TITLE: BLASTOCYST REBIOPSY OUTCOMES AFTER AN INTIAL INCONCLUSIVE PGT-A RESULT BASED ON THE DAY OF INITIAL BIOPSY

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BACKGROUND: Despite technological advancements in preimplantation genetic testing for aneuploidy (PGT-A) platforms, some embryos fail to yield a conclusive result after trophectoderm biopsy for PGT-A. Prior studies have shown re-biopsy can identify usable blastocysts, however little is known about whether the chance of identifying a euploid embryo on re-biopsy is modified based on the day of initial biopsy (day 5, 6, or 7).

OBJECTIVE: The objective of this study is to assess outcomes of repeat biopsy on embryos with initial inconclusive results and to determine if the proportion of inconclusive results differs based on day of biopsy (5, 6, or 7).

MATERIALS AND METHODS: A retrospective cohort study was conducted of all embryos that underwent PGT-A testing and received an initial inconclusive result, on day 5, 6, and 7 from autologous cycles of patients aged 30 to 44 years old between January 1, 2021, and December 31, 2022. Blastocysts of patients undergoing PGT for structural rearrangements or monogenic disorders were excluded. All trophectoderm biopsies were analyzed in a single laboratory using the NextGen Sequencing (NGS) platform. Repeat biopsies were performed at the same embryology laboratory as the initial biopsy using standard biopsy technique and the cells were sent to the same genetics laboratory as the initial biopsy.

RESULTS: A total of 396 embryos underwent biopsy for PGT and received an inconclusive diagnosis. Of these, 228 (57.6%) were re-biopsied. Two-hundred and twenty-six survived the thaw (99.1%). Ninety-three were day 5 embryos (40.8%), 124 were day 6 embryos (54.4%) and 11 were day 7 embryos (4.8%). In total, 93 embryos (40.8%) were reclassified as euploid, 113 (49.6%) reclassified as aneuploid, 18 (7.9%) reclassified as mosaic, 2 (0.9%) remained inconclusive and 2 embryos (0.9%) did not survive the re-biopsy procedure. The incidence of inconclusive results did not vary with day of biopsy, both for the initial and repeat biopsy groups. The percentage of embryos found to be euploid on re-biopsy decreased from day 5 to day 7; however, this association was not statistically significant (day 5: 44%, day 6: 39%, day 7: 36%, p=0.7).

CONCLUSIONS: Our study revealed a 41% chance of euploidy after repeat biopsy of an initially inconclusive result. Rate of euploidy was not found to be statistically different between day of biopsy on repeat results, although this may be due to the small sample size. While there is a concern that an inconclusive embryo may not survive the re-biopsy thaw or may yield another inconclusive result, both were observed to be rare outcomes. Notably, the risk of thaw failure was 1%, substantially lower than the previously reported rate of 22.6% (1). These results

underscore the importance of considering re-biopsy in cases of inconclusive initial PGT-A results, regardless of day of embryo freeze.

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REFERENCES:

1. Nohales, M., Coello, A., Martin, A. *et al.* Should embryo rebiopsy be considered a regular strategy to increase the number of embryos available for transfer?. *J Assist Reprod Genet* 40, 1905–1913 (2023). https://doi.org/10.1007/s10815-023-02875-zRepeat PGT-A results of embryos initially resulted as inconclusive

Table 1. Rebiopsy PGT-A results by day of biopsy

	Normal	Abnormal	Mosaic	No Survival	Inconclusive	Total
Day 5	41 (44.1%)	36 (38.7%)	12 (12.9%)	2 (2.1%)	2 (2.1%)	93
Day 6	48 (38.7%)	70 (56.4%)	6 (4.8%)	-	-	124
Day 7	4 (36.3%)	7 (63.6%)	-	-	-	11
Total	93 (40.8%)	113 (49.6%)	18 (7.9%)	2 (0.89%)	2 (0.89%)	228