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DETECTION OF EXACT FRAGILE X CGG REPEAT SIZE OF EMBRYONIC TROPHECTODERM BIOPSIES.

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OBJECTIVE: A premutation Fragile X Mental Retardation 1 (FMR1) gene allele exhibits the presence of 55-200 repeats and is paired with an increased risk for other Fragile X (FX) associated disorders. Premature ovarian insufficiency (POI) affects about 20% of female premutation carriers and results in hindering the outcomes of in-vitro fertilization (IVF). Additionally, there is an increased risk of expansion of the unstable CGG repeat sequence when the allele reaches the premutation range. Current pre-implantation genetic diagnosis (PGD) for FXS, involves tracking the affected X chromosome using linked markers, without observation of the size of the CGG repeats. This study sought to establish a method to determine CGG repeat sizes within the FMR1 gene of trophectoderm (TE) biopsies.

DESIGN: Experimentally, blinded study

MATERIALS AND METHODS: Phase I genomic DNA (gDNA) validation: gDNAwas isolated from 17 patients with known CGG repeat sizes to validate Amplidex PCR/CE FMR1 Reagent Kit (Asuragen). Capillary electrophoresis (CE) was conducted using the 3730xl DNA Analyzer (Thermo Fisher Scientific). Phase II 6-cell samples validation: 84 samples of 6 cells were collected from cell lines with known FX repeat sizes (23- 477 CGG repeats) (Corriell Repository) to mimic a TE biopsy. These samples were amplified with the REPLI-g Single Cell Kit (Qiagen), then subjected to the Amplidex PCR and CE. Phase III TE biopsies validation: TE biopsies from five discarded aneuploid whole embryos (expected CGG repeats sizes from 29 to >200 CGG based on parental genotypes and linked markers) were blinded, then amplified and underwent the Amplidex PCR and CE.

RESULTS: Phase I: All gDNA samples exhibited the expected CGG repeat size compared to the results from two reference laboratories. Phase II: The 6-cell samples showed CGG repeat sizes as expected. Phase III: The results of TE biopsies were consistent to the parental CGG repeat sizes.

CONCLUSIONS: CGG repeat sizes and expansion can be observed using this new methodology on TE biopsies. This methodology has the potential application to assess the amount of expansion for patients with limited numbers of usable embryos. A validation with a larger sample size will be needed before clinical use. Transferring embryos with FMR1 premutation alleles will encompass more diligent genetic counseling and detailed consents.