

Day-3 versus Day-5 first vitrification in double-vitrified embryos undergoing single biopsy: impact on blastocyst viability

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Objective

Does initial vitrification at the cleavage stage (Day-3) compared with the blastocyst stage (Day-5) influence blastocyst viability following double vitrification and a single trophectoderm biopsy?

Materials and Methods

Retrospective observational cohort study including 302 IVF cycles from 2017 to 2025, contributing 1,531 embryos (D3/BL group, 156 cycles, 897 embryos; D5/BL group, 146 cycles, 634 embryos) subjected to double vitrification and single biopsy at blastocyst stage for PGT.

Embryos within a cycle were first vitrified either at the cleavage stage or at the blastocyst stage. All embryos were warmed and biopsied only at the blastocyst stage and re-vitrified. Primary laboratory outcomes included utilization rate, blastulation timing, blastocyst quality and ploidy. Analyses were stratified by PGT indication (PGT-A-only vs PGT-M/A cycles). Mixed-effects logistic regression adjusted for maternal age accounted for clustering within cycles.

Results

Survival after warming was $\geq 99\%$ across all vitrification strategies. Utilization rate per MII and per 2PN did not differ between strategies (**Figure 1**). Day-5 biopsy occurred more frequently following Day-3 first vitrification group (29.1% vs 16.7%, $P < 0.001$). Post-transfer outcomes were comparable between strategies, with similar clinical pregnancy rates (66.7% vs 62.7%, NS) and ongoing/live birth rates (50.0% vs 52.2%, NS) for D3/BL versus D5/BL transfers (**Figure 1**).

Among biopsied embryos, the initial vitrification stage (Day-3 vs Day-5) did not significantly affect ultimate Top/Good blastocyst quality in either stratum (PGT-A only: aOR 1.52, 0.85–2.74, $P = 0.159$; PGT-M/A: aOR 1.31, 0.78–2.20, $P = 0.307$), neither euploidy rates (**Figure 2**). Increasing maternal age was independently associated with reduced odds of achieving Top/Good blastocyst quality in the PGT-M/A cohort (aOR 0.94 per year, 0.90–0.99, $P = 0.011$), but not in PGT-A-only cycles ($P = 0.550$) (**Figure 2**).

Figure 1.

Comparison of embryo utilization, development, and clinical outcomes

* $P < 0.001$ for Day-5 biopsy rate

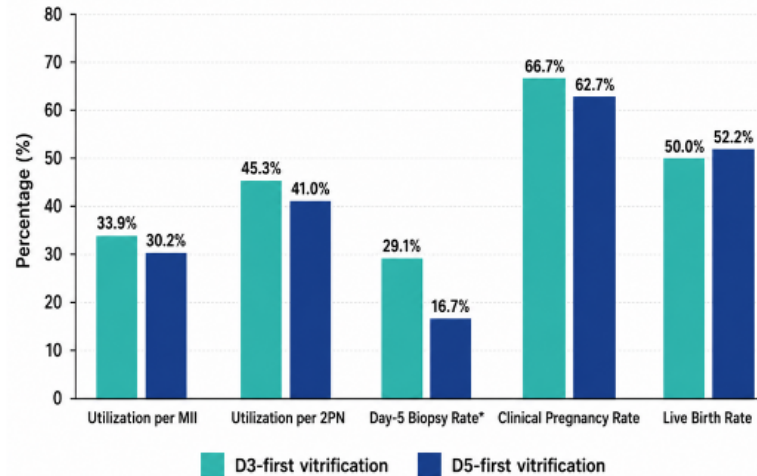
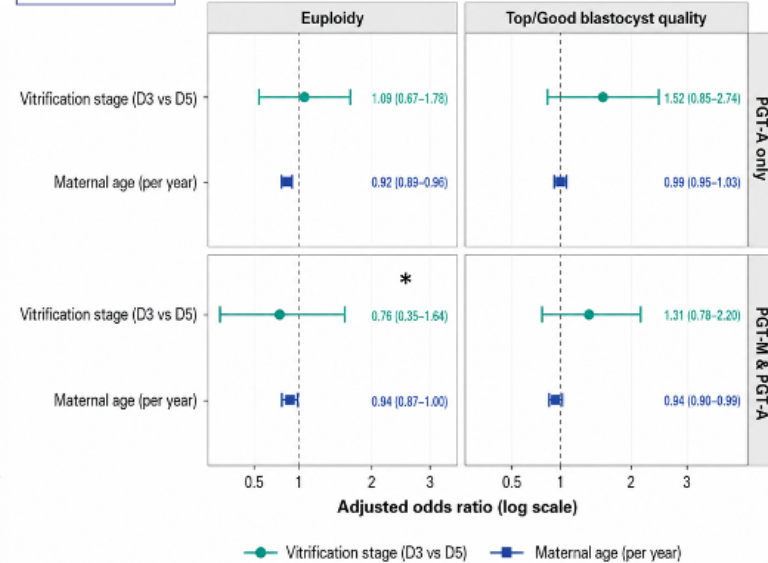


Figure 2.



Conclusion

Initial vitrification at Day 3 or Day 5 resulted in comparable blastocyst quality, embryo utilization, euploidy, and pregnancy rates, suggesting that both strategies are equally effective. Maternal age remained an important determinant of embryo quality and euploidy, particularly in PGT-M/A and PGT-A cycles, respectively. The higher proportion of blastocysts available for Day-5 biopsy in the D3/BL vitrification group may be relevant when considering biopsy timing strategies.