Clinical Outcomes of Embryos with Atypical Pronuclear Classified as Euploid by PGT-A: A Comparison of WGA-Based and Targeted NGS with SNP Profiling

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Abstract

Background and Aims: Normal fertilization is defined by the presence of two pronuclei (2PN); however, atypical pronuclear (PN) patterns (0PN, 1PN and 3PN) are frequently observed during routine embryological assessment. Atypical PN Embryos are often deprioritized due to abnormal chromosomal concerns, although PN morphology alone may not reliably reflect true ploidy status. Whole-genome amplification (WGA)–based preimplantation genetic testing for aneuploidy (PGT-A) has limitations in identifying certain ploidy abnormalities. Targeted next-generation sequencing (tNGS) with single-nucleotide polymorphism (SNP) profiling may improve ploidy assessment. This study aimed to compare clinical outcomes following transfer of atypical PN embryos classified as euploid using WGA-based PGT-A versus tNGS with SNP profiling.

Methods: This prospective single-center study assessed pronuclear status 16–18 hours post-insemination. Atypical PN patterned blastocysts underwent trophectoderm biopsy and were analyzed using either WGA-based PGT-A or tNGS combined with SNP profiling. Embryos classified as euploid by the respective testing platform were selected for transfer. Clinical pregnancy outcomes were recorded.

Results: Among transfers involving euploid embryos derived from atypical PN fertilization, no pregnancies were achieved following transfer of embryos screened using WGA-based PGT-A (0/4). In contrast, following transfer of euploid embryos screened using tNGS plus SNP profiling, pregnancies were achieved (2/4). One pregnancy resulted in a twin gestation following combined transfer of a 1PN-derived and a normally fertilized (2PN-derived) embryo. The second pregnancy was a singleton gestation followed transfer of a euploid 3PN-derived embryo, with fetal cardiac activity confirmed.

Conclusions: Clinical outcomes of embryos with atypical pronuclear patterns and classified as euploid differed according to the PGT-A methodology applied. Pregnancies were observed only following evaluation with tNGS combined with SNP profiling, including a successful singleton pregnancy from a 3PN-derived embryo. These findings highlight the limitations of pronuclear morphology alone for embryo selection and support cautious clinical consideration of embryos with atypical PN patterns when assessed using advanced PGT-A approaches.

Keywords: Preimplantation genetic testing for aneuploidy (PGT-A), Atypical pronuclear, Targeted next-generation sequencing with SNP profiling