WHY DO EMBRYOS FAIL WARMING?
IDENTIFYING PROGNOSTIC FACTORS OF EMBRYO SURVIVAL AFTER VITRIFICATION

Amalia Namath MD[1,2], Jing Wu MD[2,3], Tex VerMilyea PhD, HCLD[3], Anabel Thurman BS[4], Phillip Romanski MD MSc[5], Jeanne E. O'Brien MD[2]

[1]National Institutes of Health, Bethesda, MD [2]Shady Grove Fertility, Rockville, MD [3]US Fertility

[4]George Washington University School of Medicine, Washington DC [5]Reproductive Medicine Associates of New York, NYC, New York

BACKGROUND

- Freezing of embryos has improved over time due the development of vitrification, with more embryos surviving warming after vitrification compared to the previous practice of slow freeze (1).
- Most practices cite high blastocyst survival rates after warming, but some embryos still fail (1).
- A prior study (2021) found that with euploid embryos, those that were vitrified on day 7, fully hatched, from a higher ovarian reserve cohort, and/or with multiple trophectoderm biopsies were less likely to survive (1).
- **However, protective factors against embryo warming failure for vitrified, untested blastocysts has yet to be fully explored.**

HYPOTHESIS

Primary:

Vitrified embryos that survive warming compared to those that do not survive warming are more likely to be from younger patients (younger oocyte age).

Secondary:

Embryos that had multiple forms of micromanipulation (assisted hatching, PGT trophectoderm biopsies) were more likely to fail warming compared to those without micromanipulation techniques performed.

MATERIALS & METHODS

- Retrospective cohort study from 2010 to 2024 from a multi-center practice
- **Inclusion criteria:** Embryos who were warmed after vitrification
- **Exclusion criteria:** Embryos that underwent slow freeze, cleavage stage embryos
- **Primary outcome:** Embryo survival after warming
- **Secondary outcomes:** PGT, assisted hatching, day of freeze
- **Statistical analysis:** All comparisons of continuous factors were evaluated via generalized estimating equation models (GEE) with Poisson regression given multiple cycles per patient, and adjusted *a priori* for patient and partner age as well as BMI.

Figure 1: Embryo Survival and Failure, Separated by Age

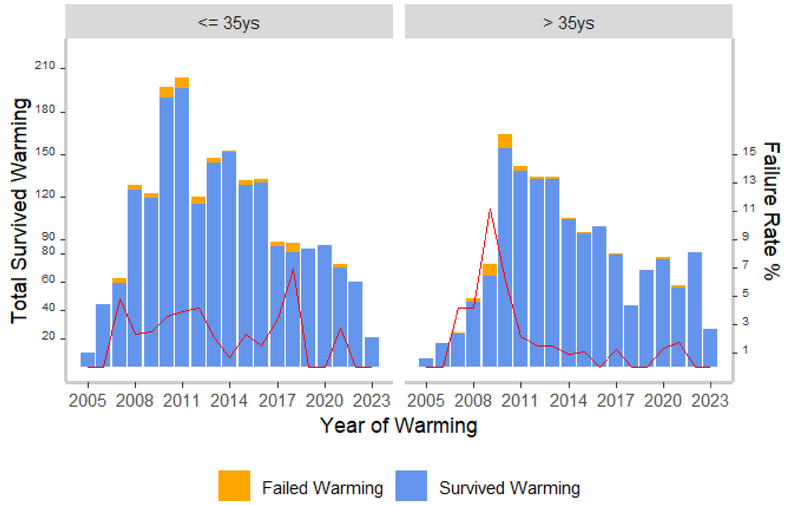
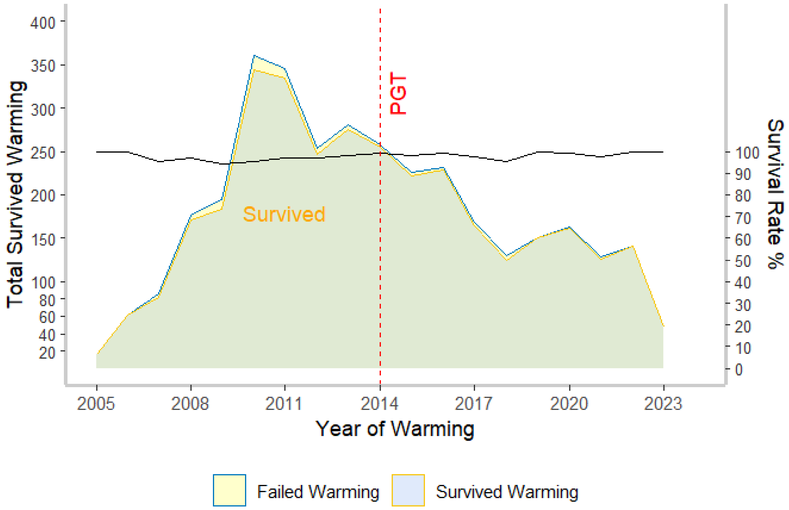


Figure 2: Embryo Survival Over Time



RESULTS

- In total, 3,336 embryos survived from 3,418 attempted warming (**97.6% survival, 2.4% failed**)
- Average age of patient/oocytes was 35 years old and partner/sperm was 39 years old, with 44.8% of embryos stored for less than 6 months
- Most were **vitrified on day 5** (61.3%), and most used ICSI (86%)
- From these, 436 embryos were PGT-tested (all ICSI)
  - 12% underwent assisted hatching for planned PGT after warming
  - 16% were vitrified after PGT was performed
- PGT-embryos had older oocytes (35.5 vs 34.8 non-PGT, p=0.002)
- No significant difference in survival between embryos with vs without PGT (99% survival with PGT vs 98.7% non-PGT, p=0.09)
- When comparing embryos that survived to those that did not, **younger age was protective to survival** (aRR 1.03, 95% CI 1.02-1.04)
- Embryos vitrified on **Day 6 or 7 were less likely to survive** vs Day 5 (aRR 0.97, 95% CI 0.96-0.99)
- Embryos from patients over 35 with PGT were more likely to survive than those without PGT (aRR 1.02, 95% CI 1.01-1.04), but this relationship was not consistent for patients 35 years or younger with PGT (Figure 1)
- **Assisted hatching was not significantly associated** with survival
- There was no difference in survival based on month; embryos vitrified from cycles in **2014 or later were more likely to survive** vs earlier cycles (aRR 1.02, 95% CI 1.01-1.03) (Figure 2)

CONCLUSIONS

Embryos **vitrified at the Day 5 blastocyst stage are more likely to survive warming compared to Day 6 and Day 7**. Overall, there has been significant improvement in vitrification and warming techniques, and **embryo survival has increased over time.**

REFERENCES

1. Oliva M, Briton-Jones C, Gounko D, Lee JA, Copperman AB, Sekhon L. Factors associated with vitrification-warming survival in 6167 euploid blastocysts. J Assist Reprod Genet. 2021 Oct;38(10):2671-2678. doi: 10.1007/s10815-021-02284-0. Epub 2021 Jul 26. PMID: 34309745; PMCID: PMC8581086.