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Chromosomal Abnormalities in Preimplantation Embryos and Detection Strategies in PGD and PGS

Pinar Tulay

Abstract

Structural and numerical chromosomal abnormalities are common in early developing embryos, and these abnormalities may cause spontaneous abortions and implantation failure. The reproductive risk of carriers with structural chromosomal abnormalities depends on the breakpoint positions, the segregation patterns and the sex of the carrier. These carriers have a lower chance of producing normal or balanced gametes due to abnormal segregation of chromosomes at meiosis leading to repeated spontaneous abortions and infertility. Preimplantation genetic diagnosis (PGD) is offered to couples who have already been diagnosed with a single gene disorder or a chromosome imbalance to select an embryo free from the mutation or an embryo with a balanced karyotype prior to implantation and pregnancy. PGS is applied to patients experiencing repeated implantation failures or spontaneous abortions with normal karyotypes. Translocations are the most common type of structural chromosome rearrangement. Both reciprocal and Robertsonian translocations are phenotypically normal. PGD for translocations was initially performed by fluorescence in situ hybridization (FISH) at cleavage stage embryos. However, with the recent developments, many centers have opted for the use of array comparative genomic hybridization (aCGH), single-nucleotide polymorphism (SNP) arrays and next generation sequencing (NGS).

Keywords: chromosomal abnormalities, PGD, FISH, aCGH

1. Introduction

Preimplantation embryo development follows a series of critical events. These events start at gametogenesis and lasts until parturition. Gametogenesis is a process of gamete formation. Male and female gametes are derived from primordial germ cells (PGCs) by the processes of spermatogenesis and oogenesis, respectively. PGCs have unique properties of

gene expression, epigenetics, morphology and behavior. Once the PGCs undergo mitosis, spermatogenesis and oogenesis progress differently. In spermatogenesis, spermatogonia undergo mitosis starting at puberty until death, and each primary spermatocyte produces four spermatids at the end of meiosis. In oogenesis, PGCs differentiate into oogenia, and they enter meiosis and arrest until puberty. Unlike meiosis II in spermatogenesis, secondary oocyte and first polar body do not undergo meiosis II until fertilization. After fertilization, meiosis II starts and each oogenia produce a single viable oocyte [1].

At fertilization, the oocyte completes meiosis, and the fertilized oocyte is called the zygote. Oocyte and sperm nuclei fuse resulting in syngamy. The zygote undergoes series of cleavage divisions, forming 2-cell, 4-cell, 8-cell, morula and blastocyst stages [2]. During cleavage stage divisions programming of maternal and paternal chromosomes takes place to create the embryonic genome (embryonic genome activation, EGA) and to start the preimplantation embryo development. If the EGA fails, the development does not continue because of the inability of the embryo to have cellular functions [3]. This activation is initiated by degradation of maternal nucleic acids, specific RNAs stored in oocytes, proteins and other macromolecules [4]. Upon EGA, which starts at the 2-cell stage in mouse and 4–8-cell stage in human [5], remarkable reprogramming of expression occurs in the preimplantation embryo. These reprogramming events are controlled by DNA methylation, histone acetylation, transcription, translation and miRNA regulation [6].

Both conception and embryonic developments during pregnancy are vulnerable processes since a large number of the conceptions are chromosomally abnormal. Chromosomal imbalances, gains or losses of segments/whole chromosomes, are common in human, and they are observed in 1/380 live births [7]. Chromosomal imbalances have been observed in preimplantation embryos mostly in the form of aneuploidies and translocations, and they may lead to embryo death or development of an affected embryo [8, 9]. The incidence of chromosomally abnormal embryos increases vividly with advanced maternal age [10–12]. The main causes of spontaneous abortions and repeated implantation failure are these numerical and structural chromosomal abnormalities [13–17]. Therefore, in the last decades, a great focus has been put on detecting these chromosomal aberrations in preimplantation embryos. Preimplantation genetic diagnosis has been applied to patients with known structural chromosomal abnormalities as well as single gene disorders, whereas preimplantation genetic screening has aimed to detect aneuploid embryos and lower the risk of implantation failures and spontaneous abortions following in assisted reproductive technology treatments [18–20].

In this chapter, the applications of preimplantation genetic diagnosis for translocations and preimplantation genetic screening for aneuploidy testing will be discussed. Translocations are the most common type of rearrangements that we come across in fertility clinics. Different techniques that are being used currently will be thoroughly evaluated. Finally, different aspects of preimplantation genetic screening will be evaluated.

## 2. Structural chromosomal abnormalities in human embryos

Majority of conceptus with chromosomal abnormality aborts spontaneously with <1% of abnormal conceptus resulting in term pregnancy. Chromosomal abnormalities can arise at

three stages during human development, gametogenesis, fertilization and embryogenesis. Analysis of chromosomes in human gametes and embryos has become available with the development of artificial reproductive (ART) technologies developed to treat infertility.

Balanced structural chromosome rearrangements are common in human. Approximately 1/500 to 1/1000 live births carry a balanced translocation [21]. Translocations are formed due to rearrangements of nonhomologous chromosome segments. They can be caused by abnormal DNA repair, chromosome breakage, centric fission followed by malsegregation of that chromosome or through the formation of isochromosomes or terminal deletion accompanied by a duplication of the rest of the chromosome [22]. Translocations are grouped in two categories: reciprocal, the most common form, and Robertsonian. Reciprocal translocations occur due to an exchange of two ends of nonhomologous chromosomes. Robertsonian translocations involve rearrangement of two acrocentric chromosomes (chromosomes 13, 14, 15, 21 and 22) with the loss of the short arms occurring in 1/900 live births [7]. The most common Robertsonian translocation involves chromosomes 13 and 14 [23].

Although the carriers of both reciprocal and Robertsonian translocations are phenotypically normal, the reproductive risk of balanced carriers varies depending on the chromosomes involved, breakpoint positions, the segregation patterns and the sex of the translocation carrier [24]. However, they generally have a lower chance to produce normal or balanced gametes due to abnormal segregation of chromosomes at meiosis leading to repeated spontaneous abortions and infertility [21, 25]. At pachytene stage of meiosis I, chromosomes with reciprocal translocation rearrangements form quadrivalent. At the end of meiosis I, these chromosomes can segregate in four different ways: alternate (2:2), adjacent (2:2), 3:1 and 4:0 (**Figure 1a**). Alternate segregation either leads to a normal or a balanced rearrangement, and therefore, it results in a viable birth. Studies suggest that the most common segregation pattern of gametes produced by the carriers of reciprocal translocations is alternate (balanced) segregation [26]. In the case of an adjacent segregation, homologous chromosomes cause a monosomy for one centric center and trisomy for the other centric center. Studies suggest that adjacent two segregation pattern is rather uncommon [26] and may rise in cases of maternal meiotic errors [21]. Three to one segregation leads to a tertiary trisomy/monosomy or interchange trisomy/monosomy. This type of segregation can be viable. If the chromosomes fail to segregate, it leads to 4:0 segregation resulting in double trisomy or double monosomy. In case of a Robertsonian translocation, one normal with one derivative chromosome or single chromosomes of derivative or the single chromosomes of the normal chromosome can segregate resulting in an abnormal gamete (**Figure 1b**). The only way of a Robertsonian carrier can produce a normal gamete is if the two normal chromosomes segregate together at meiosis I [27].

