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Title:

**CLINICAL FACTORS ASSOCIATED WITH THAW SURVIVAL IN A COHORT OF
6167 VITRIFIED-WARMED, EUPLOID BLASTOCYSTS**

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Objective:

Embryo cryopreservation has become integral to IVF treatment. While an embryo failing to survive vitrification-warming is rare, understanding of factors that predict embryo thaw survival could allow for individualized patient counseling. Prior studies on the predictors of thaw survival have been limited by the use of slow-freeze protocols and unscreened embryos. This study analyzed embryo-related factors associated with euploid embryo thaw survival.

Design:

Retrospective, case-control

Materials and Methods:

This single center study included vitrified-warmed euploid embryos from autologous IVF-PGT-A cycles from 2010-2019. Blastocysts that did not survive warming were compared to those that survived. Independent variables: patient age, basal antral follicle count (BAFC), body mass index (BMI), stimulation protocol, cumulative gonadotropin (GND) dose, estradiol (E2) and progesterone (P4) level at surge, embryo development day, oocytes retrieved, fertilization method, cleavage stage embryo cell number/fragmentation, number of trophectoderm biopsies



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and vitrification-thawing, embryo sex, Gardner morphology. Student's t-test, chi-square, and linear regression (generalized estimating equation models) were used.

Results:

Of the euploid blastocysts thawed (n=6167), 2.8% (n=175) warmed embryos did not survive. Embryos that did not survive came from women with higher BAFC (OR 0.97, 95% CI 0.95-0.99), E2 levels at surge (p=0.03), and number of oocytes retrieved (p=0.005). Embryos cryopreserved on day 5/6 were more likely to survive than day 7 (OR 4.5, 95% CI 2.5-8.1). Embryos that underwent two trophectoderm biopsies had lower odds of survival (OR 3.2, 95% CI 1.7-5.9) than embryos that had a single biopsy. Repeat vitrification-warming was not associated with thaw survival (OR 0.26, 95% CI 0.04-1.9). While cleavage stage cell count was similar between groups, increased fragmentation was associated with reduced survival (OR 0.97, 95% CI 0.94-0.99). Embryos with expansion grade 4 (OR 4.5, 95% CI 2.5-8.1) and 5 (OR 2.1, 95% CI 1.2-3.7) had higher odds of surviving than fully hatched blastocysts. ICM grade was positive correlated with thaw survival (OR 2.2, 95% CI 1.4-3.4), whereas trophectoderm grade was not. Controlling for relevant confounders, increased BAFC, double trophectoderm biopsy, and fully hatched blastocysts remained associated with reduced thaw survival.

Conclusion:

Blastocysts that undergo a second trophectoderm biopsy, and/or are fully hatched prior to vitrification are less likely to survive warming. Embryos from 'high responders' also have reduced odds of thaw survival. These findings may be related to the link between polycystic ovarian syndrome and poor oocyte quality. Repeat trophectoderm biopsy and increased exposure of fully hatched embryos may reduce vitrification-warming tolerance. Providers can use this data to better counsel patients regarding the risk of their embryo(s) not surviving the thaw. At the molecular level, studies comparing the transcriptome of fresh and vitrified-warmed embryos may provide insights to optimize vitrification protocols.