

In recent years, infertility rates have been on the rise, with 19% of women aged 15-49 having difficulties getting pregnant and 26% of women having difficulties carrying such fetuses to term (Centers for Disease Control and Prevention, 2019). This statistic also comes in concert with recent research suggesting a rise in congenital defects and abnormalities in neonates (Silesh et al., 2021). In association with these troubling trends, however, are developments in reproductive technologies and assessments. One such of these technologies is preimplantation genetic testing, otherwise abbreviated PGD. This technique uniquely allows for the detection of chromosomal abnormalities and congenital defects and, used in conjunction with IVF treatments, has seen implementation in around 5 percent of all U.S. IVF cycles ("A Microdeletion, They Called It.," 2015). Despite the hope that the testing method allows for families around the world, PGD is not without its limitations. This paper first explores PGD, determining the testing process and pinpointing its applications' current extent. It then transitions to analyzing the current methods of sampling and analysis housed in PGD, determining their benefits as well as their detriments. It finally contrasts present methods with the many proposed advances, analyzes the feasibility of such advances, and compares the benefits and limitations of these new treatment plans to conclude the best combination of developments is Blastocyst biopsy and Next Generation Sequencing for the advancement of PGD as a diagnostic procedure.

What is PGD?

Before any analysis can be done into PGD as a procedure, its position and components must be defined. PGD is one part of the two mechanisms under the greater label of Preimplantation Genetic Testing (PGT), alongside preimplantation genetic screening (PGS). PGD in itself operates by removing a singular cell or a small number of cells from a developing embryo and testing the DNA for abnormalities. Embryos that are clear of abnormalities are placed back into the womb, where they continue development into neonates (Flinter, 2001). Furthermore, PGD may be broken down into different methods depending on the stage of embryonic development at which the test is taken. Three of these will be referenced within this paper: polar body biopsy (PB), cleavage stage/blastomere biopsy, and blastocyst/trophectoderm biopsy. The last important point to address is terminology. PGD and PGS have been relabeled as PGT-M (PGT for monogenic disorders) and PGT-A (PGT for aneuploidies) respectively, but for the purposes of this paper, they will be addressed by their previous terminology.

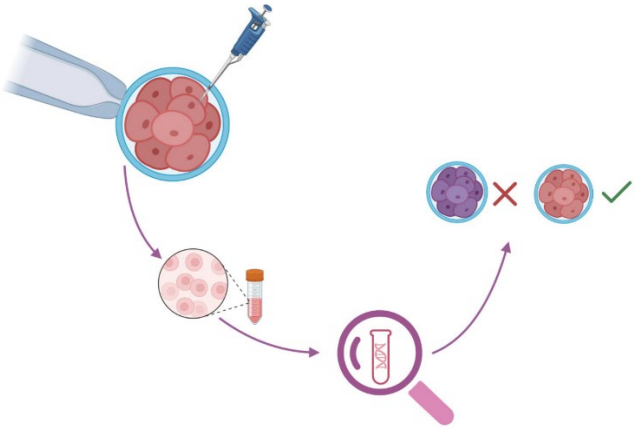

Figure 1: Image of Preimplantation Genetic Diagnosis process (Blastomere biopsy pictured above). The first step is the pipetting of the embryonic cells. Collected cells are then centrifuged and isolated. The isolated materials are then analyzed for the presence of monogenic disorders. Cells positive for monogenic disorders and identified and removed, whereas cells negative undergo further development. Created by Author with Biorender.com

What makes PGD unique?

The uniqueness of PGD as a testing option rests on its ability to detect specific abnormalities in a developing embryo, thereby providing vital information about the infant's conditions to parents and healthcare providers. Unlike its co-procedure, PGS, which detects general chromosomal abnormalities and aneuploidies, PGD is able to analyze embryonic cells to screen for the presence of far more specific hereditary disorders and abnormalities, thereby allowing couples with histories of genetic diseases to select viable embryos that have the highest chance of growing into healthy infants (Flinter, 2001). This is an especially crucial task, as recent data indicates that around one in every 33 babies in the United States have congenital defects, and around 20% of all infant deaths are tied to these congenital defects (CDC, 2018). This statistic is already at a distressing level, and the numbers will only continue to rise. In response to this, an increasing number of fertility clinics have begun to offer tests related to PGD (Stern, 2014). This much-needed response is reflective of the growing recognition of the imperativeness that proper analysis methods, such as PGD, are used as prevention to lessen the impact of birth defects on the community.

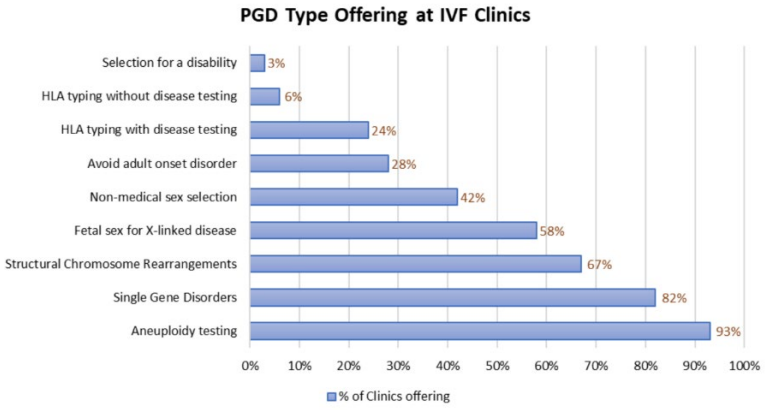

Figure 2: Graphical representation of the various PGD-related offerings at clinics. As demonstrated above, testing for aneuploidies is most commonly offered, although as research advances and public perceptions regarding PGD improve, we can expect to see a rise in the offerings of the other tests. Data taken from Stern, 2014.