Insertions can be classified as a type of translocations, and these are uncommon rearrangements. The simple insertion involves three breaks where the first two removes the part of the chromosome, and the segment is reinserted within the third break. The conceptus with a smaller insertional segment has a potential to be viable [28]. The insertions, especially the small ones, may be passed on from generations to generations without being detected. However, with the use of newer technologies, such as microarrays, more patients with insertions are likely to be detected [29]. Insertions are one the rearrangements with the highest reproductive risk, in such approximately 32% of male and 36% of female carriers are having a chromosomally abnormal child [30].
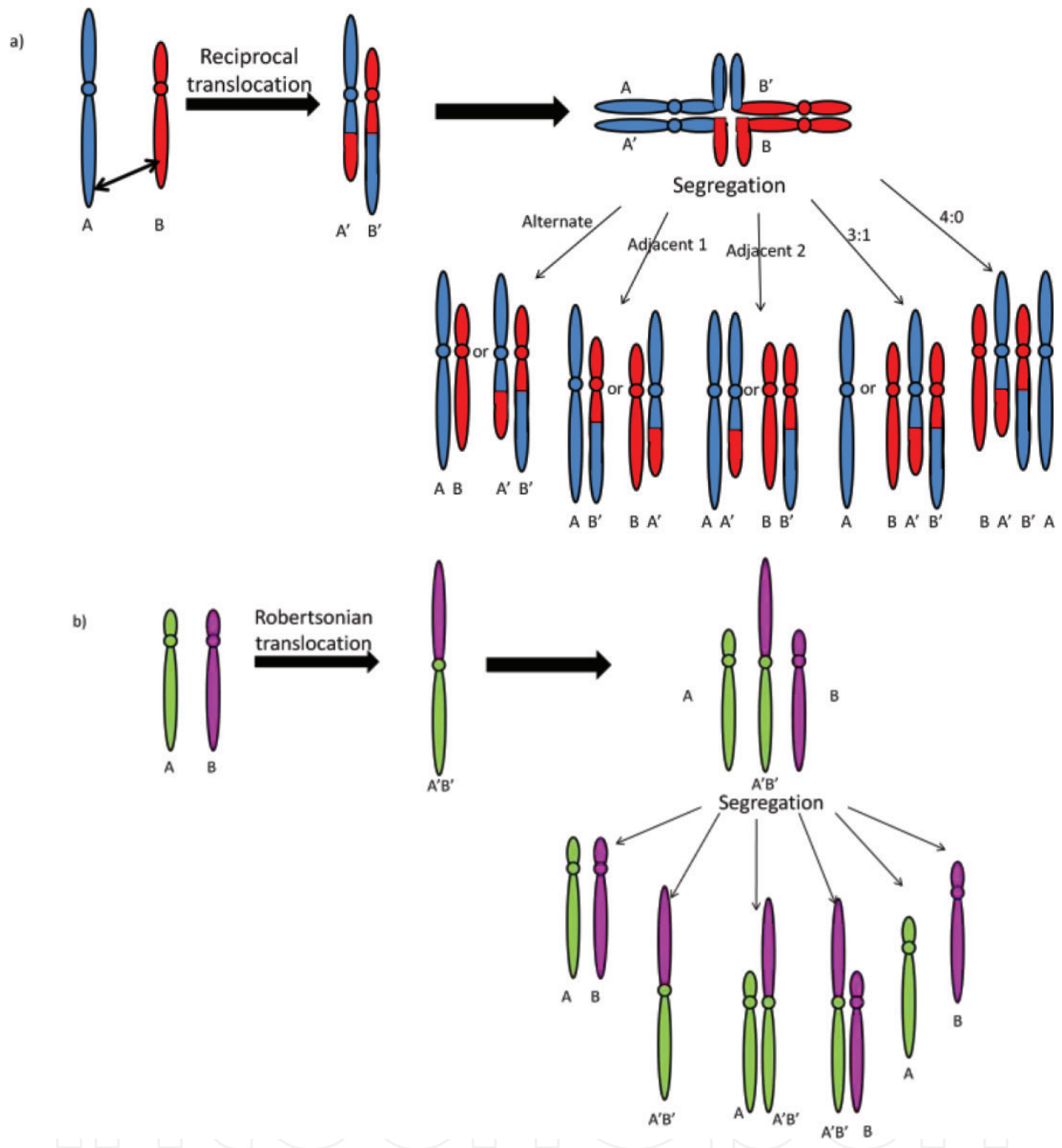
Figure 1. Segregation patterns of translocation carriers (a. Reciprocal and b. Robertsonian translocation carriers) during meiosis.

Another example of chromosomal rearrangements is inversions. These are intrachromosomal structural rearrangements. The simple inversion involves two break points within the same chromosome where the intercalcary segment gets rotated and reinserted. The inversions can be subcharacterized as pericentric, where the inverted segment involves the centromere, and paracentric, where the inverted segment is reinserted on the same chromosome arm. It is very rare that an inversion, especially pericentric inversions, would cause infertility [31]. However, abnormal synapsis of a chromosome pair may cause the development of an abnormal embryo

due to malsegregation of chromosomes during gametogenesis. Depending on the break point, whether it involves genes or not, the size of the inversion could result in detrimental effects. Therefore, the risk of an inversion carrier varies among couples, and each has their own risk. The risk estimate can be performed by family studies, literature with similar inversion break points and gamete (sperm) analysis. Sperm studies have shown that during spermatogenesis, inversions with larger segments could result in spermatogenic arrest [32].

The carriers with a chromosomal rearrangements have the option to pursue pregnancy without seeking for any medical help and wish for a chromosomally normal child. Some of these carriers may have had an abnormal child due to the chromosomal abnormalities, and some of these carriers, especially translocation carriers, may have experienced repeated spontaneous abortions. Therefore, these patients may choose to seek for different options to avoid such experiences. These couples may opt for donor gametes, prenatal diagnosis or preimplantation genetic diagnosis.

3. Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is offered to couples who have already been diagnosed with a single gene disorder or a chromosome imbalance to select an embryo free from the mutation or an embryo with a balanced karyotype prior to implantation and pregnancy [9]. The first application of PGD was performed for a couple with X-linked recessive disorder almost a quarter century ago by Handyside and colleagues [33].

PGD is a highly invasive technique that requires IVF and biopsy of the polar body of the mature oocyte or the developing embryo (cleavage stage or blastocyst stage). Polar body biopsy involves biopsying the first only or the first and the second polar bodies. Neither the first polar body nor the second polar body is required for fertilization or a normal embryonic development [34]. Polar body biopsy is advantageous since it provides sufficient time for analysis. First polar body biopsy alone only allows analysis of meiotic errors (maternal origin only), and it does not give any information on the mitotic errors. Although the biopsy of both polar bodies provides information on both the meiotic and mitotic errors, it is still limited to detect the maternal errors only [35].

