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Title:

Does Vitrification Day Matter: Euploid Embryos With Identical Day 5/6 Morphology Grade and Frozen Embryo Transfer Cycle Outcomes

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Objective:

When a patient has two or more euploid, cryopreserved embryos and chooses to proceed with a single embryo transfer (SET) in a frozen embryo transfer (FET) cycle, embryologists must choose which embryo to thaw. Although the cryopreserved embryos may have the same morphological score at the time of cryopreservation, the stage of development (Day 5 vs. 6 vs 7) may differ. When presented with this unique circumstance, the clinical standard within this study's site is to select day 5 embryos. We sought to determine whether the day of cryopreservation of embryos of similar morphology at the time of cryopreservation correlated to IVF outcomes.

Design:

Retrospective cohort analysis

Materials and Methods:

All patients who underwent a euploid, single, FET cycle between 2010 and 2016 were analyzed. Ploidy was determined by trophectoderm biopsy and analyzed via SNP analysis, qPCR, or aCGH. Cycles were segregated on the context of the day they were vitrified: Day 5, Day 6 or Day 7. Embryos were only frozen once they achieved at least expansion 3. The main outcome measure was implantation rate. Bivariate associations were examined using Pearson's Chi-square test, independent samples t-test, as appropriate. All statistical tests were two-sided and P value <0.05 was considered statistically significant. Clopper-Pearson interval was used to calculate binomial confidence intervals (CI) for all reported proportions. Adjusted odds ratio (OR) and its 95% CI for aneuploidy rate was calculated.

Results:

A total of 1822 patients underwent 2336 SET FET cycles. Cycles were binned in 6 sub-categories based on ICM and trophectoderm criteria of "high quality", "medium quality", and "low quality" and further sorted by expansion (Table 1) (modified Gardner classification). When raw data were analyzed, all sub-

categories achieved higher implantation rates when frozen on Day 5 (highest 100%, lowest 66.7%) as compared to Day 6 (highest 61.6%, lowest 36.8%) or Day 7 (highest 33.3%, lowest 25.0%). Embryos 3BB to 6AA frozen on Day 5 had statistically higher implantation rates than those frozen on Day 6 ($p<0.05$). Any embryo frozen on day 5 showed higher implantation rates than those frozen on day 6, although statistical significance was not achieved in all groups.

Conclusions:

Blastocyst-stage embryo transfer has progressively become the norm in many ART clinics. Advancements in extended culture methods have successfully enabled the development of embryos to day 5, 6, or 7 in vitro, which has given embryologists more insight into implantation potential. This study suggests that faster developing euploid blastocysts cryopreserved on Day 5 showed a trend towards higher clinical outcomes following a frozen-thawed transfer cycle. Further large randomized control trials are needed to confirm these findings.

Support:

None.

Table:

	6 SUB-CATEGORIES				
Day of cryo	Day 5	Day 6	Day 7	Day 5 vs. Day 6	
5-6AA, 5-6AB, 5-6BA, 5-6BB	75.8% (226/298) (95% CI 70.6-80.6)	61.6% (360/584) (95% CI 57.6-65-6)	30.0% (3/10) (95% CI 6.7-65.2)	p<0.05	OR 1.9 (95% CI 1.4-2.6)
3-4AA, 3-4AB, 3-4BA, 3-4BB	74.3% (417/561) (95% CI70.5-77.9)	59.1% (282/477) (95% CI 55.8-64.8)	25.0% (1/4) (95% CI 0.6-80.6)	p<0.05	OR 4.0 (95% CI 2.9-5.4)
5-6AC, 5-6BC, 5-6CA, 5-6CB	71.4% (15/21) (95% CI47.8-88.7)	49.3% (37/75) (95% CI 37.6-61.1)	33.3% (1/2) (95% CI1.3-98.7)	NS	OR 2.6 (95% CI 0.9-7.3)
3-4AC, 3-4BC, 3-4CA, 3-4BC	67.0% (65/97) (95% CI56.7-76.2)	36.8% (67/182) (95% CI 29.8-44.3)	33.3% (1/3) (95% CI 0.8-90.6)	p<0.05	OR 3.4 (95% CI 2.1-5.9)
5-6CC, 5-6CD, 5-6DC, 5-6DD	100.0% (3/3) (95% CI 29.2-100)	50.0% (1/2) (95% CI1.3-98.7)	n/a	NS	OR 7.0 (95% CI 0.2-291.3)
3-4CC, 3-4CD, 3-4DC, 3-4DD	66.7% (2/3) (95% CI 9.4-99.2)	46.2% (6/7) (95% CI 42.1-99.6)	n/a	NS	OR 0.3 (95% CI 0.01-8.2)