What are the external uses of PGD?

Although its main applications appear to derive from human conceptional therapies, PGD has also seen use in livestock production. According to research conducted analyzing this sphere, using PGD in pigs could aid in greater control over artificial selection. For example, selection for traits that confer greater muscle content in pigs could provide food for a much larger scope of people, without the time length that traditional artificial selection practices entail. Additionally, the processes for PGD are used in humans, reducing the likelihood of a disease-carrying embryo, and could also be applied to farm animals, thereby reducing the risk of disease transmissions within flocks. Research into this cause doesn't just impact the livestock themselves, however, it also has vast implications for climate change and the future of organ donations. As the world population grows ever larger, the need for more products with less waste becomes more pressing; livestock, such as pigs, greatly influence the release of greenhouse emissions into the atmosphere, meaning that proper PGD methods could lead to this increase in food products with less pollution overall. Coupling this with the fact that pigs share many genetic, anatomical, and physiological similarities with humans, which could see advances in the fields of pathology and transplantation, research into PGD advances will have a positive ripple effect on our society (Fowler et al., 2018).

Methods

Peer-reviewed articles published in reputable medical journals or governmental agencies and accessible through scholarly databases, such as EBSCO, were used to analyze the question within this paper. The results of research papers focused specifically on the use of PGD methods (current and advanced) and their relation with diagnostic and implantation potential were integrated into a comprehensive base to determine the most effective methodic combination.

Current Sampling Methods

PGD is a developing technique that still carries both benefits and limitations with it, which vary considerably depending on the stage at which the diagnosis is undertaken. This section will discuss the three main techniques, and their associated stages, that are currently used to sample embryos.

Polar Body Biopsy

At the polar stage (PBB), clear benefits are noted, as this method is less harmful to the developing embryo and provides the patient with ample time for further testing. Additionally, due to the fact that polar bodies are present in a woman before fertilization, the specific oocytes that have the highest developmental potential can be selected for further development. Even though the matter of timing provides credence to PBB as a sampling method, there are still significant drawbacks to this method. First, PBB cannot track paternally-derived disorders and can only detect monogenic disorders to a limited extent. This means a reduced scope for the procedure in general, and in the case of affected paternal genetic contribution, a 25% chance of an incorrect diagnosis with the use of the polar body method. Venturing once more to embryonic aneuploidies, the polar body method typically requires the extraction of two polar bodies and may show distorted results of aneuploidies should the second polar body not be extracted (Ven et al., 2008). This would almost certainly culminate in a doubled cost for the patient performing PBB, with reduced certainty in results (Stern 2014). Nevertheless, the earliness at which PBB is conducted allows individuals the time necessary to consider their available options, an especially useful consideration with reproductive laws today.

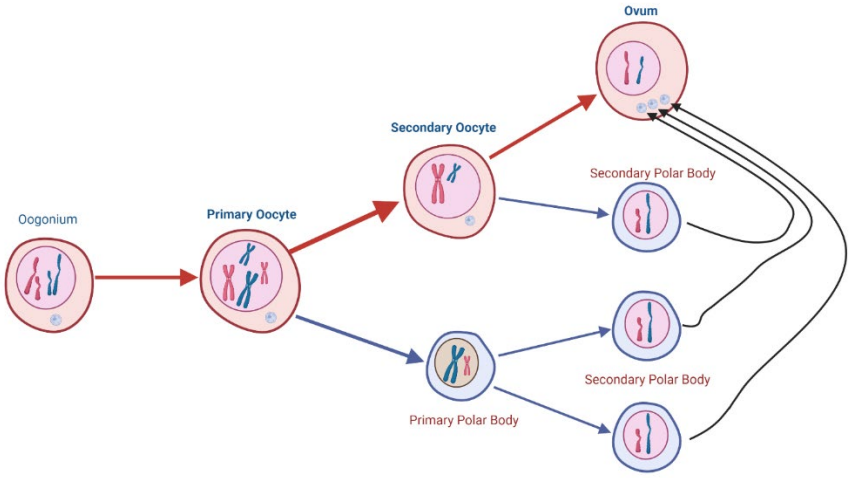

Figure 3: Image of Polar body differentiation within female meiosis. Polar bodies contain nucleic DNA, but very little cytoplasm and other materials, as a result of uneven meiotic division, where these materials are shunted to the ovum. Female meiosis can result in up to three polar bodies, which are extracted and tested in PBB sampling. Created by Author with Biorender.com

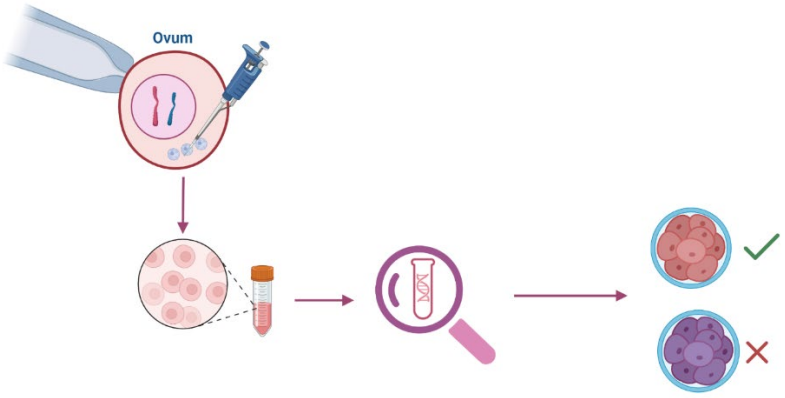

Figure 4: Image of the Polar body method of sampling. Polar bodies are extracted from the ovum, centrifuged, and then analyzed to determine the genes present. This analysis can provide insight into the genomic makeup of the ovum itself. Created by Author with Biorender.com

