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Short Communication

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Evaluating the Plausibility of Euploid Embryos Transfer on Day-5 by Reanalysis of Day-3 Single Aneuploid Embryos: A Case Series

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Abstract

Background: During preimplantation development, single aneuploidies are more commonly tolerated than complex aneuploidies. Some studies have reported that blastocysts with aneuploid karyotypes on Day-3 embryo biopsy can exhibit a normal karyotype on Day-5 rebiopsy, suggesting that single aneuploidies may have a higher likelihood of presenting a normal karyotype on Day-5. The purpose of the current study was to assess the benefit of reanalyzing the karyotypes of Day-3 single aneuploid embryos on Day-5.

Methods: Day-3 and Day-5 biopsies of preimplantation embryos were subjected to array comparative genomic hybridization (aCGH). A proof of concept case series study was conducted involving 13 Day-5 embryos from 4 couples across 3 ART centers, collected between October 2019 and June 2020. Each center provided one normal embryo and 3-4 embryos with single aneuploidy based on Day-3 aCGH results. The karyotype of each Day-5 embryo was compared with its corresponding Day-3 karyotype.

Results: Among the 10 embryos with single aneuploidy on Day-3, 3 (30%) exhibited discordant karyotypes on Day-5, while the remaining 7 single aneuploid embryos and 3 normal embryos maintained the same karyotype from Day-3 to Day-5. None of the Day-3 single aneuploid embryos displayed a normal karyotype on Day-5.

Conclusion: Contrary to previous reports suggesting the potential correction of single aneuploidies in some embryos, the findings of this study did not support such a possibility in the analyzed embryos. Genomic reanalysis of Day-3 single aneuploid embryos on Day-5 does not appear to be a reliable method for identifying euploid embryos suitable for transfer.

Keywords: Aneuploidy, Biopsy, Blastocyst, Cleavage stage, Preimplantation.
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Introduction

Despite decades of development in assisted reproductive technologies, improving success rates remains a challenge. Preimplantation genetic testing for aneuploidy (PGT-A) is a topic of debate, although it is widely used in certain circumstances, such as in cases with advanced maternal age (1). Mosaic embryos, which contain a mixture of aneuploid and euploid cells, have been associated with healthy live births, potentially due to the depletion of aneuploid cells during development (2, 3). Previous studies have reported that a selection process occurs during preim-

plantation development, specifically acting against complex aneuploidies, particularly those originating from mitotic errors (4). Furthermore, it has been observed that blastulation rates decline in a linear fashion from euploid to aneuploid embryos, with a distinction between single chromosomal errors and multiple chromosomal errors (5). These findings suggest that there is a higher tolerance for single aneuploidies compared to complex aneuploidies. Moreover, several studies have indicated that some blastocysts, which exhibit aneuploid karyotypes during Day-3 embryo biopsy, may have a normal karyotype during Day-5 rebiopsy. Notably, two of these studies with substantial sample sizes (6, 7) suggest that single aneuploidies may have a higher likelihood of presenting a normal karyotype during Day-5 rebiopsy. The purpose of the current study was to assess the feasibility of reanalyzing the karyotypes of embryos with Day-3 single aneuploidy on Day-5.

Methods

This descriptive case series study involved the reanalysis of Day-5 embryos with Day-3 single aneuploidy between October 2019 and June 2020. Embryo culture and biopsy were performed following a previously described protocol (8). Briefly, the embryos were cultured in sequential media (Vitrolife, Sweden) and placed under mineral oil (Origio, Denmark). After routine assisted reproductive technology (ART) treatments, two pronucleate (2PN) zygotes were transferred to fresh microdrops of G-1TM V5 medium (Vitrolife, Sweden) supplemented with 10% human serum albumin (Vitrolife, Sweden). To perform the embryo biopsy, the embryos were incubated for 1-2 *min* in Ca/Mg free G-PGDTM biopsy medium (Vitrolife, Sweden). Following the biopsy, the embryos were transferred to G-2TM medium (Vitrolife, Sweden) for further culture. On Day 3, one blastomere was biopsied, while several cells were separated for Day 5 trophectoderm biopsy. The Day-3 and Day-5 biopsies were analyzed using the 24sure array comparative genomic hybridization (aCGH) platform (Illumina, US), following the manufacturer's instructions. Whole genome amplification was conducted according to the 24sure V3 protocol. Samples showing successful amplification were studied using the 24sure array CGH platform (Illumina, US). The amplified samples were loaded onto a 1% agarose gel, and those with successful amplification were further analyzed. The hybridization images were scanned using a laser scanner (InnoScan 710; Innopsys, France) and analyzed using the BlueFuse Multi Software version 4.1 (Illumina, US), which is a specialized software for array CGH data analysis.

