Shallow whole genome sequencing is well suited for the detection of chromosomal aberrations in human blastocysts

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Objective: To add evidence that massive parallel sequencing (MPS) is a valuable substitute for array comparative genomic hybridization (arrayCGH) with a resolution that is more appropriate for preimplantation genetic diagnosis (PGD) in translocation carriers.
Design: Study of diagnostic accuracy.
Setting: University hospital.
Patient(s): Fifteen patients with a balanced structural rearrangement were included in the study: eight reciprocal translocations, four Robertsonian translocations, two inversions, and one insertional translocation.
Intervention(s): Trophectoderm biopsy was performed on 47 blastocysts.
Main Outcome Measure(s): In the current study, shallow whole genome MPS on a NextSeq500 (Illumina) and Ion Proton (Life Technologies) instrument was performed in parallel on 47 whole genome amplified trophectoderm samples. Data analyses were performed using the QDNAseq algorithm implemented in Vivar.
Result(s): In total, 5 normal and 42 abnormal embryos were analyzed. All aberrations previously detected with arrayCGH could be readily detected in the MPS data using both technologies and were correctly identified. The smallest detected abnormality was a ~4.5 Mb deletion/duplication.
Conclusion(s): This study demonstrates that shallow whole genome sequencing can be applied efficiently for the detection of numerical and structural chromosomal aberrations in embryos, equaling or even exceeding the resolution of the routinely used microarrays. (Fertil Steril® 2015;104:1276–85. ©2015 by American Society for Reproductive Medicine.)
Key Words: Massive parallel sequencing, preimplantation genetic diagnosis, whole genome amplification, blastocyst biopsy, chromosomal rearrangements

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Chromosomal abnormalities in early embryonic development can give rise to implantation failure, early spontaneous abortions and/or fetuses with multiple congenital anomalies. For couples with a known balanced or unbalanced chromosomal rearrangement, preimplantation genetic diagnosis (PGD) can be offered against unbalanced chromosomal aberrations in the embryo during assisted reproductive technology (ART) (1). At the same time, it is well established that early-stage embryos suffer from a high rate of (mosaic) aneuploidy, reducing embryo survival, implantation potential, and hence the success rate of ART (2).

In the past, fluorescent in situ hybridization (FISH) has been widely used to screen for chromosomal

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imbalances in embryos (3). With FISH the chromosomes involved in the translocation can be evaluated for unbalanced rearrangements at the single cellular level. However, for every single case, an individual laboratory genetic work-up is needed to optimize the protocol before starting the PGD procedure (3, 4).

In recent years, array comparative genomic hybridization (arrayCGH) has replaced fluorescent in situ hybridization for PGD in translocation carriers. The arrayCGH is a valuable technology in the context of PGD, as all chromosomes can be interrogated in a single experiment. There is no need for optimization of the PGD test in advance and besides chromosomal aberrations due to the (balanced) chromosomal rearrangement in the parent, also other aneuploidies or large copy number variations (CNV) can be detected. The arrayCGH and other comprehensive screening methods, such as real-time polymerase chain reaction (PCR) and single nucleotide polymorphism arrays, allow improved embryo diagnosis before transfer to the uterus and have proven their clinical utility (5–8).

Embryonic aneuploidy detection is not only valuable in a PGD setting. Recent evidence suggests that also patients with normal karyotypes, who have recurrent miscarriages, can benefit from comprehensive chromosome screening of the embryo (8). At present, randomized controlled trials are ongoing to evaluate the use of preimplantation genetic screening in those patients and these studies might prove that comprehensive chromosome screening could increase the implantation and pregnancy rate (7, 8).

