P-540 A feasible diagnostic approach for the cryptic subtelomeric traslocations in early recurrent miscarriage patients by preimplantation genetic testing (PGT).

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Study question: Could cryptic subtelomeric traslocations in early recurrent miscarriage patients be diagnosed by preimplantation genetic testing?
Summary answer: PGT is a powerful tool to detect subtelomeric cryptic traslocations identifying the cause of early recurrent miscarriage and allowing subsequent genetic counselling. What is known already: Chromosome translocations are frequently associated with birth defects, spontaneous early pregnancy losses and infertility. However, submicroscopic traslocations (so-called cryptic traslocations) are too small to be detected by conventional karyotyping.. Due to balanced status, high resolution molecular techniques as arrayCGH are not able to detect it. Thus, cryptic traslocations detection is challenging. PGT is able to detect CNVs at higher resolution than routine karyotyping. Therefore, the recurrent diagnosis of CNV at embryo level could suggest a subchromosomal parental traslocation. The aim of this study is to investigate the feasibility of using PGT as an indicator of parental balanced cryptic traslocations.
Study design, size, duration: We included three couples who underwent PGT for unexplained repeated pregnancy loss (RPL) in our clinic from February 2020 to November 2020. Common established causes of RPL (uterine anomalies, antiphospholipid syndrome, immunological, hormonal and metabolic disorders) were previously rouled-out. Even couple karyotypes were normal. Twenty-three embryos from those couples were biopsied at blastocyst and analysed for CNVs detection using low coverage whole genome NGS.
Participants/materials, setting, methods: PGT by NGS was performed by Veriseq-NGS (Illumina), with previous whole genome amplification. Fluorescence in situ hybridization (FISH) using parental blood samples were performed to validate the origin of subchromosomal number variation. Commercially available subtelomeric specific probes were selected according to the CNV identified and the procedures were performed according to the manufacturer's protocols.
Main results and the role of chance: Overall, CNVs of terminal duplication and deletion that imply unbalanced traslocation derivatives were detected in the 43.5% of biopsied embryos. For couple 1, 4 out of 5 embryos (80%) carried deletion of telomeric region on chromosomes 5 and 21. Three out of 6 biopsed embyos (50%) were diagnosed with subchromosomal copy variants at telomeric region on chromosomes 6 and 16 for couple 2. In the case of couple 3, three out of 12 embryos (25%) were carriers of CNV at subtelomeric region on chromosomes 2 and 6. The size of CNVs detected ranges from 8Mb to 20Mb. Accurate diagnosis with the parental study was made by FISH. The combination of probes to detect the structural chromosome alteration were: Tel5qter-LSI21q, Tel6pter-CEP16 and Tel6pter-CEP6 for each couple respectively. The FISH studies reveal that CNVs were inherited from one parent carrying the balanced cryptic traslocation. Ultimately, the abnormal karyotype from the carrier parent were 46,XY,t(5;21)(q33.2;q21.2) for couple 1, 46,XY,t(6;16)(p22.3;q22.1) for couple 2 and 46,XY,t(2;6)(p25.1;p24.2) for couple 3. Finally, each couple performed a cryotransfer of a single normal balanced embryo. Two pregnancies are ongoing.
Limitations, reasons for caution: The main limitation of this approach is the NGS- PGT resolution. CNVs smaller than 5Mb could not be detected.
Wider implications of the findings: This study shows the value of PGT for unexplained RPL, followed by parental FISH to better characterize CNVs and identify couples in whom one partner carries a cryptic translocation. Accurate diagnosis of parental chromosome translocation can achieve with FISH only, but FISH would not be performed unless PGT showed CNVs.
Trial registration number: Not applicable