Comparison of the results of preimplantation genetic screening obtained by a-CGH and NGS methods from the same embryos

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Abstract
Chromosomal aneuploidies are known for being the main cause of abnormal development of embryos with normal morphology, their implantation failure and early reproductive losses in IVF treatments. Preimplantation genetic screening (PGS) allows selecting embryos with normal chromosomal content and increases IVF treatment efficiency due to higher implantation rates and less frequent early pregnancy losses. New technologies used for PGS allow making genome-wide analysis of the presence of all chromosomes in embryos. This article presents our study of evaluation of two techniques used for PGS: previously developed and used in our laboratory a-CGH assay based on Agilent technology and newly tested semi-conductive NGS technique (Torrent technology).

Introduction
The success of infertility treatments using IVF largely depends on the quality of cultured embryos and their implantation potential. Modern molecular genetic approaches make it possible to obtain a “genetic profile”, which gives the opportunity to select for transfer embryos without chromosome aneuploidies and big structural rearrangements, avoiding the live birth of children with severe chromosomal abnormalities [1]. As it was demonstrated, idiopathic recurrent miscarriages are caused mostly by aneuploid embryos [2–4].

According to some investigations, 25% of oocytes in women above 35 years old were found to contain chromosome aberrations (mostly aneuploidy) with the frequency of aneuploidy being directly dependent on age, hence making it possible to rise up to 80% in women over 40 years old [5–6]. Preimplantation genetic screening (PGS) was developed as a method to improve IVF outcomes by selecting chromosomally normal embryos from those which have potentially lethal forms of aneuploidy. PGS can improve IVF success by increasing the implantation rate and reducing pregnancy losses. Fast development of molecular biology methods made it possible to rapidly improve the methods of chromosome screening of embryos during the preimplantation stage.

First generation of PGS techniques involved the use of fluorescent in-situ hybridization (FISH) analysis to screen for chromosome abnormalities in polar bodies and blastomeres from cleavage stage embryos. However, FISH method had its limitations as it could only screen from 5 to 12 chromosomes in each embryo biopsy specimen. Screening usually required the selection of chromosomes 13, 18, 21, X and Y, respectively, and only in some methods up to 12 chromosomes were analyzed by using repeated cycles of hybridization. However, as was shown by the large randomized clinical trials (RCTs), FISH method on a limited number of chromosomes does not allow for a significant improvement of IVF treatment efficiency [7]. Because of the mosaicism of embryos in this stage and the result obtained from one blastomere, this method fails to provide a complete picture of the whole chromosomal content of an embryo [8]. But more likely, the explanation for this fact is not a complete detection of aneuploidy in embryos, because of the limited number of analyzed chromosomes of the embryo. Recent studies have demonstrated that the implantation potential of cleavage-stage embryos can be impaired by the biopsy procedure itself, especially if it was performed under the suboptimal conditions or by insufficiently skilled lab personnel.

During the last decade, new technologies for aneuploidy screening have been introduced in practice. They allow carrying out the screening of all 24 chromosomes in human embryos. Among them, microarray comparative genomic hybridization (a-CGH), single nucleotide polymorphism microarrays (SNP arrays) and quantitative polymerase chain reaction (qPCR) have been validated and successfully applied clinically [3,9]. Comparative genome hybridization assay was the first technology to allow PGS of all 24 types of chromosomes. In this method, DNA is hybridized on relatively short DNA probes, specific to DNA sequences of investigated cells. First tests of this technique...
demonstrated a successful use of screening of all 24 chromosomes in human oocytes and embryos [10]. Today, array-CGH technology is accepted as a reliable and safe method for PGS and it is widely used in many IVF centers around the world [11, 12].

Array-CGH also detects all mitotic and meiotic abnormalities present in one cell or even in a group of cells. Unlike single nucleotide p. (SNP arrays), array-CGH does not require testing of the couple or the couple’s parents prior to an IVF cycle. Array CGH technique can be used not only for testing chromosomal aneuploidy, but also for diagnostics of some translocations, inversions and other chromosomal abnormalities – both gains and losses of chromatin.

The high efficient sequencing technology called next-generation PGS from 2010 [13–15] was implemented into the practice of IVF. A sufficient difference of this technology was the flexibility and preciseness of the test. Depending on the number of investigated chromosome locuses, it was possible to change the precision and purpose of investigation – from aneuploidy to medium size deletions or insertions in chromosomes. Provided new equipment begins to allow the investigation of dozens of samples in the same run and at the same time to reduce the cost of investigation compared to array-CGH, which makes this method more attractive for practical PGS.