Biopsy at the cleavage stage on day 3 postfertilization provides more complete diagnosis than polar body biopsy and with enough time to finish the diagnosis before the embryo transfer [36, 37]. However, mosaicism (presence of at least two cell lines) at this stage is a major issue for PGS. In mosaic embryos, one or two cells may not represent the rest of the embryo due to different cell types in every cell [38–43].

Blastocyst biopsy has been applied more frequently in PGS in the last years. Biopsy of trophectoderm cells provides more number of cells for diagnosis and therefore overcomes the trouble of the single cell diagnosis [44]. Even though some studies report mosaicism at the blastocyst stage [45–47], due to the activation of cell cycle control points by the 8-cell stage embryo, many mosaic embryos are arrested or are repaired [48]. The lower rates of mosaicism in addition to analysis of several cells instead of just one provide less diagnostic errors. Conversely, blastocyst stage biopsy is limited before the procedure can even begin as it depends on the development of the embryo into a blastocyst [49].

4. Numerical chromosomal abnormalities in embryos and preimplantation genetic screening

Since the initial applications of PGD for sex-linked disorders and monogenic diseases, the indications have been expanded to aneuploidy screening by preimplantation genetic screening (PGS). PGS is applied to patients with advanced maternal age, recurrent miscarriages, repeated in vitro fertilization (IVF) failures or male infertility. Unlike PGD, patients undergoing PGS do not carry a genetic disorder and they have a normal karyotype. The main goal of PGS is to test embryos for aneuploidies that arise spontaneously in human gametogenesis, more prevalent in female meiosis, or early embryonic development [50, 51]. Aneuploidy is a common feature in preimplantation embryos causing the low success rates and high miscarriage rates in assisted reproductive technology (ART) treatments [52, 53]. Although embryos with autosomal monosomies are mainly lethal; embryos with some trisomies (13, 18 and 21) have higher chances of survival with the risk of developing genetic disorders [54], and some trisomies (15, 16 and 22) can cause embryonic developmental arrest or implantation failures [55, 56]. Therefore, selecting an embryo with a normal chromosomal complement helps to improve the implantation rates and increases the chances of birth of a healthy child.

The first PGS was performed by fluorescence in situ hybridization (FISH) in polar bodies and cleavage stage embryos in 1995 [57–59]. Up until recently, FISH was the preferred method of analysis in cleavage stage embryos [60]. As discussed earlier, although at cleavage stage, both maternal and paternal errors can be analyzed, it is complicated by high levels of mosaicsism. Mosaicsism is rare for monogenic diseases; however, it is very common for aneuploidies in the embryos at cleavage stage. There are more than ten randomized control trials showing that cleavage stage biopsy and FISH analysis does not improve the delivery rates [61–71]. In 2010, European Society of Human Reproduction and Embryology (ESHRE) reported that cleavage stage biopsy using FISH is not recommended for PGS [72]. The majority of the aneuploidies in the embryos affecting the pregnancy rates are believed to occur in the oocyte, and therefore, polar body (PB) biopsy may have an added advantage in PGS, especially since PBs are not affected by mosaicim arising in mitosis [73]. However, biopsy of the first PB does not provide a complete aneuploidy screening since biopsy of first PB only gives errors occurring in meiosis I, and it does not reveal any information about meiosis II. Therefore, performing both PB I and PB II biopsies are recommended for better analysis. Biopsy of PBs is considered less invasive than biopsy of a blastomere or trophectoderm, and the use of aCGH in PB biopsy was shown to have improved implantation rates [74]. Furthermore, the multicenter randomized control trial set by the ESHRE Task force reported that PGS using the first and the second PB by aCGH increases the delivery rates significantly in patients with advanced maternal age [75]. One of the pitfalls of polar body biopsy is that oocytes diagnosed as aneuploid may actually form a euploid embryo due to a chromatid predivision error in MI with a balanced segregation at MII [75, 76]. Moreover, Geraedts and colleagues (2011) reported that at least 1 in 10 oocytes biopsied do not provide a diagnostic result [75]. Therefore, embryos with no diagnostic results and developed normally are either discarded or biopsied at a later stage. This increases the labor for both embryology and genetics teams, and it causes an added economical burden to the patients.

With the recent improvements in IVF laboratories, blastocyst biopsy has become the preferred method for PGS. In the past, one of the main problems of performing blastocyst biopsy was the limited time allowed for the diagnosis since the embryonic cells are either biopsied on day 5 or on day 6 for the slow developing embryos. With the use of vitrification, high embryo survival rates were reported [77–80], and many centers have opted performing PGS at blastocyst stage [81]. Furthermore, vitrifying embryos provide chance of an embryo transfer during an unstimulated cycle that was shown to result in high pregnancy rates [82–84]. In good prognosis patients, a pilot randomized clinical trial showed that trophectoderm biopsy and use of aCGH for PGS increases the implantation and ongoing pregnancy rates [85]. The pitfall of trophectoderm biopsy is that some embryos may not reach to the blastocyst stage in vitro that may be viable in utero [86]. As an added evaluation of aneuploidy screening, mitochondrial DNA (mtDNA) copy number has been investigated in euploid embryos showing that high mtDNA copy number indicates lower embryo viability and implantation [87, 88].

In addition to the array comparative genomic hybridization (aCGH) platforms, validation and the initial applications of single-nucleotide polymorphism (SNP) arrays [22, 76, 89–91] and next generation sequencing (NGS) platforms [92–96] showed promising results for their use in PGS. With the use of SNP arrays, the aneuploidies including monosomies or partial deletions as well as parental origin of any chromosomal abnormality can be identified [97].

Although PGD is widely accepted and applied throughout the world, there is still an ongoing debate on whether PGS is beneficial to infertile couples due to variable success rates depending on the maternal age, the technique used and the time of biopsy. Therefore, more and more studies are being developed for indirect aneuploidy assessment of the embryos.

5. PGD for translocations

Up until recently, the most common technique used to detect translocations in PGD was FISH. Polymerase chain reaction (PCR), which has been widely used to detect monogenic disorders, has also been used to detect translocations in PGD [98]. Other techniques that have been introduced to detect translocations in PGD are aCGH and more recently SNP arrays and NGS platforms.

5.1. PGD for translocations by FISH

FISH is a cytogenetic technique that had been used to detect structural chromosome analysis for patients with translocations and X-linked disorders. FISH is based on the hybridization of interphase chromosomes on specific DNA probes [99]. Although FISH is a rapid and accurate technique, it is limited as only a few chromosomes can be examined in a single cell. Moreover, it is restricted to analyze only the regions known to have imbalances. Signal interpretation is very important for correct diagnosis since the hybridization efficiency with each successive round could be lowered due to signal splitting and signal overlap. Additionally, loss of micronuclei during fixation of the blastomere causes difficulties in diagnosis [100].

