P- 137

Effective Universal Warming-Dilution of Blastocysts Vitrified on an Open Device System

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Objective

Universal warming protocols for vitrified embryos have been previously validated for a closed vitrification system (microSecure VTF). **The goal of this study was to verify the effectiveness of different non-permeating sugar solutions** for post-warming dilution of blastocysts vitrified on an open system when alternatives to their typical commercial products are needed.

Experimental Design and Methods

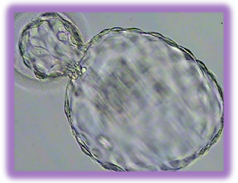
A **prospective, randomized study** of patient consented research discard blastocysts (>2BB quality) was conducted in an apriori arrangement of 4 sugar solution treatments post-warming: **A) fresh** Irvine Sci./FF NX Vit Warm kit solutions (TS/DS); **B) frozen-thawed** Irvine Sci./FF TS/DS solutions (i.e., flash frozen in LN_2); **C) 1M sucrose**; and **D) 10% honey** (commercial grade, multi-floral). It was conducted in duplicate (60 embryos each, 15/treatment) by two junior Embryologists. Our null hypothesis was that fresh commercial thaw solutions are required to maximize the viability of ultra-rapid vitrified blastocysts.

Blastocysts vitrified on Cryolocks in Irvine Sci./FF NX Vit kit solutions (30% EG/DMSO) were rapidly warmed in 1 of 4 solution treatment groups. Solutions A and B followed commercial protocol. Solutions C and D were lab-made, filtered stock LG-H+additive solutions, both requiring heat and agitation over a 30 min period to completely mix into suspension. The latter treatments involved a 4-step dilution (3 min/step, 50% reduction/step, 21°C) prior to isotonic equilibration (LG-H+additives; 5 min at 37°C). All embryos underwent 18-24 hr in vitro group culture/microdroplet (5 embryos/drop). Survival, based on osmotic responsiveness and cellular integrity/fullness was visually assessed at 0 and +2 hr, followed by overnight observations of blastocyst expansion and continued development.

Results

There was no difference in blastocyst survival, as they were 100% intact and osmotically responsive. Furthermore, there was no difference in post-warming/elution treatment viability, as shown in the Table below**: #BL Developing**

TECH/Replicate	Solution: A	B	C	D
I	13	14	13	15
II	14	14	15	14
TOTAL (%)	27 (90%)	28 (93%)	28 (93%)	29 (96%)



This study represents an excellent training exercise, while simultaneously validating scientific questions. In this case, both Tech's achieved similar outcomes with 90-95% post-culture development (93% overall).

Conclusion & Impact

This study confirms that various sugar-based dilution solutions work equally well to elute cryoprotectants from vitrified blastocysts independent of ultra-rapid cooling on Cryolocks. 1M sucrose is a known universal diluent, whereas 10% honey (composed predominantly of fructose and glucose) is a readily available natural product for any lab around the world to safely use when a commercial solution is unavailable or cost prohibitive. We further verified that commercial thaw solutions stored in cryovials can be frozen shipped with embryos in LN_2 dry-shippers, if the preferred commercial solution is needed by the recipient lab, as previously documented for a closed device.

Upon rapid warming, independent of the device system used, the viability of vitrified blastocysts is not dependent on a commercial source or particular type the non-permeating sugar solution used to elute cryoprotectants from blastomeres.

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