Blastomere Biopsy

The next major method of PGD is the Blastomere, or Cleavage Stage, biopsy. According to Harvey Stern, this method extracts a cell from the embryo during the 6-8 cell stage and is currently the most popular method of PGD (2014). Unlike the polar body method, blastomere biopsy can detect maternal and paternal defects, a wider and much-needed scope (Bar-El et al., 2016). The major pitfall of this practice is not due to issues with detection, however, but rather another embryonic practice: mosaicism. According to Sina Abhari and Jennifer Kawwass (2021), mosaicism is a process by which multiple cells with different genotypes are present in an embryo; a bridge between euploid and aneuploid embryos. Embryonic mosaicism is a common process, affecting around 15-80% of all embryos during the cleavage stage, and blastomere biopsy can raise questions about whether the extracted blastomere is an accurate representation of the entire embryo in cases of mosaicism (Stern 2014). While this detriment to analysis is severe enough

on its own, blastomere biopsy may also reduce the compaction and blastulation of an embryo, a necessary process in a successful pregnancy (Bar-El et al., 2016). These limitations are significant, and once again, could result in large amounts of investment into a sampling method with no corresponding increase in effectivity. Despite this, the timing at which the procedure takes place could provide patients with a "happy medium" in terms of proper analysis and sufficient time for decision-making.

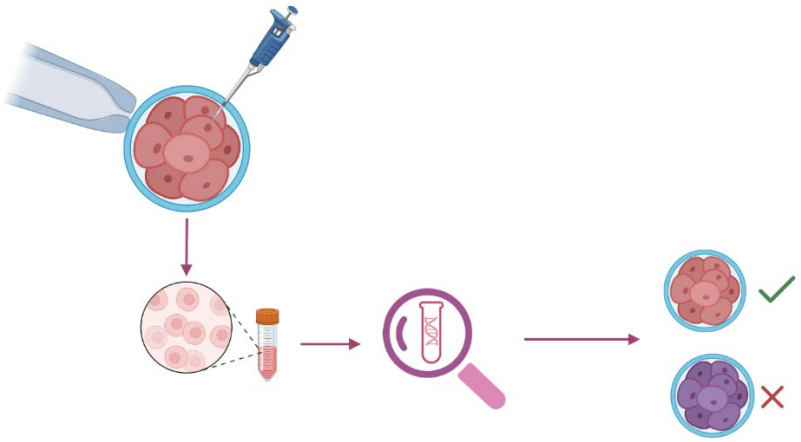

Figure 5: Image of the Blastomere biopsy method of sampling. One or two cells are extracted from the blastomere, centrifuged, and tested for the presence of monogenic disorders and defects. Despite its popularity, it is a highly invasive procedure that can severely impact the viability of a pregnancy. Created by Author with Biorender.com

Blastocyst Biopsy

The last major method of PGD is trophectoderm, or blastocyst, biopsy. In a study conducted by Harvey Stern, it was noted that, in contrast to the relationship between blastomere biopsy and mosaicism, blastocyst biopsy is able to mitigate the effects by collecting multiple cells, thereby providing a more accurate depiction of the developing embryo (2014). This collection of multiple cells also helps blastocyst biopsy combat a process known as allelic dropout, whereby an allele is inadequately amplified during the analysis procedure, and thus not categorized. In addition to these helpful additions in accuracy, blastocyst biopsy is also believed to cause the least harm to the embryo, due to the fact that only trophectoderm cells are removed and the inner cell mass is avoided. The only major issue regarding blastocyst biopsy is the fact that, as it is much later in embryonic development than both polar body testing and blastomere biopsy, there are a reduced number of viable embryos to biopsy; this lateness not only contributes to a reduced ability to culture embryos, but the detection process itself leads to a reduced number of embryos viable for transfer to the uterus (Stern, 2014). While this testing plan may result in a more effective screening of the embryo, its limitation of reducing viable embryos works counter to the goals of IVF treatment. Still, the major benefits of this procedure should be considered for patients seeking the best sampling method of PGD.