This study obtained approval from both the Institutional Review Board and the Institutional Ethics Committee, under the approval code IR.ACECR.ROYAN.REC.1397.210. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, which provides ethical guidelines for research involving human subjects. Informed consent was obtained from all participants, ensuring that they were fully informed about the study's purpose, procedures, and potential risks, and that they voluntarily agreed to participate. Given the study's descriptive design and the nature of the results, no statistical analysis was performed. The primary focus of the study was to provide a descriptive account of the observed data rather than analyzing it quantitatively.

Results

Among the 10 embryos identified with single aneuploidy on Day-3, 6 were found to be monosomic and 4 were trisomic. Out of these Day-3 single aneuploid embryos, 3 (30%) exhibited discordant karyotypes on Day-5. Conversely, the remaining 7 single aneuploid embryos and 3 normal embryos maintained the same Day-3 karyotype on Day-5. None of the Day-3 single aneuploid embryos exhibited a normal karyotype on Day-5, as indicated in table 1.

Discussion

Preimplantation aneuploidy is a significant factor contributing to human infertility. Several reports have highlighted inconsistencies in aneuploidy screening results between Day-3 and Day-5 preimplantation embryos (3, 6, 7, 9, 10). Notably, two studies with substantial sample sizes (6, 7) have indicated that Day-3 embryos with single aneuploidies have a higher likelihood of exhibiting a normal karyotype on Day-5.

It is worth noting that the rates of aneuploidy can vary across different assisted reproductive technology (ART) centers, even among egg donors (10), due to the influence of ART procedures. To address potential center-related variations, this pilot study included single aneuploid embryos obtained from three different ART centers. While blastocyst biopsy for PGT-A is widely used in centers associated with the latest ESHRE PGT Consortium (1), Day-3 biopsy continues to be pre-

Table 1. Comparison of Day-3 and Day-5 karyotypes of the embryos using array CGH

Center	Day-3 PGT-A	Day-5 developmental status	Day-5 PGT-A	Concordant
1	47, XY +2	Hatching	47, XY +13, +19, -20	No
1	45, XX -13	Hatching	45, XX -13	Yes
1	45, XY -4	Midblastocyst	45, XY -4	Yes
1	46, XY	Hatching	46, XY	Yes
2	46, XY	Hatching	46, XY	Yes
2	45, XY -4	Midblastocyst	45, XY -4	Yes
2	47, XX +15	Hatching	47, XX +15	Yes
2	47, XX +22	Midblastocyst	47, XX +22	Yes
3	45, XY -22	Compact morula	48, XY +1, +7, +8, -22	No
3	47, XXY	Compact morula	46, XO with multiple aneuploidy	No
3	47, XY +22	Early blastocyst	47, XY +22	Yes
3	46, XX	Early blastocyst	46, XX	Yes
3	45, XY -2	Early blastocyst	45, XY -2	Yes

valent in certain ART centers worldwide. In this study, reanalysis of single aneuploid embryos did not reveal any cases of normal karyotypes. A previous experiment involving 30 blastocysts and utilizing the fluorescence in situ hybridization (FISH) technique yielded similar results, with the exception of one embryo (8). Given the limitations of FISH for aneuploidy screening, our study focused on using array comparative genomic hybridization (aCGH) as a robust technique, specifically targeting single aneuploidies that have a higher probability of exhibiting normal karyotypes on Day-5.

A limitation of our study is the small sample size. Given the inherent challenges in accurately estimating the correction rate of single aneuploidies within a larger sample size, our study was primarily designed to evaluate the cost-effectiveness of rebiopsy and reanalysis on Day-5 for patients, rather than to address a specific research question. If the preliminary findings of our study had indicated a normal karyotype for Day-3 single aneuploid embryos, it would have been reasonable to investigate a larger sample size of Day-3 single aneuploid embryos to assess the potential benefits of Day-5 rebiopsy and karyotyping for couples facing similar situations. However, further analyses were not pursued due to the absence of any instances of single aneuploidy correction among the 10 embryos that were analyzed.

Conclusion

The findings of the current study indicated that the correction of single aneuploidies in embryos was not observed. These results from the proof of concept study indicate that rebiopsy of single aneuploid embryos does not typically reveal a normal chromosomal status suitable for embryo transfer.

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Conflict of Interest

The authors declare that they have no competing interests.

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