The aim of this investigation was to evaluate the possibilities of embryo investigation results by a new method using semi-conductive NGS technology and to compare these with results obtained with array-CGH based on Agilent platform.

The design of the study: this is a prospective parallel investigation, which includes an aneuploidy investigation of human embryos on the blastocyst stage by using both NGS and array-CGH assays.

Criteria for investigation: embryos, which were derived from IVF cycles and were allocated for PGS aneuploidy screening were investigated using two techniques – array-CGH based on Agilent platform and NGS using Torrent technology.

Materials and methods

For investigation, we used samples of trophectoderm cells from 38 biopsied embryos taken from patients undergoing infertility treatments by the IVF method using PGS. After the controlled ovarian hyperstimulation, a follicular aspiration was performed 35 h after the injection of human chorionic gonadotropin for ovulation induction. It was followed by ICSI and 35 h after the injection of human chorionic gonadotropic for embryo transfer time after completed PGS diagnostics.

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A comparative genomic hybridization of the genetic material in embryos was carried out with the use of C Scanner (Agilent, Santa Clara, CA). Full-genomic amplification of the DNA in researched cells was carried out by WGA-PCR PicoPlex SingleCell WGA Kit (Rubicon Genomics) and MDA GenetiSure Prescreen Amplification and Labeling Kit (Agilent). The quality and quantity of DNA received during amplification were controlled by electrophoresis using 1.2% agarose gel. The labeling of amplification products was carried out by means of the SureTag DNA labeling Kit Agilent according to the enclosed instruction. The labeled amplification products were applied on the Sure Print G3 8x60 a-CGH Agilent array. Interpretation of the received results was carried out by means of the Agilent CytoGenomics.

Fragmented sequencing libraries were prepared using Ion Xpress Plus Fragment Library Kit according to the user guide. The multiplexed sequencing was performed using Ion Proton, Ion PI™ Sequencing 200 Kit v3 and Ion PI™ Chip v2. Primary data analysis (base calling, trimming, filtering and alignment to hg19 reference) was performed with Torrent server 4.4.3 software.

Subsequent data analysis was performed according to the in-house developed pipeline. For analysis, we only used not duplicated reads mapped to unique genome regions with mapping quality value ≥10. After LOESS GC correction, the presence of aneuploidy was estimated using Z score.

Table 1. Comparison of results using NGS and Agilent a-CGH.

| # of embryo | a-CGH (Agilent) NGS investigation | Amplification method |
|-------------|----------------------------------|---------------------|
| 1           | +10,+12,XY                       | WGA-PCR             |
| 2           | XX/XY mos                        | WGA-PCR             |
| 3           | N,XY                             | WGA-PCR             |
| 4           | XX, +1+4 mos                     | WGA-PCR             |
| 5           | N,XX                             | WGA-PCR             |
| 6           | N,XX                             | WGA-PCR             |
| 7           | N,XX                             | WGA-PCR             |
| 8           | N,XX                             | WGA-PCR             |
| 9           | N,XX                             | WGA-PCR             |
| 10          | N,XX                             | WGA-PCR             |
| 11          | XX    YY                         | WGA-PCR             |
| 12          | +4,XY                            | WGA-PCR             |
| 13          | N,XY                             | WGA-PCR             |
| 14          | XX    N,XX                       | WGA-PCR             |
| 15          | N,XY                             | WGA-PCR             |
| 16          | N,XY                             | WGA-PCR             |
| 17          | +16,XX (q arm)                   | WGA-PCR             |
| 18          | N,XX                             | WGA-PCR             |
| 19          | N,XX                             | WGA-PCR             |
| 20          | Chaotic                          | WGA-PCR             |
| 21 (rebiopsy of 9) | N,XX                          | MDA                 |
| 22 (rebiopsy of 10) | N,XY                           | MDA                 |
| 23 (rebiopsy of 13) | N,XY                            | MDA                 |
| 24 (rebiopsy of 15) | N,XY                           | MDA                 |
| 25          | –16,XY                           | MDA                 |
| 26          | N,XX                             | MDA                 |
| 27          | N,XX                             | MDA                 |
| 28          | N,XX                             | MDA                 |
| 29          | N,XX                             | MDA                 |
| 30          | N,XX                             | MDA                 |
| 31          | N,XX                             | WGA-PCR             |
| 32          | N,XX                             | WGA-PCR             |
| 33          | Chaotic                          | WGA-PCR             |
| 34          | Chaotic                          | WGA-PCR             |
| 35          | Chaotic                          | WGA-PCR             |
| 36          | –13, +14, XY                     | WGA-PCR             |
| 37          | –19, –22, XY                     | WGA-PCR             |
| 38          | –15, –22, XY                     | WGA-PCR             |