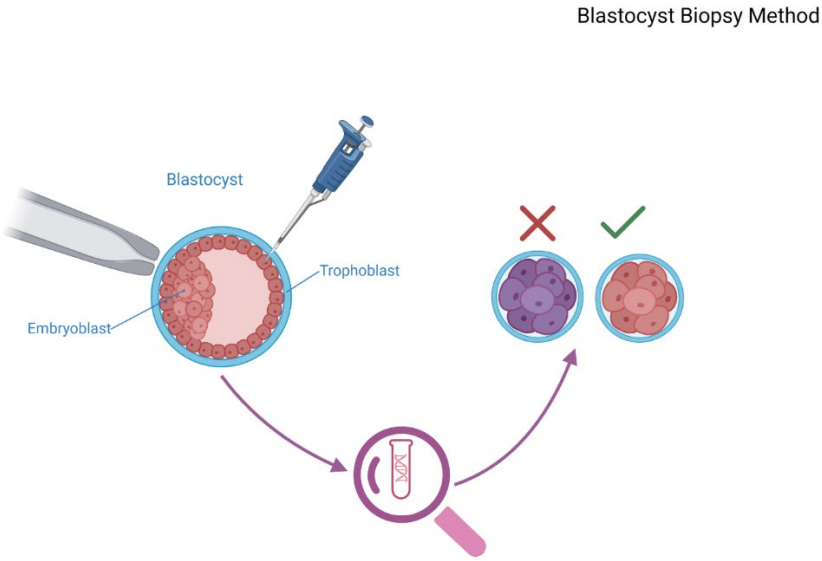

Figure 6: Image of the Blastocyst biopsy method of sampling. A small hole is incised in the trophectoderm (outside layer of the blastocyst), from which multiple cells are extracted. The cells are then centrifuged, and isolated genes are screened for irregularities. Created by Author with Biorender.com

Advances in Methods of Sampling

Though blastocyst biopsy is currently considered the most successful method of PGD, the general PGD process still has limitations that have the potential to be improved upon. One of these such issues is with sampling; a field where three significant improvements have been made.

Blastocoel Fluid Sampling

The first potential sampling-method development is called BF sampling. Unlike blastocyst biopsy, BF sampling relies on the aspiration of blastocoel fluid (BF) from the blastocyst, rather than the cells located within the complex, as noted by Shi et al. (2022). This fluid was proved to contain genomic materials, opening up the possibility of the extraction method providing information regarding embryonic sex and aneuploidies, all while presenting a less-invasive method of PGD than blastocyst biopsy. However, the method by which blastocoel is extracted, blastocentesis, has a high failure rate. Additionally, the efficiency and accuracy rates of BF sampling vary wildly between culturation studies, leaving the treatment practically ineffective for true use (Shi et al., 2022). Despite these inconsistent results, the diagnosis plan does hold promise, should more research and progress be made on the procedure before its implementation.

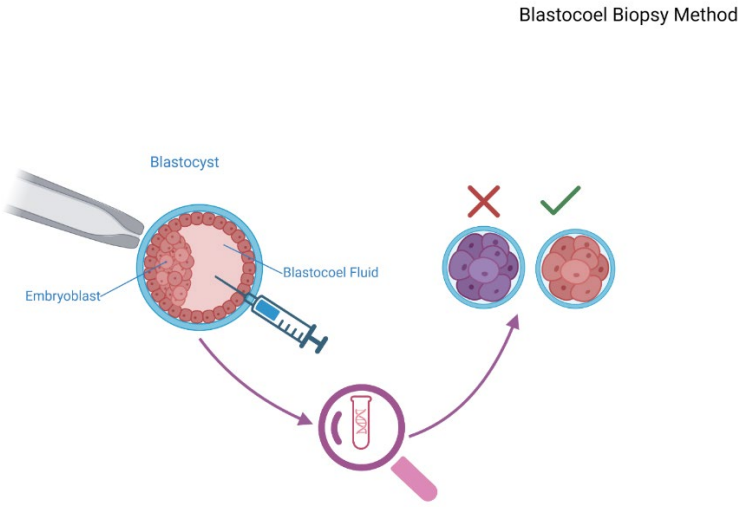

Figure 7: Image of the Blastocoel Fluid method of sampling. BF is extracted from the blastocyst, centrifuged, and then isolated for its genetic material. The genetic material is then extracted from the substance and screened for abnormalities. Created by Author with Biorender.com

PCR Testing of Cell-Free Nucleic Acids

The second potential advancement in sampling techniques is PCR testing of the cell-free nucleic acids, a substance released by embryos into the culture medium during the IVF procedure. According to Lu et al. (2016), this non-invasive procedure, according to a study conducted on the method's detection of α-thalassemias^{SEA}, yielded a higher diagnosis efficiency rate than fluorescent PCR analysis of a traditional biopsy. The method also presents a highly accurate tool for sex determination, and further tests will specify the treatment in determining X-linked disorders. Though this method does appear to significantly push forward the field of PGD, there is concern about the efficiency of the method data stemming from contamination concerns of the fluids (Lu et al., 2016). Though it is still in its beginning stages, this method yields high potential, and further testing could anchor its future in PGD testing.

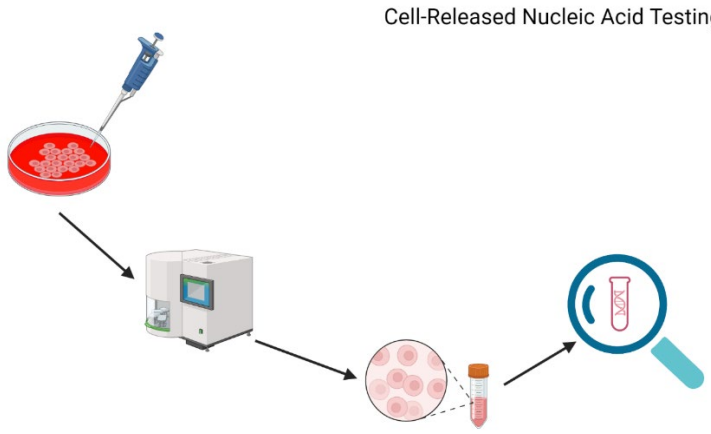

Figure 8: Image of the cell-released nucleic acid method of sampling. This substance, which can be extracted from the cell medium in IVF, is centrifuged, isolated, and then screened for irregularities. This method is currently tied to a PCR assay, though further research could venture into its applications with other analysis methods. Created by Author with Biorender.com

