Validation of a Universal Warming/Dilution Protocol for Frozen Embryo Transfers Independent of Vitrification Device/Cryoprotectant

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Objective: Validate a universal warming/dilution approach for all vitrification (VTF) solutions associated with various device systems.

Design: Using a 2x2 factorial arrangement of treatments, 124 blastocysts derived from 363 research consented slow frozen embryos were vitrified in Glycerol/ethylene glycol (EG) solution (≥7.9M; I.C.E.) or a 15%DMSO/15%EG solution, and subsequently rapidly warmed and diluted by standard operating procedures (SOP) or a universal sucrose step-down dilution procedure (n=31 embryos/group). Furthermore, we performed a retrospective analysis of our routine success with microSecure VTF (µS-VTF with Glycerol/EG)/warming to applying a universal sucrose dilution protocol, independent of vitrification device/solution, to blastocysts shipped in from outside facilities between 2015-2017. In validating our clinical approach, we contrasted live birth rates achieved in the latter groups (A and B, respectively; see Table) to outcomes reported in the 2015 CDC data, including the national average (C) and four local IVF labs/clinics (D-H).

Materials and Methods: All research blastocysts were vitrified by µS-VTF using solutions and dilution treatments described above. Conversely, all internal FET cycles implemented a standardized sucrose step-down dilution using I.C.E. diluents (T1 -T4; estimated to possess 1.0M, 0.5M, 0.25M and 0.125M sucrose concentrations; 3 min/step), independent of the VTF device/cryoprotectant, upon rapid warming and blastocyst isolation. All blastocysts underwent isotonic equilibration in Hepes buffered media for 5 min at 37ºC before being in-vitro cultured until ET or research developmental assessment. Our live birth pregnancy rates for FETs (A,B; autologous oocytes, <43 years old) were compared to both national (C) and local clinic success (D-H).

Results: No statistical differences in survival or sustained development was observed between the combined dilution treatments of vitrified research blastocysts. SOP thawing (93.5%) or standard sucrose dilution (90.3%) outcomes were similar. Mean live births (see Table) following our universal sucrose dilution approach (B) revealed similar or improved outcomes to those reported in the 2015 CDC summary (C-G). The apparent reduced live birth success compared to µS-VTF (A) and a local group (H) is likely due to differences in embryo production (i.e., lab effect), not to a warming-dilution effect post-VTF.

	A	B	C	D	E	F	G	H
# VFET cycles	384	54	36,365	87	38	424	74	164
% Live birth	71%	52%	41%	39%	45%	56%	59%	70%

Conclusion: A universal sucrose, step-down dilution approach has proven to be a simple, cost-effective and highly successful procedure in our treatment for vitrified blastocysts derived from outside IVF programs, thus alleviating the need to purchase and maintain various commercial thaw solutions.

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