5.2. PGD for translocations by PCR

PCR, a technique to amplify DNA by in vitro enzymatic replication, is mainly used to detect monogenic disorders [37] and recently, to detect translocations [98]. PCR is a technically demanding procedure, especially single cell PCR for PGD. The most important issues with PCR are the high risk of contamination, allele dropout (ADO) and amplification failure. ADO, which occurs when one of the alleles fails to amplify in a heterozygote cell for that particular region and is usually caused by a low amount of DNA in single cell PCR procedures, incomplete lysis or imperfect denaturation temperature [101], could lead to a misdiagnosis [101]. Fluorescent-PCR and multiplex PCR, which are more sensitive than conventional PCR, can be used to lower the ADO risk and amplification failure [102]. Although PCR has its limitations, it has the potential to conquer the drawbacks of FISH in detecting translocations.

Quantitative fluorescent PCR (QF-PCR) has been incorporated to the analysis of chromosomal imbalances. Studies have shown that QF-PCR is a sensitive, rapid and accurate technique that has been applied to study chromosomal abnormalities in spontaneous miscarriages [103] and in prenatal diagnosis [104]. Not only the parental and meiotic origin of aneuploidy can be detected by QF-PCR by using semi/fully informative short tandem repeat (STR) markers, but also the possible recombination events can be analyzed using informative STR markers. QF-PCR results of this study are preliminary, and more studies must be carried out.

5.3. PGD for translocations by array comparative genomic hybridization

aCGH, which is a similar technique to metaphase CGH, is used to determine total or partial aneuploidy by detecting chromosomal gains and losses of the entire genome [105]. Manual identification of chromosomes is not required with aCGH, and this technique has higher sensitivity and specificity for small genomic changes [106]. aCGH not only is used in prenatal diagnoses for identification of translocations [107, 108] and being reported as a rapid technique to detect de novo chromosome imbalances [109] but also is used to detect translocations in PGD clinically [110–112].

The comprehensive chromosome screening using aCGH has an added advantage to FISH in detecting aneuploidies and interchromosomal effect. Interchromosomal effect is the phenomenon known as the interference of chromosomes involved in rearrangement with the segregation of the structurally normal chromosomes [113–115]. Twenty-four chromosome aneuploidy screening revealed that segregation errors occur at high frequency even for the chromosomes not tested by FISH [8, 9, 20, 100, 116–120]. Furthermore, aCGH can detect copy number differences more precisely compared to FISH and PCR analyses since these methods are at much lower resolution than aCGH [121]. However, one of the limitations of aCGH is its inability to detect ploidy [122].

The methodology of aCGH is similar to metaphase-CGH, such that the only difference is that aCGH does not require metaphase chromosomes, and it can use target DNA for hybridization from cloned DNA segments, such as PCR-generated sequences, bacterial artificial chromosome (BAC) and cDNA clones [49]. More importantly, aCGH is much faster technique

compared to metaphase CGH in detecting chromosomal abnormalities within less than 1 day [123]. The test and reference DNA are labeled with green and red fluorochromes, respectively. After hybridizing the labeled test and reference DNA on the array covered with BAC/cDNA clones or PCR-generated sequences, an array scanner captures the scanned image and computer systems are used to analyze the ratio of green to red fluorescence. If the test DNA is normal, the ratio of green to red signal should be 1:1. If the test DNA is monosomic, the green labeled chromosome will be less compared to the red labeled, and therefore, the ratio of green to red ratio is decreased and vice versa [124].

One of the most important advantages of aCGH is that it requires a small amount of genomic DNA for hybridization, as low as 2–4 µg [44]. However, in PGD/PGS, WGA, a technique used to amplify the whole genome for molecular analysis using small amounts of DNA [125], is fundamental. This technique can abolish DNA as being a limiting factor for genetic analysis by generating large quantities of DNA from starting material as small as 6pg, such as from a single blastomere [126]. Multiple WGA techniques have been used in the past, such as primer extension preamplification [127], linker-adaptor PCR [128], degenerate oligonucleotide primed-PCR [9, 129–131] and multiple displacement amplification [132–134].

5.4. PGD for translocations by single nucleotide polymorphism arrays

SNP arrays consist of oligonucleotide probes and most of them examine between 10,000–500,000 SNPs with high accuracy and reproducibility. SNP arrays utilize an approach similar to metaphase-CGH, such that the labeled test sample is hybridized separately on a different area of the array than the reference sample that is analyzed in parallel. The alleles detected at each SNP locus for the embryo are compared with the SNPs detected for the parents, and then, fluorescence intensities obtained for the test (embryo) and reference samples are analyzed by the brightness of the signals obtained. Brighter signals of the test sample indicate excess of that chromosome and *vice versa* [135].

The main advantage of SNP arrays is that they can determine the inheritance of genes that can allow simultaneous analysis of monogenic diseases and chromosome rearrangements, such as translocations including the balanced translocations unlike aCGH or FISH [100]. The drawback of SNP arrays is their high susceptibility to noise and bias, especially with the amplified single cell samples. SNP arrays cannot detect duplications. When SNP arrays are used in PGD/PGS, vitrification of embryos is necessary to enable enough time to complete the procedure [135].

SNP arrays have been applied in research to detect total aneuploidy and structural chromosomal imbalances to identify disease risks such as for type-2 diabetes, prostate cancer, glaucoma and some cardiovascular conditions [136]. SNP arrays were shown to analyze the copy number differences and chromosomal instability in studies following WGA of cells from cell lines [100] and amplified blastomeres from human cleavage-stage embryos [22, 137, 138]. Clinical applications of SNP arrays have been reported for several cancers [139] and for Gaucher disease and Marfan syndrome following blastomere biopsy [140]. SNP arrays have also been clinically applied in PGD and PGS [141–143].

5.5. PGD for translocations by next-generation sequencing

NGS is a technology that is used to sequence the nucleotides in a massively parallel manner. With the use of NGS, higher throughput data with lower cost can be obtained in a faster way compared to Sanger sequencing. Furthermore, for NGS platforms, bacterial cloning procedures are not required. On the other hand, NGS technologies require complex alignment algorithms in order to assemble and map the genome using short reads [144]. Up until this year, three main NGS platforms have been introduced, Roche (454), Life technologies and Illumina. Roche (454) generates 700 base pair fragments of approximately 1 million reads [145]. With the Life Technologies platform, semiconductor-sequencing technology has been used with solid-state pH meter. In this platform, proton generates up to 200 bp fragments of about 60–80 million reads. This technology generates up to 10 Gb of sequence in every run [145]. Illumina's platform generates up to 150 bp fragments of about 6 billion reads with approximately 1.8 Tb of sequence in each run over a period of 3 days [145]. Since NGS has become cost-effective, this comprehensive analysis has been applied in assessment of numerical and structural chromosomal abnormalities in PGS and PGD [96, 141, 146–149].