Time-Lapse Imaging

The third and final sampling advancement that this paper will cover is time-lapse imaging of embryos. As shown by Lemmen et al. (2008), this procedure cultures embryos and identifies the suitable embryos for single embryo transfer by monitoring them for set time parameters and logging when events in embryonic development occur (e.g. early cleavage, blastocyst formation). A distinct difference can be noted between when events occur and the relative abnormality of an embryo, thereby providing crucial information about embryo viability in selection. While this is a significant advancement, time-lapse imaging has the potential for technical and statistical errors, casting doubt upon the results that the process yields (Lu et al., 2016). With the understanding of these breakthroughs and limitations in the field, it is a requirement that more research be done before it can be truly used in common PGD testing.

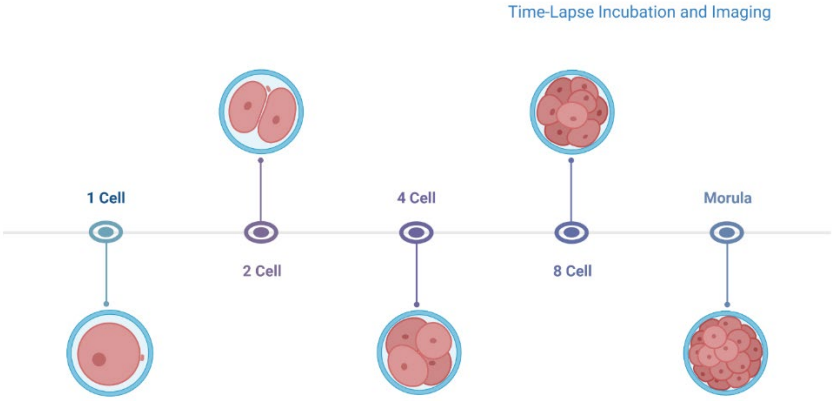

Figure 9: Image of the time-lapse imaging method of sampling. Potential embryos are incubated and their developmental progress is tracked to make estimations of their abnormality (or lack thereof). This is a non-invasive method, though it does not particularly allow for gene analysis. Created by Author with Biorender.com

Current Analysis Methods:

As with the sampling methods of PGD, analysis methods also play a significant role in the PGD process. There are currently three techniques in use for genetic analysis in PGD.

Fluorescence in Situ Hybridization

The first analysis method currently used in PGD is Fluorescence in situ Hybridization, abbreviated as FISH. As said in *Fluorescence in Situ Hybridization Fact Sheet* (n.d.), the FISH method is particularly useful in determining the location of abnormal genes on a person's chromosomes, providing valuable information for researchers about relations between clusters of genes. The FISH method works to isolate the location of an abnormal gene by preparing small strands of DNA, termed probes, that match the nucleotide sequence of the gene in question. These probes are then labeled with a fluorescent dye and introduced to a strand of the embryo's DNA. Due to the pairing nature of DNA, the strands will link together, indicating the presence and location of any abnormalities. There are three types of FISH probes, all used for a specific purpose. Locus-specific probes are constructed of a specific gene-unique sequence and are used to determine where an abnormality is located or its commonality throughout the genome. Alphoid probes, on the other hand, utilize repeating sequences that are found on every chromosome to detect aneuploidies within an embryo's DNA. The last type of probe, whole chromosome probes, is a compilation of smaller probes, used to screen

for chromosomal abnormalities. Despite these features, the FISH method has mostly fallen into the shadow of micro-array-based methods. Still, it remains useful in some regards, such as cross-species comparisons (*Fluorescence in Situ Hybridization Fact Sheet*, n.d.).

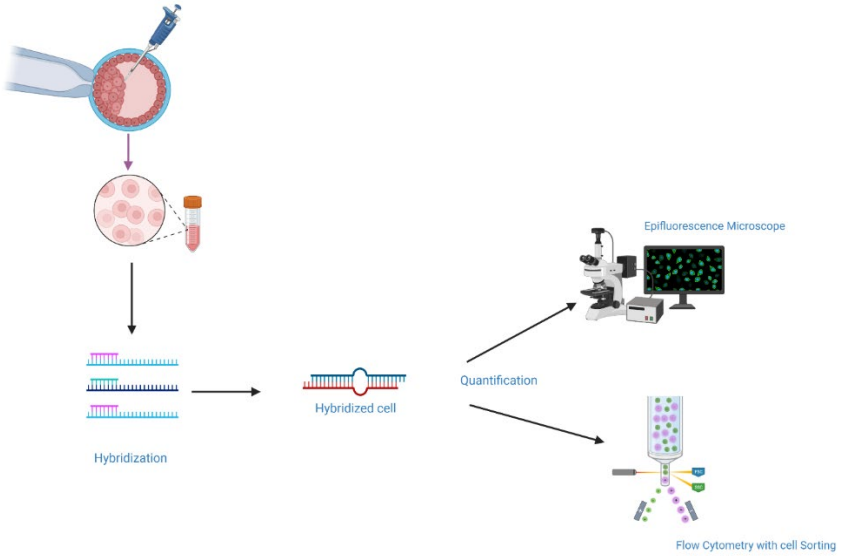

Figure 10: Image of the FISH testing. FISH testing uses probes and fluorescent dyes to target specific DNA strands and identify potential sources of misrepresentation and abnormality. It is fairly outdated, however, and it is being overtaken by other newer, and more advanced, methods. Created by Author with Biorender.com

