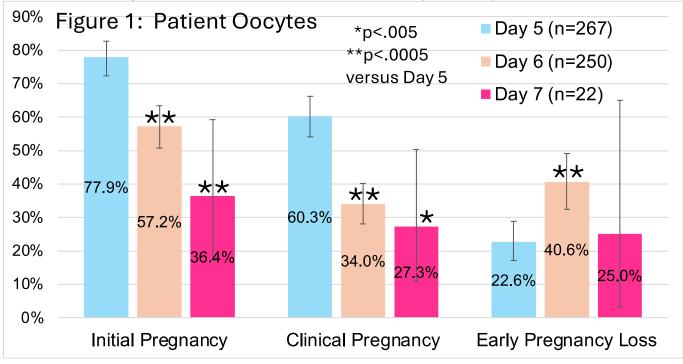
## PREGNANCY OUTCOMES ACCORDING TO TIMING OF EXPANSION AND VITRIFICATION AMONG 967 TRANSFERS OF SINGLE CRYOPRESERVED NON-BIOPSIED BLASTOCYST STAGE IN VITRO FERTILIZED (IVF) EMBRYOS

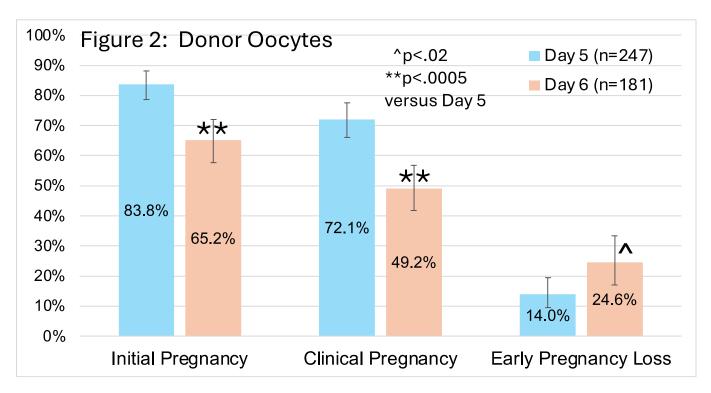
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**Background:** Single blastocyst transfer only recently became the norm for in vitro fertilization (IVF) in the United States. Before 2016, most IVF transfers were of multiple embryos. Multiple embryos were transferred in fewer than 20% of all US IVF cycles for the first time in 2020. Unlike multiple embryo transfer, single embryo transfer allows for complete and unambiguous data regarding outcomes of every transferred embryo, including initial uterine implantation as evidenced by fetally produced hCG in the maternal bloodstream. The newly standard practice of single embryo transfer thus provides unprecedented ability to reevaluate factors associated with successful embryo implantation. **Objective:** To evaluate post-transfer embryo viability among cryopreserved blastocysts not undergoing preimplantation genetic testing (PGT) according to blastocyst expansion time.

**Materials and Methods:** All single non-biopsied vitrified blastocyst transfers performed at a single IVF center (2021-2023) were reviewed. Patient and donor oocyte cycles were evaluated separately. Transfers were categorized according to the day after oocyte retrieval on which blastocysts expanded and were cryopreserved. Initial implantation (positive serum hCG) and clinical pregnancy (ultrasound confirmation of a gestational sac with heartbeat) per transfer, and early losses per initial pregnancy, were compared by  $\chi^2$  (or Fisher's exact test, for comparisons including Day 7). Error bars indicate 95% binomial confidence intervals.

**Results:** Among untested autologous IVF cycles in which patients used their own eggs (Figure 1), blastocyst expansion and vitrification occurred on Day 5 after oocyte retrieval in nearly half (49.5%). Blastocyst expansion and vitrification occurred on Day 5 for most transfers (57.7%) of untested blastocysts derived from donor eggs (Figure 2). Regardless of the egg source, Day 6 blastocysts resulted in significantly lower initial pregnancies (by 19% to 21%) and clinical pregnancies (by 23% to 26%) per transfer, and significantly higher (by 11% to 18%) early pregnancy losses per initiated pregnancy, when compared to Day 5 blastocysts. Day 7 blastocysts (present only among transfers using patients' own eggs) also resulted in a significant 42% decrease in initial pregnancy and 33% decrease in clinical pregnancy per transfer compared to Day 5 blastocysts. There were too few Day 7 transfers to confirm statistical significance of observed differences versus Day 6 blastocysts.





**Conclusions:** Regardless of oocyte source, blastocysts that expanded and were vitrified by Day 5 after oocyte retrieval were significantly more successful, by clinically substantial margins, compared to slower developing embryos. Day 6 blastocysts were associated with significantly lower initial pregnancy, as indicated by detection of fetal hCG in maternal serum. Day 6 blastocysts were also associated with significantly higher early pregnancy loss, defined as the proportion of these initiated pregnancies (with positive hCG) that failed to develop a gestational sac with a heartbeat. The combined result was a significantly and substantially lower (by 23% to 26%) ongoing clinical pregnancy per transfer for Day 6 versus Day 5 blastocysts. Our observed Day 7 blastocyst transfer results reinforce previous advocates of their clinical utility [1-3]. Although of markedly poorer quality, blastocysts not expanding until Day 7 are still potentially viable and worthy of consideration when better embryos are unavailable

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## **References:**

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