The Importance of Extending Individualized Patient Care to the Embryo Level: Vitrification at the Time of Full Blastocyst Expansion is Critical in Maximizing Clinical Success.

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BACKGROUND

Embryo developmental timing is a well-established determinant of implantation potential and overall clinical success. Blastocysts that reach full expansion by Day 5 typically demonstrate greater developmental competence compared with embryos that expand later, reflecting both intrinsic embryo quality and culture efficiency. While embryo competence is influenced by biological factors, laboratory workflow and operational constraints may also contribute to variability in the timing of vitrification. In busy clinical settings, vitrification may occasionally occur beyond the optimal developmental window despite appropriate embryo quality. Although the association between delayed blastocyst development and reduced implantation potential has been described, the clinical impact of delayed vitrification at the stage of full blastocyst expansion remains incompletely characterized. Understanding whether extended timing to full blastocyst expansion affects pregnancy and loss outcomes is essential to optimizing embryo-level decision-making and individualized patient care.

OBJECTIVE

To evaluate the clinical impact of delayed blastocyst vitrification at the time of full expansion on embryo competence and transfer outcomes. Specifically, we aimed to compare pregnancy rate, biochemical pregnancy loss, and clinical pregnancy loss across vitrification timing groups, using Day 5 full expansion (D5→D5) as the reference.

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MATERIALS AND METHODS

Study Design: Retrospective cohort study of 3,018 frozen single embryo transfers (January 2020-July 2025) at a U.S. fertility center.

Embryo Culture & Assessment: EmbryoScope+ time-lapse culture; Gardner grading system. Fully expanded blastocysts underwent laser-assisted collapse prior to vitrification (Irvine Scientific; Cryolock).

Vitrification Timing Groups:

D5→D5: Expanded & vitrified on Day 5;

D5→D6: Expanded on Day 5, vitrified on Day 6;

D6→D6: Expanded & vitrified on Day 6

Transfer Distribution by Group:

- **Full cohort (n = 3,018):** D5→D5 (n = 1,471), D4→D5 (n = 620), D5→D6 (n = 535), D6→D6 (n = 392)
- **PGT-tested cohort (n = 2,074):** D5→D5 (n = 982), D4→D5 (n = 413), D5→D6 (n = 405), D6→D6 (n = 274)

Outcomes: Pregnancy rate (positive β-hCG ≥ 5 mIU/mL), Biochemical pregnancy loss, and Clinical pregnancy loss

Statistical Analysis: Maternal age-adjusted logistic regression with pairwise Ors (95% CI); FDR correction.

Blastocyst Development (Days 4-6)

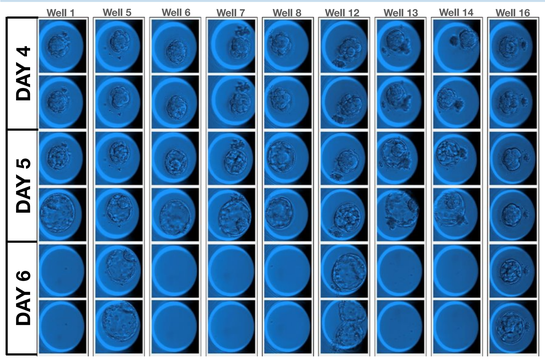
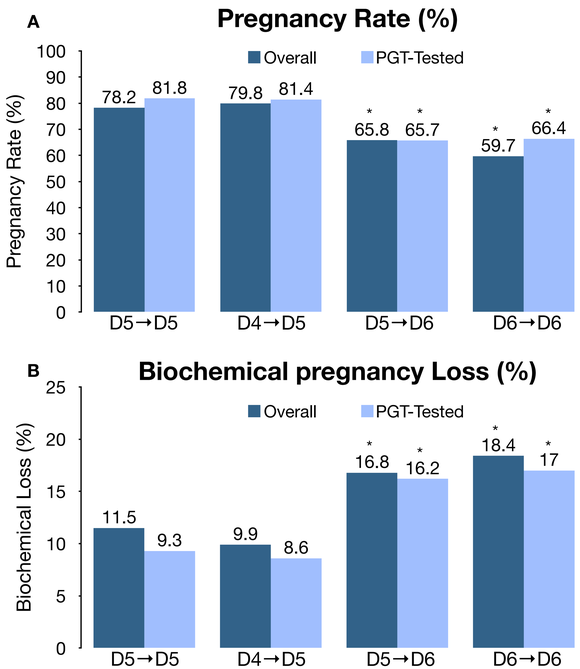


Figure1. Representative **Embryoscope+** time-lapse images illustrating blastocyst development across **days 4-6**.

RESULTS

Pregnancy rate was highest in D5→D5 and was significantly lower in D5→D6 and D6→D6 after FDR correction. Biochemical pregnancy loss increased in D5→D6 and D6→D6 compared with D5→D5. Clinical pregnancy loss was significantly higher in D5→D6 relative to D5→D5. D4→D5 outcomes were comparable to D5→D5 across endpoints. In the PGT-A tested cohort, D5→D5 embryos likewise demonstrated the highest pregnancy rates. Delayed vitrification (D5→D6) remained associated with reduced pregnancy and increased loss, indicating that the adverse impact of delayed vitrification persists even after controlling for aneuploidy.



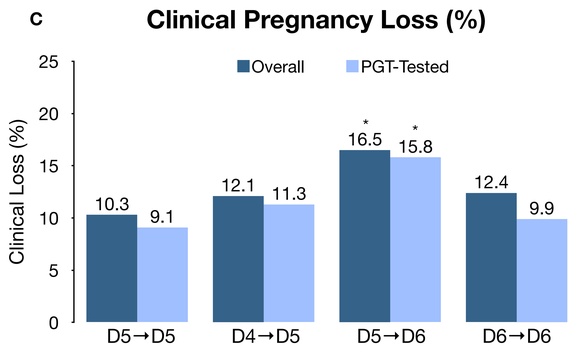


Figure 2. Transfer outcomes by vitrification timing in all blastocysts (n = 3,018) and PGT-Tested blastocysts (n = 2,074). (**A**) Pregnancy rate per transfer. (**B**) Biochemical pregnancy loss per pregnancy. (**C**) Clinical pregnancy loss per pregnancy. Comparisons were performed against D5→D5 using logistic regression with FDR correction. Significant vs D5→D5 (FDR-adjusted $p < 0.05$).

CONCLUSION

- Vitrification at full blastocyst expansion is associated with optimized implantation and sustained clinical pregnancy outcomes.
- Delayed vitrification beyond Day 5 full expansion is associated with reduced pregnancy rates and increased biochemical and clinical loss.
- This adverse effect persists in PGT-tested embryos, suggesting an impact beyond aneuploidy alone.
- Timely vitrification represents a modifiable laboratory factor to improve overall clinical success.