6. Conclusion

One of the most important reasons of the development of PGD was to avoid termination of pregnancy or avoid a severe congenital abnormality. Soon after the PGD implications have developed; PGS has been introduced aiming to select a euploid embryo to improve the implantation rates and avoid spontaneous abortions. However, PGD is not an easy reproductive option especially since there is no guarantee of pregnancy or even an embryo transfer in cases where all the embryos have the mutation or chromosomal imbalance [72]. Complex and multidisciplinary approaches are required for a successful PGD cycle combining the expertise of geneticists, embryologists and fertility doctors. Each PGD cycle starts with genetic counseling, fertility assessment, hormonal ovarian stimulation, development of embryos in vitro, biopsy of these embryos and preimplantation genetic testing of the embryonic samples. Initial studies were performed by a molecular cytogenetic technique, FISH, with some limitations including problems with fixation of the nucleus, hybridization problems and intensity of the fluorescence of the probes. As the newer technologies have been introduced, the fields of PGD and PGS have also improved. In the last past few years, with the development of aCGH, SNP arrays and NGS technologies, precise and reliable results have obtained from embryo biopsies with improvements in the implantation and take home baby rates.

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References

[1] Jaroudi, S. and S. SenGupta, DNA repair in mammalian embryos. Mutat Res, 2007. **635**(1): pp. 53-77.

[2] William, L., Human Embryology. Vol. 2nd edition. Churchill Livingstone, New York. 1997.

[3] Schultz, R.M., The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum Reprod Update, 2002. **8**(4): pp. 323-331.

[4] Moore, K. and T.V.N. Persaud, The Developing Human. Saunders Company, Philadelphia. Vol. 6. 1998.

[5] Telford, N.A., A.J. Watson, and G.A. Schultz, Transition from maternal to embryonic control in early mammalian development: a comparison of several species. Mol Reprod Dev, 1990. **26**(1): pp. 90-100.

[6] Bell, C.E., M.D. Calder, and A.J. Watson, Genomic RNA profiling and the programme controlling preimplantation mammalian development. Mol Hum Reprod, 2008. **14**(12): pp. 691-701.

[7] Traversa, M.V., L. Carey, and D. Leigh, A molecular strategy for routine preimplantation genetic diagnosis in both reciprocal and Robertsonian translocation carriers. Mol Hum Reprod, 2010. **16**(5): pp. 329-337.

[8] Voullaire, L., et al., Chromosome analysis of blastomeres from human embryos by using comparative genomic hybridization. Hum Genet, 2000. **106**: pp. 210-217.

[9] Wells, D. and J.D. Delhanty, Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Hum Reprod, 2000. **6**: pp. 1055-1062.

[10] Kuliev, A., et al., Chromosomal abnormalities in a series of 6,733 human oocytes in pre-implantation diagnosis for age-related aneuploidies. Reprod Biomed Online, 2003. **6**(1): pp. 54-59.

[11] Pellestor, F., et al., Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. Hum Genet, 2003. **112**(2): pp. 195-203.

[12] Fragouli, E., et al., Increased susceptibility to maternal aneuploidy demonstrated by comparative genomic hybridization analysis of human MII oocytes and first polar bodies. Cytogenet Genome Res, 2006. **114**(1): pp. 30-38.

[13] Kim, J.W., et al., Chromosomal abnormalities in spontaneous abortion after assisted reproductive treatment. BMC Med Genet, 2010. **11**(153). pp. 1-6

[14] Hodes-Wertz, B., et al., Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. Fertil Steril, 2012. **98**(3): pp. 675-680.

[15] Raziel, A., et al., Increased frequency of female partner chromosomal abnormalities in patients with high-order implantation failure after in vitro fertilization. Fertil Steril, 2002. **78**(3): pp. 515-519.

[16]   Munne, S., et al., Outcome of preimplantation genetic diagnosis of translocations. Fertil Steril, 2000. **73**(6): pp. 1209-1218.

[17]   Verlinsky, Y., et al., Preimplantation testing for chromosomal disorders improves reproductive outcome of poor-prognosis patients. Reprod Biomed Online, 2005. **11**(2): pp. 219-225.

[18]   Munne, S., et al., Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. Fertil Steril, 2005. **84**(2): pp. 331-335.

[19]   Liang, L., et al., Identification of chromosomal errors in human preimplantation embryos with oligonucleotide DNA microarray. PLoS One, 2013. **8**(4): pp. e61838–e61838.

[20]   Fiorentino, F., et al., PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. Hum Reprod, 2011. **26**: pp. 1925-1935.

[21]   Simopoulou, M., et al., Preimplantation genetic diagnosis of chromosome abnormalities: implications from the outcome for couples with chromosomal rearrangements. Prenat Diagn, 2003. **23**(8): pp. 652-662.

[22]   Vanneste, E., et al., Chromosome instability is common in human cleavage-stage embryos. Nat Med, 2009. **15**(5): pp. 577-583.

[23]   Anton, E., et al., Sperm FISH studies in seven male carriers of Robertsonian translocation t(13;14)(q10;q10). Hum Reprod, 2004. **19**(6): pp. 1345-1351.

[24]   Faraut, T., et al., Cooperation of selection and meiotic mechanisms in the production of imbalances in reciprocal translocations. Cytogenet Cell Genet, 2000. **88**: pp. 15-21.

[25]   Scriven, P.N., A.H. Handyside, and C.M. Ogilvie, Chromosome translocations: segregation modes and strategies for preimplantation genetic diagnosis. Prenat Diagn, 1998. **18**(13): pp. 1437-1449.

[26]   Estop, A.M., V. Van Kirk, and K. Cieply, Segregation analysis of four translocations, t(2;18), t(3;15), t(5;7), and t(10;12), by sperm chromosome studies and a review of the literature. Cytogenet Cell Genet, 1995. **70**(1-2): pp. 80-87.

[27]   Gardner, R.J.M. and G.R. Sutherland, Chromosome Abnormalities and Genetic Counselling. 2nd ed. Oxford University Press, New York. 1996.

[28]   Doheny, K.F., et al., Segregation of a familial balanced (12;10) insertion resulting in Dup(10)(q21.2q22.1) and Del(10)(q21.2q22.1) in first cousins. Am J Med Genet, 1997. **69**(2): pp. 188-193.

[29]   Kang, S.H., et al., Insertional translocation detected using FISH confirmation of array-comparative genomic hybridization (aCGH) results. Am J Med Genet A, 2010. **152A**(5): pp. 1111-1126.

[30]   Van Hemel, J.O. and H.J. Eussen, Interchromosomal insertions. Identification of five cases and a review. Hum Genet, 2000. **107**: pp. 415-432.

[31] De Braekeleer, M. and T.N. Dao, Cytogenetic studies in male infertility: a review. Hum Reprod, 1991. **6**: pp. 245-250.