Polymerase Chain Reaction

The second analysis method currently used is Polymerase Chain Reaction (PCR). As illustrated in the National Human Genome Research Institute (2020), much like the FISH method's use of probes, PCR utilizes synthetic segments called primers to target sequences of interest on a strand of DNA. The major difference between the procedures is in PCR's duplicative and magnifying ability. Taking advantage of the process of DNA replication, PCR can duplicate the targeted strand of DNA to nearly a billion copies, thus allowing researchers to study the genes of interest better. The main ingredients of such a reaction include DNA polymerase, primers, nucleotides, and a sequence of interest. The first step of PCR is the denaturation of the DNA strands via heat. This is followed by DNA synthesis by Taq duplicating the strand. This process is repeated multiple times to achieve the desired replication amount (National Human Genome Research Institute, 2020). This constant replication provides embryologists with ample supply for the detection of mutations or genetic diseases. Though this method is simplistic and plentiful, like any form of DNA replication, pre-knowledge is required on certain genetic diseases to identify their chromosomal location, and the PCR process is prone to mutations that can permeate through the entire sample, rendering it useless (NCBI, 2017).

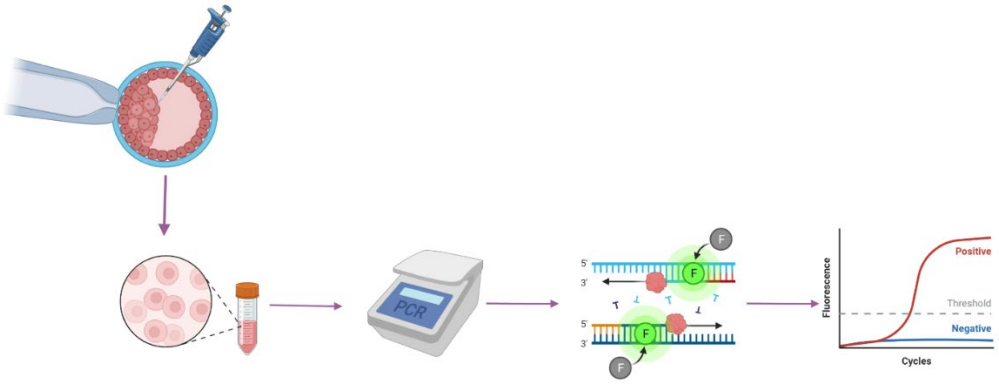

Figure 11: Image of the PCR testing. PCR testing uses primers to target specific DNA strands for duplication which, through the repeated process of denaturation, annealing, and synthesis, can then be analyzed to identify potential sources of misrepresentation and abnormality. Created by Author with Biorender.com

Comparative Genomic Hybridization

With similarities to FISH and PCR, Comparative Genomic Hybridization, or CGH, demonstrates its uniqueness as a comparison technique that can be used to evaluate chromosomal abnormalities at a speed unmatched. As demonstrated by Pinkel and Albertson (2005), to operate, the technique uses two strands of DNA, one in the control group and one from the patient. Similar to the use of probes and primers in FISH and PCR respectively, strands are labeled with brightly-colored fluorophores to identify sights of miss-replication in the DNA during CGH. Unlike the FISH method and PCR, however, CGH can cross-reference multiple transcription and translation changes across the entire genome, in comparison to the individualized approach of PCR and FISH. This does have limitations though, with larger sequences somewhat corresponding to less resolution in analysis, leading to some uncertainty about results and a requirement for double-checking with the FISH method. In current studies, CGH has proved particularly promising with a cancer diagnosis as well as with chromosomal abnormality detection, particularly single copy changes, and heritable diseases; a cornerstone for PGD (Pinkel & Albertson, 2005). CGH is quite a bit more efficient than the FISH and PCR methods, and highly accurate, but further research is still needed to develop this technique.

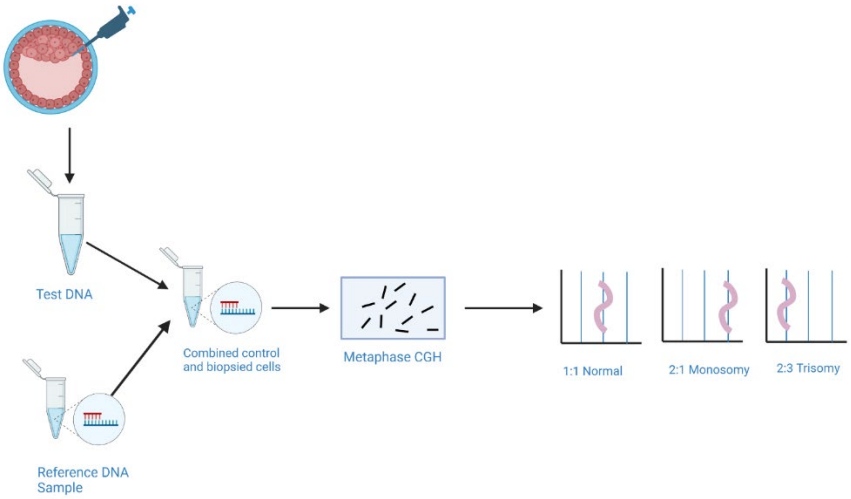

Figure 12: Image of the CGH testing. CGH testing uses the hybridization of whole DNA strands and identifies potential sources of misrepresentation and abnormality, at a larger, though less accurate scale. It is perhaps the best analysis currently in use, though it may be overshadowed by aCGH. Created by Author with Biorender.com

Advances in Analysis Methods

Aside from improvements in sampling techniques, significant developments have been made in the field of gene analysis for PGD. The current methods of PGD analysis include FISH and PCR, methods considered outdated and less-than-effective. As such, researchers have made numerous developments in diagnostic techniques. This paper will discuss five of these developments.

