Next-generation sequencing enabled chromosomal analysis for 2Mb of chromosomal segment in preimplantation embryos

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[Introduction] The chromosome analysis for preimplantation genetic testing for structural rearrangement (PGT-SR) has changed from fluorescence in situ hybridization (FISH) to next-generation sequencing (NGS). It is reported that accurate chromosome analysis cannot be performed for small chromosomal segment of 10 Mb or less by NGS, compared to FISH that was easy to diagnose using telomere probes.
Here, we report a reciprocal translocation couple who has 120kb and 2.57Mb of chromosome segments and could obtain a baby by PGT-SR using the modified NGS method.

[Case]
37-year-old woman, gravida 5 para 1. In her daughter with developmental delay, chromosome was diagnosed as normal by G-banding, but duplication of the long arm of chromosome 17 (17q25.3) was detected by FISH. Reciprocal translocation of chromosomes 2 and 17 in one of the couple was revealed by FISH, suggesting that the microduplications in their offspring were derived from the parental translocation. Preliminary chromosome analysis using NGS was carried out in order to request PGT-SR for the couple after four miscarriages. Accurate chromosome analysis of the offspring using microarray method revealed a copy number loss of 120 kb at the short arm of chromosome 2 and a copy number gain of 2.57 Mb at the long arm of chromosome 17. It was difficult to detect unbalanced translocations by conventional NGS analysis with 1 million leads and 1 Mb window width. Modified NGS, which increased the number of reads to 4 million and reduced the window width to 100-500 kb, allowed unambiguous detection of the 2.57 Mb duplication of chromosome 17, but failed to detect a small deletion of chromosome 2. The preliminary results showed that NGS could be used for PGT-SR in this case. PGT-SR was conducted after approval from the Japan Society of Obstetrics and Gynecology. Six blastocysts were first analyzed by conventional NGS. Two of the three blastocysts with balanced chromosomes had aneuploidy and the remaining three were determined to have unbalanced chromosomes due to adjacent I. In addition, modified NGS was performed on 4 blastocysts excluding 2 aneuploid ones to verify the results obtained by conventional NGS. The modified NGS showed that microduplication of chromosome 17 was clearly detected in all unbalanced blastocysts and any deletion or duplication was not detected in one blastocyst to be diagnosed as balanced. The balanced and euploid blastocyst was transferred, leading to

pregnancy.

[Conclusions]

Adjustment of the analysis conditions in NGS allowed the diagnosis of the small chromosomal segment of about 2 Mb in PGT-SR. Before conducting PGT-SR for small chromosomal segments, it is necessary to conduct preliminary inspection to confirm that accurate analysis by NGS is possible.