The results differ for a-CGH and NGS are highlighted in bold.
Results

According to a-CGH investigation 25 (65.7%) out of 38 embryos were euploid and 13 (34.3%) were aneuploid (Table 1). In 36 samples, we obtained concordant results for both NGS and a-CGH analysis. Discordant results were obtained only for 2 (5.2%) of all investigated samples.

In order to compare Whole Genome Amplification technique, four samples were biopsied two times and amplified using WGA-PCR and MDA. In all cases, the results were concordant. The results of investigation using our two methods for PGS are presented in Table 1.

Discussion

The Array-CGH method is routinely used for PGS in most IVF clinics today. At the same time, the Array-CGH technique cannot be used to test balanced translocations (reciprocal or Robertsonian translocations, inversions and insertions) and some unbalanced translocations like point mutations, trinucleotide expansions, small deletions and duplications because they are beyond the resolutions of the method.

After the first successful IVF birth where PGS was performed using the NGS technique in 2013 in Philadelphia, some large genetic laboratories reported preclinical studies and have demonstrated that NGS is an efficient and safe technique, which can be used for preimplantation screening [13]. The NGS technique in 100% of cases allows to reveal the aneuploidies and it does so with 99.98% compliance with the array-CGH technique.

Our study, conducted on a rather small number of samples (38 biopsied embryos) has revealed coincidence of results of array-CGH and NGS in 94.8% cases. Samples 11 and 14 according to Agilent a-CGH had a karyotype 47, XXY, however, according to NGS they had a normal set of chromosomes. These distinctions in data interpretation results can be connected with the low level of a fluorescent signal according to a-CGH technique. Most likely, it is connected with insufficient product labeling efficiency of amplification when passing array CGH. It is also worth noting differences in the received results when using various methods of nucleic acid amplification. During this research we have found that carrying out MDA is more preferable than WGA-PCR, because of more considerable signal/noise level at measurement of fluorescence on the microarray. Increase in this value simplifies the interpretation of received results, and does so more objectively and reliably. Other important circumstance of preference of MDA before WGA-PCR is that the amplified products present studied genome more completely reducing allele drop-out chance. This fact can have crucial importance in case products of amplification have to be used for further research, for example, carrying out preimplantation genetic diagnostics.

Thus, distinctions in the results can speak both higher resolution of the used NGS method, and different approaches for interpretation of the received results including the results received by different kits for full-genomic amplification. It is necessary to continue research for the purpose of choosing the most optimal method of full-genomic amplification, an assessment of an opportunity and need of detection of mosaic samples, and also an assessment of minimal sites for sequencing, which is necessary to cover full human genome.

Besides aneuploidy, NGS may also be used for the simultaneous evaluation of single-gene disorders and abnormalities of the mitochondrial genome, from the same biopsy material, without the need for multiple technological platforms [16]. PGS for chromosome aneuploidy cannot somehow improve the health of an embryo. The technique is used for more accurate selection of embryos with normal number of chromosomes for transfer and to reduce the treatment time to achieve a healthy live birth and to reduce the risk of miscarriage or a profoundly disabled child due to an abnormal number of chromosomes. NGS technique is offering some advantages to array-CGH, including the reduction of cost for DNA sequencing that makes it possible to provide an extensive use of this technology in selecting the best embryo for implantation.

The way to demonstrate a clinical effectiveness of NGS is to perform a well-designed and well-executed prospective randomized controlled trial, showing actual improvements in clinical outcomes.

In conclusion, the results achieved in this study demonstrate the reliability of the NGS-based protocol for the detection of whole chromosome aneuploidies and segmental changes in embryos. NGS methods may ultimately lead to reduced costs per patient, allowing IVF couples a wider use of PGS for choosing the most competent embryo(s) for transfer. NGS-based PGS represents a valuable alternative to other currently available CCS techniques, ready to find a place in routine clinical practice in IVF treatments.

Declaration of interest

The authors report no conflicts of interest.

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