Multiplex qPCR

The first advancement this paper will cover is multiplex qPCR. As touched on by Lu et al. (2016), Multiplex qPCR is, in itself, two advancements with PCR: in compilation and efficiency. In contrast to regular PCR, multiplex qPCR is able to amplify multiple genomic targets in a single reaction, thereby reducing the time requirements for analysis in comparison to regular PCR, while also reducing the risk of cross-contamination (Kralik & Ricchi, 2017). While this does represent a significant advancement in testing, and the process is being advanced in terms of implantation and live birth, it is far from perfect. Multiplex qPCR has proven reliable in detecting aneuploidy, but its unreliability in detecting chromosomal abnormalities and uniparental disomy, a process in which the embryo receives chromosomes (or parts of chromosomes) from one parent and nothing from the other parent (Lu et al., 2016; Yang et al., 2015). Additionally, according to statistics by Yang et al. (2015), only 53.8% of reimplanted embryos were able to thrive until the second trimester. Altogether, while multiplex qPCR does advance the field of PGD, it does not carry with it significant beneficial changes like many of the other analysis methods mentioned in this paper.

Microarray Comparative Genomic Hybridization

Microarray Comparative Genomic Hybridization, abbreviated aCGH, is an advancement of the previous method of CGH. As mentioned by Lu et al. (2016), much like multiplex qPCR, aCGH is far more efficient than normal CGH. It is able to achieve this by labeling the DNA strands and proceeding to hybridize the strands with DNA probes or long oligonucleotides (Bejjani & Shaffer, 2006; Lu et al., 2016). This process allows for the assessment of various abnormalities, including copy number variations (CNV) and unbalanced translocations, where the embryo receives a chromosome with extra or missing genetic material from the parent. This method can be successfully integrated with the in-use methods of sampling, alongside the advanced method of BF sampling. Additionally, the implementation of aCGH in a research study found the successful detection of chromosomal abnormalities and delivery of healthy offspring (Lu et al., 2016). Again, while it does not necessarily represent a newly different method, the innovative advancement in the process does accelerate success within embryology and PGD.

Single-Nucleotide Polymorphism Microarray

While it has been around for some time, Single-nucleotide polymorphism microarray has only recently discovered its niche as a potential analysis method in PGD. Single-nucleotide polymorphisms are a common genetic variation, due to the small scale at which the mutation occurs (LaFramboise, 2009). Although most of these mutations are generally harmful, breakthroughs have been made in our understanding of SNPs which has catapulted our abilities to detect genomic disorders. As covered in LaFramboise (2009), SNP microarray works by hybridizing various fragments of single-stranded DNA. Using probes linked to targeted DNA sequences, SNP can detect single-gene disorders, imbalance translocations, and various aneuploidies (Lu et al., 2016). This is a major development that could potentially remove some of the uncertainty that comes with PGD for its clients.

Karyomapping

Karyomapping is a method of analysis that can be paired with aCGH and SNP microarray in successful births and could potentially be integrated with multiplex qPCR. In a study conducted on the transmission of Cystic Fibrosis transmembrane receptor mutations, quoted in Lu et al. (2016), karyomapping was able to utilize parental chromosome analysis to determine if an embryo was unaffected, a carrier, or affected by a disease. It did so by analyzing which chromosome from each parent contains the gene of interest and whether the embryo has inherited the chromosome in question from the parent. Apart from Cystic Fibrosis, it has also seen success in PGD of Marfan syndrome and PGD of a mutation related to Tuberculosis sclerosis. Its compatibility with a variety of sampling methods suggests its revolutionary applications within the field of PGD, especially when it is linked with other sampling methods (Lu et al., 2016).

