STANDARDIZATION OF ESTABLISHED PROTOCOLS AND THE QUALITY CONTROL OF PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A)

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Abstract Body

Introduction: The accuracy of PGT-A using massive parallel sequencing entirely depends on the effectiveness of quality control of embryological, genetic and analytical stages. The aim of this study was the development of intra-laboratory standards at every stage to optimize the working protocols, as well as participation in the inter-laboratory comparative tests of international consortiums (UK NEQAS, GenQA) on PGT.
Materials and methods: PGT-A was performed in more than 5,000 trophectoderm samples on the MiSeq instrument using VeriSeq PGS kit (Illumina). Quantitative and qualitative assessment of libraries was performed on the Fragment Analyzer (Advanced Analytical) applying a High Sensitivity NGS Fragment Analysis Kit (DNF-474-1000).
Results and summary: Intra-laboratory validation of the library preparation methodology for sequencing was carried out in order to obtain optimal sequencing parameters. Fragment analysis made it possible to accurately assess the quality of libraries and to recalculate the length of their fragments for subsequent normalization and uniform distribution of passing filter reads (PF) in terms of the sample. However, with optimal sequencing quality, difficult-to-interpret genomic profiles were obtained in about 10% of cases. Segmental aneuploidies in the mosaic variants, which are often evaluated by an expert as artifacts, are suspicious. The recognition of artifact false-mosaicism structural rearrangements can help, in particular, the absence of break points, the stereotypical pattern of copy number variation (CNV) in certain regions of chromosomes that are associated with the difficulties of reading GC-rich DNA regions. As a result of participation in the program of external quality assessment of PGT-A consortium UK NEQAS we found that partial degradation of DNA in the sample makes it impossible to detect trisomies. Moreover, we have recently started a research project (Vitrolife financial support) to verify how various technical manipulations carried out at the embryological stage can affect the CNV quality data and their interpretation.