IS DAY 3 (D3) VS. DAY 5 (D5) ASSISTED HATCHING (AH) ASSOCIATED WITH INCONCLUSIVE PREIMPLANTATION GENETIC TESTING (PGT) RESULTS?

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Background:

There has been a nationwide increase in the use of preimplantation genetic testing for aneuploidy (PGT-A) but there is no consensus whether assisted hatching (AH) at cleavage (D3) or blastocyst stage (D5) is preferred. Furthermore, the impact of AH on indeterminate PGT-A has not been investigated.

Objective:

To evaluate the rate of indeterminate PGT-A results amongst embryos that undergo day 3 (D3) or day 5 (D5) assisted hatching.

Materials and Methods:

Design: Retrospective cohort.

Setting: Academic fertility center.

Patients: 5,063 PGT-A tested blastocysts from 856 patients.

Intervention: D3 vs. D5 AH.

Outcomes: Blastocysts with inconclusive 1st and 2nd PGT-A results [absolute numbers and percentage (%) of embryos tested].

Statistics: Parametric and non-parametric tests were used. Generalized estimating equations (GEE) linear regression was used to account for multiple embryos per patient and adjust for maternal age, infertility diagnosis, and fertilization method.

Results:

The unadjusted analysis showed that the mean number and rate of inconclusive 1st PGT-A results per retrieval were similar in both D3 and D5 AH groups (Mean±SD: 0.2±0.7 vs. 0.1±0.4, p=0.08 and Rate±SD: 5.1%±14.3% vs. 3.1%±11.7%, p=0.09). However, D3 AH blastocysts accounted for more inconclusive 2nd PGT-A results per retrieval (Mean±SD: 0.1±0.3 vs. 0.0±0.1, p=0.01), and comprised a larger percentage of inconclusive 1st PGT-A (Rate±SD: 3.1%±4.6% vs. 0.3%±1.1%, p=0.01). The D3 AH blastocysts also contributed a greater total percentage of all embryos initially tested (Mean±SD: 1.7±8.1 vs. 0.2±2.3, p=0.01, for D3 vs. D5 AH, respectively).

When adjusted for maternal age, infertility diagnosis, and fertilization method, there was a higher total number of inconclusive 1st PGT-A results per oocyte retrieval

among D3 AH blastocysts (Adj β 0.12, 95%CI [0.03,0.21], p=0.01), while there were no differences in the % of inconclusive 1st PGT-A results out of total embryos tested (Adj β 2.11, 95%CI [-0.51,4.74], p=0.12). Adjusted analysis revealed inconclusive 2nd PGT-A results were higher following D3 AH. Specifically, the absolute number of inconclusive 2nd PGT-A results (Adj β 0.1, 95%CI [0.03,0.10], p=0.001), percentage of inconclusive 2nd PGT-A out of inconclusive 1st PGT-A results (Adj β 1.98, 95%CI [0.29,3.66], p=0.02), and percentage of inconclusive 2nd PGT-A of all embryos tested (Adj β 1.19, 95%CI [0.48,1.90], p=0.001).

Conclusions:

There were no significant differences in inconclusive 1st PGT-A results between D3 and D5 AH. Interestingly, inconclusive 2nd PGT-A results seem to be higher following D3 AH, with these embryos comprising a majority of the total number of embryos that returned with indeterminate results despite multiple test attempts.

As clinics move toward increased freeze-all/PGT-A cycles, factors such as timing of assisted hatching should be evaluated to assess for any possible impact on the validity of PGT-A testing. This retrospective study shows that there may be an increased likelihood of obtaining inconclusive PGT-A results with Day 3 AH, compared to Day 5 AH. These findings are an important step towards developing universal embryology protocols that can improve the efficiency of PGT-A testing.

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