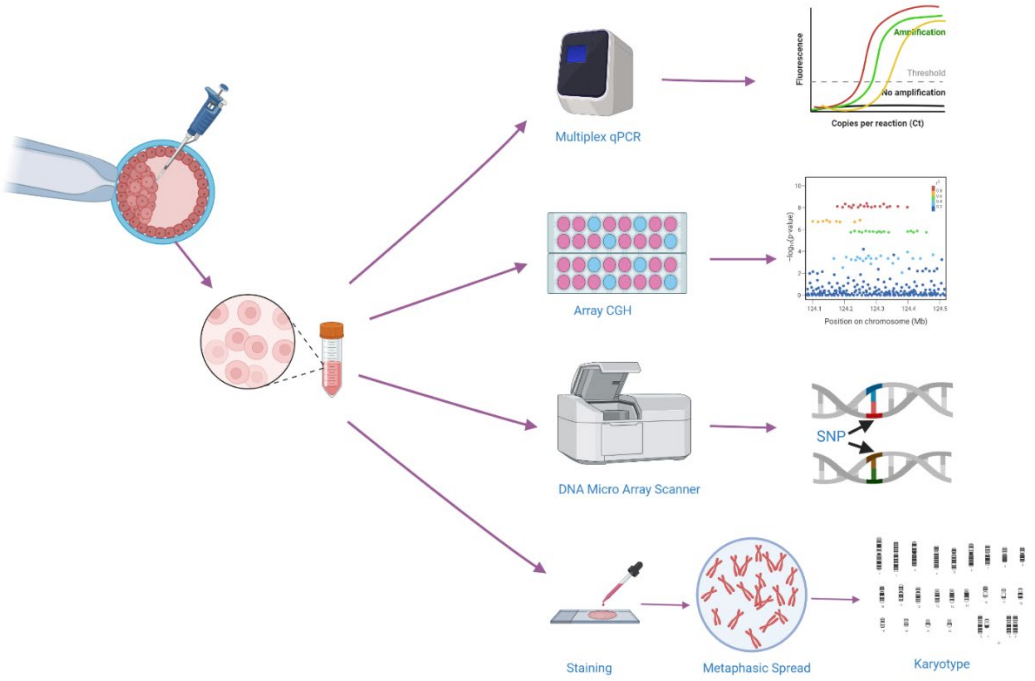

Figure 13: Image of 4 advances in sampling methods. (1) Multiplex qPCR, (2) aCGH, (3) SNP Microarray, (4) Karyomapping Created by Author with Biorender.com

Next-Generation Sequencing

The last method of analysis, and a particularly new and promising technique for PGD is Next-Generation Sequencing (NGS). Within the research by Alekseyev et al. (2018), it is seen that, unlike PCR, which only allows for singular gene analysis within each test, NGS is able to detect thousands of genetic abnormalities within each test run. It is able to achieve this through spatial separation of the genetic components during each step, not physical separation into different test tubes like many of the other procedures mentioned. Like FISH, NGS can also be utilized with three mechanisms: whole genome sequencing, whole exome sequencing, and targeted sequencing. Whole genome sequencing, as its name suggests, is extremely efficient in analyzing the entire genome and pinpointing even the smallest of variants. Whole exome sequencing, on the other hand, is faster and quite a bit more cost-efficient than whole genome sequencing, on the account that it only analyzes protein-coding regions of the genome, an area that contains most of

the mutations responsible for genetic diseases. The final method, targeted sequencing, is even more specific, and like PCR, analyzes a specific genomic area of interest. In regards to detecting hereditary disorders, NGS provides a wealth of information, though there may be difficulties in interpreting said information. Still, NGS is a lower-cost and easier process that allows targeted analysis of the molecular basis in disorders, a valuable advancement in the field of embryology (Alekseyev et al., 2018).

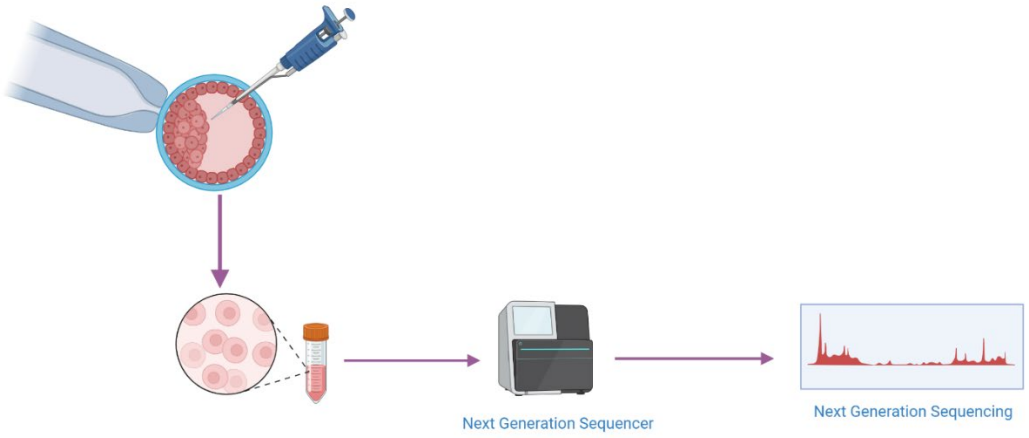

Figure 14: Image of the NGS analysis. NGS testing combines all the current methods (CGH, PCR, and FISH) to analyze the genome and identify potential sources of misrepresentation and abnormality. Though it is considered by some to be research-based rather than practical, it has been extremely successful in implementation and has tremendous potential. Created by Author with Biorender.com

Discussion

Based on the data explored within this research paper, and looking at the criteria of effectiveness in diagnosis as well as in implantation potential, the evaluation suggests that testing of cell-free nucleic acids yields the most promise in terms of sampling advances. Testing of cell-free nucleic acids, however, does not hold as much weight in its early stages due to its high risk of cross-contamination. For these reasons, the already present method of Blastocyst biopsy is a safer alternative for PGD sampling. In terms of analysis advances, Next Generation Sequencing is the most promising and its current implementations have shown its reliability in documentation and diagnosis. The potential capabilities of NGS far surpass current methods of analysis, which are less efficient and prone to faults, and similar advances in analysis, which are less reliable and accurate.

Conclusion

PGD has certainly developed as a diagnostic tool, but therein lies the question of its impact For the thousands of couples who have used the testing option and the thousands of healthy babies who have been born as a result of the procedure, there is no doubt. PGD provides a source of hope to families struggling to conceive or those facing the cruel reality that their genomic disorders might be passed on to their children. As our world progresses we will be forced to make tough decisions where our happiness and health may be on different sides, however, thanks to PGD, we may have one less tough decision to make.

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