

Abstract

Preimplantation genetic testing for aneuploidies (PGT-A) is a widely used approach in assisted reproductive technologies (ART) that aims to improve implantation and pregnancy rates by selecting embryos with a normal chromosomal complement. Accurate and timely identification of chromosomal abnormalities is especially critical when working with limited DNA quantities from embryo biopsy samples. This study aims to evaluate the diagnostic performance of quantitative fluorescent PCR (QF-PCR) in detecting chromosomal aneuploidies from trophoctoderm biopsy samples, using next-generation sequencing (NGS) as the reference method to assess sensitivity, specificity, and overall concordance.

QF-PCR is widely validated in prenatal diagnostics. In this study, our aim is to implement this method and to conduct its analytical and clinical validation in preimplantation testing (PGT-A) for the analysis of cells from trophoctoderm biopsies. Next-generation sequencing (NGS)-based preimplantation testing for aneuploidy often requires more time and financial resources, increasing the per-test cost; therefore, a rapid, informative, and cost-effective alternative is needed. QF-PCR delivers results in a short time and, with high accuracy, detects whole-chromosome aneuploidies on the most clinically relevant chromosomes—21 (Down syndrome), 18 (Edwards syndrome), and 13 (Patau syndrome).

QF-PCR was performed for chromosomes 13, 18, 21, X, and Y using STR markers, and results were analyzed with GeneMapper® software. Peak patterns, allele dosage ratios, and number of alleles were used to identify disomies and full aneuploidies. Each sample was then analyzed with NGS-based PGT-A to enable direct comparison. The goal was to assess how well QF-PCR can detect full aneuploidies and sex chromosome abnormalities which provides high-resolution chromosomal analysis.

The results show that QF-PCR is highly accurate in detecting full aneuploidies across the five target chromosomes, reliably identifying disomies, trisomies, and monosomies. The specificity of the method was particularly high, with minimal false-positive results observed. Concordance with NGS was strong in most cases involving full chromosomal gains or losses,

while discordant results were mainly limited to suspected mosaic patterns or chromosomal aberrations.

This study confirms the effectiveness of QF-PCR as an independent, efficient, and cost-effective diagnostic method for detecting aneuploidies in the selected five chromosomes. Bottom of Form