[32] Gabriel-Robez, O. and Y. Rumpler, The meiotic pairing behaviour in human spermato-cytes carrier of chromosome anomalies and their repercussions on reproductive fitness I: Inversions and insertions. Ann Génét, 1994. **37**: pp. 3-10.

[33] Handyside, A.H., et al., Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature, 1990. **344**: pp. 768-770.

[34] Kaplan, B., et al., Viability of embryos following second polar body removal in a mouse model. J Assist Reprod Genet, 1995. **12**(10): pp. 747-749.

[35] Dreesen, J.C., et al., RS46(DXS548) genotyping of reproductive cells: approaching preim-plantation testing of the fragile-X syndrome. Hum Genet, 1995. **96**(3): pp. 323-329.

[36] Goossens, V., et al., Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. Hum Reprod, 2008. **23**(3): pp. 481-492.

[37] Goossens, V., et al., ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007. Hum Reprod, 2009. **24**(8): pp. 1786-1810.

[38] Munne, S., et al., Diagnosis of major chromosome aneuploidies in human preimplanta-tion embryos. Hum Reprod, 1993. **8**(12): pp. 2185-2191.

[39] Delhanty, J.D., et al., Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation, (FISH). Hum Mol Genet, 1993. **2**(8): pp. 1183-1185.

[40] Harper, J.C., et al., Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic preimplantation human embryos. Prenat Diagn, 1995. **15**(1): pp. 41-49.

[41] Delhanty, J.D., et al., Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. Hum Genet, 1997. **99**(6): pp. 755-760.

[42] Munne, S., et al., Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. Prenat Diagn, 1998. **18**(13): pp. 1459-1466.

[43] Los, F.J., D. Van Opstal, and C. van den Berg, The development of cytogenetically nor-mal, abnormal and mosaic embryos: a theoretical model. Hum Reprod Update, 2004. **10**(1): pp. 79-94.

[44] Basille, C., et al., Preimplantation genetic diagnosis: state of the art. Eur J Obstet Gynecol Reprod Biol, 2009. **145**(1): pp. 9-13.

[45] McArthur, S.J., et al., Blastocyst trophectoderm biopsy and preimplantation genetic diagnosis for familial monogenic disorders and chromosomal translocations. Prenat Diagn, 2008. **28**(5): pp. 434-442.

[46] Sandalinas, M., et al., Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. Hum Reprod, 2001. **16**(9): pp. 1954-1958.

[47] Bielanska, M., et al., Diploid-aneuploid mosaicism in human embryos cultured to the blastocyst stage. Fertil Steril, 2005. **84**(2): pp. 336-342.

[48] Richeldi, L., et al., T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med, 2004. **170**(3): pp. 288-295.

[49] Wells, D. and B. Levy, Cytogenetics in reproductive medicine: the contribution of comparative genomic hybridization (CGH). Bioessays, 2003. **25**(3): pp. 289-300.

[50] Wells, D. and J.D. Delhanty, Preimplantation genetic diagnosis: applications for molecular medicine. Trends Mol Med, 2001. **7**(1): pp. 23-30.

[51] Hassold, T., H. Hall, and P. Hunt, The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet, 2007. **16 Spec No. 2**: pp. R203–R208.

[52] Spandorfer, S.D., et al., Relationship between maternal age and aneuploidy in in vitro fertilization pregnancy loss. Fertil Steril, 2004. **81**(5): pp. 1265-1269.

[53] Menasha, J., et al., Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med, 2005. **7**(4): pp. 251-263.

[54] Munne, S., et al., Improved implantation after preimplantation genetic diagnosis of aneuploidy. Reprod Biomed Online, 2003. **7**(1): pp. 91-97.

[55] Macklon, N.S., J.P. Geraedts, and B.C. Fauser, Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update, 2002. **8**(4): pp. 333-343.

[56] Iwarsson, E., et al., A high degree of aneuploidy in frozen-thawed human preimplantation embryos. Hum Genet, 1999. **104**(5): pp. 376-382.

[57] Munne, S., et al., The use of first polar bodies for preimpantation diagnosis of aneuploidy. Hum Reprod, 1995. **10**: pp. 1015-1021.

[58] Munne, S., et al., Assessment of numeric abnormalities of X, Y, 18, and 16 chromosomes in preimplantation human embryos before transfer. Am J Obstet Gynecol, 1995. **172**(4 Pt 1): pp. 1191-9; discussion 1199-201.

[59] Verlinsky, Y., et al., Pregnancies following pre-conception diagnosis of common aneuploidies by fluorescent in-situ hybridization. Hum Reprod, 1995. **10**(7): pp. 1923-1927.

[60] Goossens, V., et al., ESHRE PGD Consortium data collection XI: cycles from January to December 2008 with pregnancy follow-up to October 2009. Hum Reprod, 2012. **27**(7): pp. 1887-1911.

[61] Hardarson, T., et al., Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. Hum Reprod, 2008. **23**(12): pp. 2806-2812.

[62] Staessen, C., et al., Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod, 2004. **19**(12): pp. 2849-2858.

[63] Staessen, C., et al., Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. Hum Reprod, 2008. **23**(12): pp. 2818-2825.

[64] Mastenbroek, S., et al., What next for preimplantation genetic screening? More randomized controlled trials needed? Hum Reprod, 2008. **23**(12): pp. 2626-2628.

[65] Mastenbroek, S., et al., Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update, 2011. **17**(4): pp. 454-466.

[66] Mastenbroek, S., et al., In vitro fertilization with preimplantation genetic screening. N Engl J Med, 2007. **357**(1): pp. 9-17.

[67] Debrock, S., et al., Preimplantation genetic screening for aneuploidy of embryos after in vitro fertilization in women aged at least 35 years: a prospective randomized trial. Fertil Steril, 2010. **93**(2): pp. 364-373.

[68] Schoolcraft, W.B., et al., Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. Fertil Steril, 2009. **92**(1): pp. 157-162.

[69] Blockeel, C., et al., Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. Reprod Biomed Online, 2008. **17**(6): pp. 848-854.

[70] Mersereau, J.E., et al., Preimplantation genetic screening to improve in vitro fertilization pregnancy rates: a prospective randomized controlled trial. Fertil Steril, 2008. **90**(4): pp. 1287-1289.

[71] Meyer, L.R., et al., A prospective randomized controlled trial of preimplantation genetic screening in the "good prognosis" patient. Fertil Steril, 2009. **91**(5): pp. 1731-1738.

[72] Harper, J.C., et al., ESHRE PGD Consortium data collection X: cycles from January to December 2007 with pregnancy follow-up to October 2008. Hum Reprod, 2010. **25**(11): pp. 2685-2707.

[73] Hens, K., et al., Dynamics and ethics of comprehensive preimplantation genetic testing: a review of the challenges. Hum Reprod Update, 2013. **19**(4): pp. 366-375.

[74] Magli, M.C., et al., Polar body array CGH for prediction of the status of the corresponding oocyte. Part II: technical aspects. Hum Reprod, 2011. **26**(11): pp. 3181-3185.

