IVF WORLDWIDE ONLINE CONGRESS

Comprehensive PGT using fragment STR-analysis after full-genomic amplification

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Introduction

Chromosomal abnormalities in embryos, in particular aneuploidy, are a leading factor in failed ART programs and subsequent pregnancy losses.

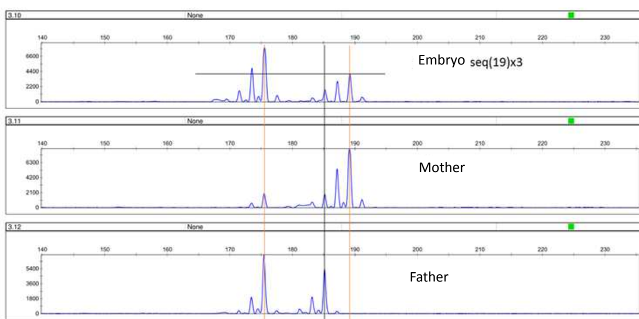
Aim of the study

Creation of a methodological approach for further study of the mechanism of the occurrence of aneuploidy in embryos taking into account the parental contribution.

Matherials and methods

63 pairs of patients were enrolled in the study. They applied for an ART program with PGT-A with the following indications: the mother's age is over 35; repeated unsuccessful IVF attempts in history (2 or more); habitual miscarriage; severe spermatogenesis disorders; the patient's desire.

The embryos biopsies were examined using both comparative genomic hybridization on chips (Agilent) and high-throughput sequencing (ReproSeq, Thermo Fisher Scientific). To carry out genome-wide amplification, two principal approaches were used: WGA-PCR using kits from Rubicon and MDA using kits from Qiagen. The resulting genome-wide amplification was used for fragment analysis using the original primers for STR analysis developed in this study. The obtained fragments were analyzed using an ABI-3500 capillary phoresis instrument.



Result

In general 155 embryos were examined among them 52% were with aneuploidy. All human chromosomes, including sex ones, were involved in the development of these aneuploidies. As part of this study, primers were selected and synthesized for the analysis of chromosomes 1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 19, 22, and X. It has been shown that both the product obtained using WGA-PCR (with and without barcodes for NGS) and the products obtained using MDA can be used to successfully conduct STR analysis and determine the parental contribution to the development of aneuploidy in embryos. At the same time, reliable results can be obtained only if the STR markers of the parents do not coincide, since the size of the peak and the area under the curve can be affected by non-equilibrium whole genome amplification of different regions of different chromosomes.

Conclusion

This approach allows preimplantation screening and diagnostics (PGT-A and PGT-M) in conjunction with embryo genotyping without the need for repeated biopsies, which can negatively affect their viability.

Table 1

Chromosome	Genes
1	GBA, RHD
3	GLB1
4	PKD2
5	SMN1
6	CYP21, HFE, PKHD1
7	CFTR, SLC26A4
9	GALT, FXN
11	HBB, ATM, TYR
12	PAH
13	ATP7B, GJB2
14	GALC
15	OCA2, HEXA
16	HBA1, HBA2, PKD1, MEFV
19	GCDH
22	ARSA
X	DMD, F8, F9, FMR, WAS

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