

Letter to Editor

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Timing of assisted hatching affects the mosaicism rate in embryos

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Assisted hatching (AH) is commonly performed before trophectoderm (TE) biopsy for preimplantation genetic testing for aneuploidy (PGT-A), yet whether AH timing influences the detection of chromosomal mosaicism remains uncertain. Beyond timing, procedural choices in PGT-A must balance diagnostic yield against potential harm from additional handling (*e.g.*, repeat TE biopsy or cryopreservation steps), as underscored by a recent systematic review and meta-analysis[1]. Early AH at the cleavage stage (Day 2–3) may facilitate TE herniation and simplify later biopsy, whereas AH at the expanded blastocyst (Day 5–6) is physiologically aligned but technically demanding. Prior reports are conflicting: Lee *et al* observed no difference between early and late AH, while Xiong *et al* reported higher mosaicism when AH was performed on Day 3[2,3]. We retrospectively analyzed 1354 blastocysts biopsied at our center (2023–2024) using two routine protocols—McArthur 2005 (Day-3 zona breaching; biopsy at blastocyst) and Capalbo 2014 (breaching and biopsy at blastocyst)—to test whether AH timing is associated with mosaicism while accounting for laboratory changes and patient characteristics. Inclusion criteria: Blastocysts derived from embryos cultured on Day 5-6, or Day 6; Embryos biopsied using either the McArthur (2005) protocol (zona breaching on Day 3, biopsy at blastocyst stage) – group 1 or Capalbo (2014) protocol (zona breaching and biopsy at the blastocyst stage) – group 2; Embryos that underwent PGT. Exclusion criteria: Embryos subjected to two freeze-thaw cycles; Cases with incomplete clinical or laboratory data, or canceled embryo transfer cycles; Maternal uterine abnormalities, including uterine malformations and endometrial pathologies.

Using retrospective data collected between 2023 and 2024, a total of 1354 blastocysts were analyzed and divided into two groups: Group 1 included 725 blastocysts AH on Day 3, and Group 2 included 629 blastocysts AH on Day 5-6. Embryo characteristics by AH day are shown in Table 1. The mean maternal age at oocyte retrieval was significantly lower in Group 2 compared to Group 1 [(34.1±5.3) *vs.* (35.4±4.3) years, *P*<0.05]. The mean maternal age in our cohort appears to be younger than that reported in several previously published studies[4,5]. This difference may reflect socio-cultural factors, as Vietnamese women tend to marry and attempt conception at a younger age compared to women in many other countries.

The distribution of embryo morphological grades in our study was as follows: good quality embryos accounted for 58.0%, fair for 26.4%, and poor for 15.6%. When stratified by biopsy protocol, the proportion of good-to-fair quality embryos in the Day 3 AH group (McArthur 2005) was 81.5%, which was lower than the 87.7% observed in the Day 5-6 AH group (Capalbo 2014). Furthermore, the proportion of poor-quality embryos was significantly lower in Group 2 (6.3%) compared to Group 1, with a statistically significant difference (*P*=0.02).

In this cohort, AH on Day 5–6 was associated with higher mosaicism than Day 3 [28.1% (177/629) *vs.* 21.2% (154/725); *P*<0.05], a higher euploidy rate (42.8% *vs.* 40.3%; *P*=0.02), and a lower aneuploidy rate (29.1% *vs.* 38.5%; Figure 1A). These findings contrast with Lee *et al*, who found no difference, and with Xiong *et al*, who reported higher mosaicism after Day-3 breaching[2,3].

Table 1. Embryo characteristics by assisted hatching (AH) day.