[75] Geraedts, J., et al., Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results. Hum Reprod, 2011. **26**(11): pp. 3173-3180.

[76] Scott, R.T., Jr., et al., Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril, 2012. **97**(4): pp. 870-875.

[77] Kokkali, G., et al., Birth of a healthy infant following trophectoderm biopsy from blastocysts for PGD of beta-thalassaemia major. Hum Reprod, 2005. **20**(7): pp. 1855-1859.

[78] McArthur, S.J., et al., Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril, 2005. **84**(6): pp. 1628-1636.

[79] Youssry, M., et al., Current aspects of blastocyst cryopreservation. Reprod Biomed Online, 2008. **16**(2): pp. 311-320.

[80] Tulay, P., et al., Number of embryos biopsied as a predictive indicator for the outcome of preimplantation genetic diagnosis by fluorescence in situ hybridisation in translocation cases. Zygote, 2014. **24**(1):107-14.

[81] Van Landuyt, L., et al., Influence of cell loss after vitrification or slow-freezing on further in vitro development and implantation of human Day 3 embryos. Hum Reprod, 2013. **28**(11):2943-9.

[82] Chang, E.M., et al., Use of the natural cycle and vitrification thawed blastocyst transfer results in better in-vitro fertilization outcomes: cycle regimens of vitrification thawed blastocyst transfer. J Assist Reprod Genet, 2011. **28**(4): pp. 369-374.

[83] Zhu, D., et al., Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles—time for a new embryo transfer strategy? Fertil Steril, 2011. **95**(5): pp. 1691-1695.

[84] Ku, P.Y., et al., Comparison of the clinical outcomes between fresh blastocyst and vitrified-thawed blastocyst transfer. J Assist Reprod Genet, 2012. **29**(12): pp. 1353-1356.

[85] Yang, Z., et al., Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet, 2012. **5**(1): pp. 24.

[86] Parriego, M., F. Vidal, and A. Veiga, Blastocyst Biopsy, in Preimplantation Genetic Diagnosis, J. Harper, Editor. 2009, Cambridge University Press: Cambridge.

[87] Diez-Juan, A., et al., Mitochondrial DNA content as a viability score in human euploid embryos: less is better. Fertil Steril, 2015. **104**(3): pp. 534-541.

[88] Fragouli, E., et al., Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. PLoS Genet, 2015. **11**(6): pp. e1005241.

[89] Treff, N.R., et al., Single nucleotide polymorphism microarray-based concurrent screening of 24-chromosome aneuploidy and unbalanced translocations in preimplantation human embryos. Fertil Steril, 2011. **95**: pp. 1606-1612.

[90] Forman, E.J., et al., Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod, 2012. **27**: pp. 1217-1222.

[91] Treff, N.R., et al., Accurate 23 chromosome aneuploidy screening in human blastomeres using single nucleotide polymorphism (SNP) microarrays. Fertil Steril, 2007. **86**(S217): pp. S1.

[92] Fiorentino, F., et al., Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril, 2014. **101**(5): pp. 1375-1382.

[93] Fiorentino, F., et al., Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod, 2014. **29**(12):2802-13.

[94] Wells, D., et al., Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation. J Med Genet, 2014. **51**(8): pp. 553-562.

[95] Wang, L., et al., Detection of chromosomal aneuploidy in human preimplantation embryos by next-generation sequencing. Biol Reprod, 2014. **90**(5): p. 95.

[96] Yin, X., et al., Massively parallel sequencing for chromosomal abnormality testing in trophectoderm cells of human blastocysts. Biol Reprod, 2013. **88**(3): p. 69.

[97] Brezina, P.R., et al., Single-gene testing combined with single nucleotide polymorphism microarray preimplantation genetic diagnosis for aneuploidy: a novel approach in optimizing pregnancy outcome. Fertil Steril, 2011. **95**(5): pp. 1786 e5–e8.

[98] Fiorentino, F., et al., Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations. Fertil Steril, 2010. **94**(6):2001-11.

[99] Griffin, D.K., et al., Dual fluorescent in situ hybridisation for simultaneous detection of X and Y chromosome-specific probes for the sexing of human preimplantation embryonic nuclei. Hum Genet, 1992. **89**(1): pp. 18-22.

[100] Treff, N.R., et al., Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays. Fertil Steril, 2010. **94**: pp. 2017-2021.

[101] Spits, C. and K. Sermon, PGD for monogenic disorders: aspects of molecular biology. Prenat Diagn, 2009. **29**(1): pp. 50-56.

[102] Dreesen, J.C., et al., Multiplex PCR of polymorphic markers flanking the CFTR gene; a general approach for preimplantation genetic diagnosis of cystic fibrosis. Mol Hum Reprod, 2000. **6**(5): pp. 391-396.

[103] Ban, Z., et al., Rapid diagnosis of triploidy of maternal origin using fluorescent PCR and DNA fragment analysis in the third trimester of pregnancy. Prenat Diagn, 2002. **22**(11): pp. 984-987.

[104] Hulten, M.A., S. Dhanjal, and B. Pertl, Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF-PCR. Reproduction, 2003. **126**(3): pp. 279-297.

[105] Weiss, M.M., et al., Comparative genomic hybridisation. Mol Pathol, 1999. **52**(5): pp. 243-251.

[106] Solinas-Toldo, S., et al., Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer, 1997. **20**(4): pp. 399-407.

[107] Callier, P., et al., Contribution of array CGH in prognosis and genetic counselling of prenatally diagnosed supernumerary ring chromosome 20. Prenat Diagn, 2009. **29**(10): pp. 1002-1005.

[108] Colmant, C., et al., Interstitial deletion 6p22.3-p24.3 characterized by CGH array in a foetus with multiple malformations. Prenat Diagn, 2009. **29**(9): pp. 908-910.

[109] Vialard, F., et al., Array comparative genomic hybridization in prenatal diagnosis: another experience. Fetal Diagn Ther, 2009. **25**(2): pp. 277-284.

[110] PGDIS, 10th international congress on preimplantation genetic diagnosis, in Trophectoderm biopsy and array CGH to detect unbalanced forms of t(9;15)(q22.2;q11.1) R. Edwards, et al., Editors. 2010, Reproductive BioMedicine online: Montpellier.

[111] Christodoulou, C., et al., Preimplantation genetic diagnosis for chromosomal rearrangements with the use of array comparative genomic hybridization at the blastocyst stage. Fertil Steril, 2017. **107**(1):212-219.e3.

[112] Garcia-Herrero, S., et al., Genetic analysis of human preimplantation embryos. Curr Top Dev Biol., 2016. **120**: pp. 421-447.

[113] Alfarawati, S., et al., Embryos of robertsonian translocation carriers exhibit a mitotic interchromosomal effect that enhances genetic instability during early development. PLoS Genet, 2012. **8**(10): p. e1003025.