Although arrayCGH proved its value, its rather limited resolution and the relative high cost are a disadvantage (9). For cryptic chromosomal rearrangements, the current resolution of approximately 10 Mb can be shortcoming. These issues could be addressed using massive parallel sequencing (MPS). Shallow or low-pass whole genome sequencing is used when no full genome coverage is needed. This technique can be used for detection of aneuploidy and/or chromosomal imbalances. The rapid development, dropping costs, and the possibility of screening at higher resolution, make MPS an attractive alternative to further increase the success rate of PGD and/or preimplantation genetic screening after ART.

Some proof of concept studies have shown the possibilities of shallow whole genome sequencing in a PGD/preimplantation genetic screening setting (9–12). However, most of these studies focused on aneuploidy detection and the resolution remained rather limited.

A recent important evolution in PGD is the time point for embryo biopsy. Although cleavage-stage biopsy, taking one or two blastomeres from the embryo on day 3, has long been the gold standard, recently a shift toward blastocyst trophectoderm biopsy on day 5 has occurred.

The present study evaluates the use of MPS for PGD on trophectoderm cells of blastocysts, comparing with arrayCGH in terms of accuracy. With this study, we aim to add evidence that MPS is a valuable and efficient substitute for arrayCGH, with the potential of being more cost efficient, and with a resolution that is more appropriate for PGD in translocation carriers.

MATERIALS AND METHODS

Study Design

In the present study, 15 patients with a balanced structural chromosomal rearrangement were enrolled. The aberrations included eight reciprocal translocations, four Robertsonian translocations, two inversions, and one insertional translocation (Table 1). Maternal age ranged from 27–41 years (mean, 33.5 years). Retrieved oocytes underwent intracytoplasmic sperm injection (ICSI) and trophectoderm biopsies were performed on day 5 or 6. After whole genome amplification (WGA) of the DNA from the trophectoderm cells, 24sure+ arrayCGH was performed for PGD. DNA amplified material from 47 embryos that was found abnormal with arrayCGH or did not implant after transfer, were selected for MPS. In this validation study we evaluated whether MPS is able to detect small but relevant chromosomal aberrations in a PGD setting. The 47 embryos were selected from a larger clinical cohort of 141 embryos in total and were specifically selected based on different sizes of the involved translocated segments and aneuploidy of different chromosomes. This study cohort represents a variety of clinical relevant chromosomal aberrations in a PGD setting.

After arrayCGH, we analyzed the remaining amplified DNA with shallow whole genome sequencing on a NextSeq500 platform (Illumina) and an Ion Proton sequencer (Life Technologies). Results from MPS were subsequently compared with the previously generated arrayCGH results (Fig. 1).

The arrayCGH analysis was performed between September 2014 and December 2014. Ion Proton and NextSeq500 sequencing were performed simultaneously at the beginning of 2015.

Our institutional review board approved this study (EC/UZG/2015/0108). Informed consent was obtained from all patients included in the present study.

Oocyte Retrieval and Blastocyst Biopsy

Controlled ovarian hyperstimulation (COH) of the study patients was performed according to age, antimüllerian hormone levels and previous response. The gonadotropins used were either a recombinant FSH (Gonal F, Merck Serono) or a urinary FSH (Menopur, Ferring Pharmaceuticals) at daily doses between 150 and 300 U. When an agonist protocol was followed, 0.1 mg triptorelin (Decapeptyl, Ipsen) was administered SC for 7 days starting on cycle day 1, and gonadotropins were started on cycle day 3. In case an antagonist protocol was necessary, gonadotropins were started on cycle day 3, and 0.25 mg cetrorelix (Cetrotide, Merck Serono) was injected SC as a daily dose from the sixth day of stimulation until the day of oocyte maturation triggering. The course of stimulation was followed by ultrasound monitoring. As soon as 50% of the follicles were >10–18 mm in diameter, oocyte maturation and retrieval was performed according to Vandekerckhove et al. (13).

After ICSI, oocytes were cultured in 25-μl drops of IVF-cleavage-medium (COOK) in the microwells of Embryoslides (Vitrolife) and overlaid with 1.2 mL of mineral oil.