Embryo characteristics	Group 1 (Day 3 AH)	Group 2 (Day 5 AH)	<i>P</i> -value
Number of embryos, <i>n</i> (%)	725 (53.5)	629 (46.5)	
Mean maternal age at oocyte retrieval, years	35.4±4.3	34.1±5.3	0.02
Embryo quality, <i>n</i> (%)			
Good	361 (49.8)	424 (67.4)	< 0.001
Fair	230 (31.7)	128 (20.3)	< 0.001
Poor	134 (18.5)	77 (12.2)	< 0.001
Blastocyst development day, <i>n</i> (%)			
Day 5	552 (76.1)	512 (81.4)	0.02
Day 6	173 (23.9)	117 (18.6)	0.02

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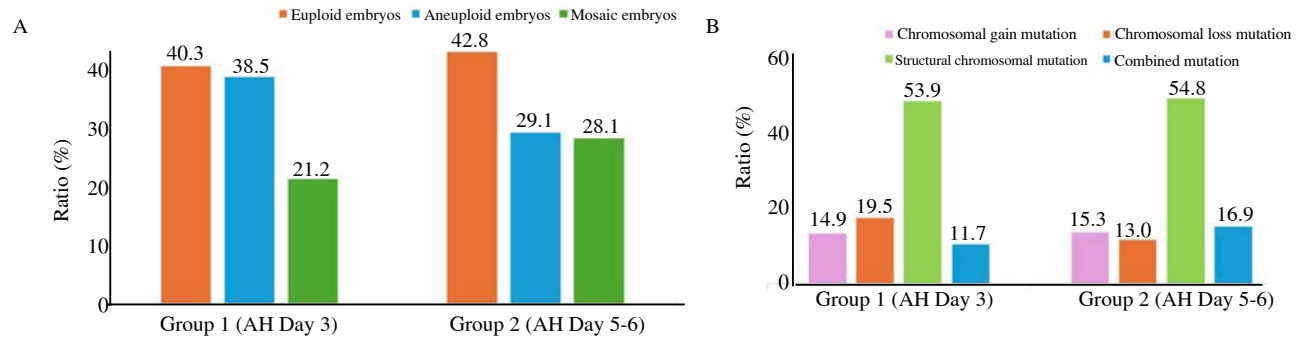


Figure 1. (A) Preimplantation genetic testing (PGT) outcomes of blastocysts following assisted hatching (AH) performed on Day 3 versus Day 5-6; **(B)** Chromosomal abnormalities by biopsy protocol.

In addition, our research demonstrates notable differences in the chromosomal abnormality profiles of blastocysts biopsied under two AH protocols (Figure 1B).

Several factors may explain the divergence. Manipulation at the expanded blastocyst can introduce stage-specific mechanical stress and sampling heterogeneity, potentially increasing mosaic calls. Detection also likely shifted with the adoption of paired-end next-generation sequencing (NGS) in September 2023 and implementation of the Capalbo protocol in January 2024, which together enhance sensitivity to small aberrations and low-level mosaicism. Baseline imbalances—lower maternal age in the Day 5–6 group [(34.1±5.3) years *vs.* (35.4±4.3) years, $P=0.02$], fewer poor-quality embryos (6.3%), and endometrial parameters (*e.g.*, endometrial thickness) known to correlate with clinical pregnancy outcomes in fresh *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles and large multicenter datasets[6,7]—further confound direct comparisons. Interpretation should therefore consider timing, technique, platform, embryo morphology[8], and patient mix rather than timing in isolation.

Taken together, our data suggest that AH timing may influence the detection of mosaicism but not necessarily in a manner that mirrors aneuploidy trends. As a substantial proportion of blastocysts can exhibit some degree of mosaicism, and platform sensitivity shapes its detection, optimization should integrate AH timing, biopsy methodology, analytic resolution, morphology-based embryo assessment[8], and endometrial-thickness-informed transfer strategies[6,7], ideally confirmed in adjusted models and prospective studies[9,10].

Conflict of interest statement

The authors declare no competing interests.

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Authors' contributions

Hoang Minh Ngan was responsible for drafting the manuscript. Tran Thi Thuy conceptualized the study design and critically revised the manuscript. Tran Quang Anh and Nguyen Dinh Tao contributed to data collection and manuscript revision. Nguyen Hong Phuc conducted data processing and analysis.

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