[114] Estop, A.M., et al., Is there an interchromosomal effect in reciprocal translocation carriers? Sperm FISH studies. Hum Genet, 2000. **106**(5): pp. 517-524.

[115] Munne, S., et al., Negligible interchromosomal effect in embryos of Robertsonian translocation carriers. Reprod Biomed Online, 2005. **10**(3): pp. 363-369.

[116] Fishel, S., et al., Live birth after polar body array comparative genomic hybridization prediction of embryo ploidy the future of IVF? Fertil Steril, 2010. **3**: pp. 1006.e7-1006.e10.

[117] Treff, N.R., et al., SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. Mol Hum Reprod 2010. **8**: pp. 583-589.

[118] Schoolcraft, W.B., et al., Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril, 2010. **94**: pp. 1700-1706.

[119] Fiorentino, F., Array comparative genomic hybridization: its role in preimplantation genetic diagnosis. Curr Opin Obstet Gynecol, 2012. **4**: pp. 203-209.

[120] Gutierrez-Mateo, C., et al., Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. Fertil Steril, 2011. **3**: pp. 953-958.

[121] Shaffer, L.G. and B.A. Bejjani, A cytogeneticist's perspective on genomic microarrays. Hum Reprod Update, 2004. **10**(3): pp. 221-226.

[122] Harper, J.C. and G. Harton, The use of arrays in preimplantation genetic diagnosis and screening. Fertil Steril, 2010. **94**(4): pp. 1173-1177.

[123] Le Caignec, C., et al., Single-cell chromosomal imbalances detection by array CGH. Nucleic Acids Res, 2006. **34**(9): p. e68.

[124] Daphnis, D.D., et al., Analysis of the evolution of chromosome abnormalities in human embryos from Day 3 to 5 using CGH and FISH. Mol Hum Reprod, 2008. **14**(2): pp. 117-125.

[125] Luthra, R. and L.J. Medeiros, Isothermal multiple displacement amplification: a highly reliable approach for generating unlimited high molecular weight genomic DNA from clinical specimens. J Mol Diagn, 2004. **6**(3): pp. 236-242.

[126] Pinard, R., et al., Assessment of whole genome amplification-induced bias through high-throughput, massively parallel whole genome sequencing. BMC Genomics, 2006. **7**: p. 216.

[127] Zhang, L., et al., Whole genome amplification from a single cell: implications for genetic analysis. Proc Natl Acad Sci U S A, 1992. **89**(13): pp. 5847-5851.

[128] Peng, W., H. Takabayashi, and K. Ikawa, Whole genome amplification from single cells in preimplantation genetic diagnosis and prenatal diagnosis. Eur J Obstet Gynecol Reprod Biol, 2007. **131**(1): pp. 13-20.

[129] Barbaux, S., O. Poirier, and F. Cambien, Use of degenerate oligonucleotide primed PCR (DOP-PCR) for the genotyping of low-concentration DNA samples. J Mol Med, 2001. **79**(5-6): pp. 329-332.

[130] Voullaire, L., et al., Detection of aneuploidy in single cells using comparative genomic hybridization. Prenat Diagn, 1999. **19**(9): pp. 846-851.

[131] Little, E.S., et al., Array CGH using whole genome amplification of fresh-frozen and formalin-fixed, paraffin-embedded tumor DNA. Genomics, 2005. **87**(2): pp. 298-306.

[132] Lasken, R.S. and M. Egholm, Whole genome amplification: abundant supplies of DNA from precious samples or clinical specimens. Trends Biotechnol, 2003. **21**(12): pp. 531-535.

[133] O'Geen, H., et al., Comparison of sample preparation methods for ChIP-chip assays. Biotechniques, 2006. **41**(5): pp. 577-580.

[134] Talseth-Palmer, B.A., et al., Whole genome amplification and its impact on CGH array profiles. BMC Res Notes, 2008. **1**(56): pp. 1-8.

[135] Wells, D., S. Alfarawati, and E. Fragouli, Use of comprehensive chromosomal screening for embryo assessment: microarrays and CGH. Mol Hum Reprod, 2008. **14**(12): pp. 703-710.

[136] Kittai, A., E. Widra, and R. Leach, SNP PGD microarray analysis from single embryonic cell. in ASHG. 2008.

[137] Handyside, A.H., et al., Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet, 2010. **47**(10): pp. 651-658.

[138] Kearns, W.G., et al. Comprehensive Preimplantation Genetin Diagnosis(PGD) by single cell SNP microarrays. in ASHG. 2009.

[139] PGDIS, 10th international congress on preimplantation genetic diagnosis, in Comprehensive microarray genetic analyses on single cell(s) from polar bodies or embryos to determine 23-chromosome aneuploidy, structural chromosome aberrations and genome-wide scans using single nucleotide polymorphisms (SNPs); to identify what partner provided the extra chromosome in aneuploid embryos; and to determine what embryo implanted following an in vitro fertilisation (IVF) cycle. R. Edwards, et al., Editors. 2010, Reproductive BioMedicine online: Montpellier.

[140] PGDIS, 10th international congress on preimplantation genetic diagnosis, in First clinical application of karyomapping for PGD of Gaucher disease combined with 24 chromosome screening. R. Edwards, et al., Editors. 2010, Reproductive BioMedicine online: Montpellier.

[141] Tan, Y., et al., Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing. Gigascience, 2014. **3**(1): pp. 30.

[142] Xu, J., et al., Comparative study of single-nucleotide polymorphism array and next generation sequencing based strategies on triploid identification in preimplantation genetic diagnosis and screen. Oncotarget, 2016. **7**(49): 81839-81848.

[143] Ottolini, C.S., et al., Karyomapping identifies second polar body DNA persisting to the blastocyst stage: implications for embryo biopsy. Reprod Biomed Online, 2015. **31**(6): pp. 776-782.

[144] Miller, J.R., S. Koren, and G. Sutton, Assembly algorithms for next-generation sequencing data. Genomics 2010. **95**: pp. 315-327.

[145] Park, S.T. and J. Kim, Trends in next-generation sequencing and a new era for whole genome sequencing. Int Neurourol J, 2016. **20** (Suppl 2): pp. S76–S83.

[146] Zhang, W., et al., Clinical application of next-generation sequencing in preimplantation genetic diagnosis cycles for Robertsonian and reciprocal translocations. J Assist Reprod Genet, 2016. **33**(7): pp. 899-906.

[147] Lukaszuk, K., et al., Healthy baby born to a robertsonian translocation carrier following next-generation sequencing-based preimplantation genetic diagnosis: a case report. AJP Rep, 2015. **5**(2): pp. e172–e175.

[148] Zhang, C., et al., A single cell level based method for copy number variation analysis by low coverage massively parallel sequencing. PLoS One., 2013. **8**(1): pp. e54236.

[149] Aleksandrova, N., et al., Comparison of the results of preimplantation genetic screening obtained by a-CGH and NGS methods from the same embryos. Gynecol Endocrinol., 2016. **32**(suppl. 2): pp. 1-4.