In-Straw Dilution of Vitrified Bovine Embryos Produced In-Vitro

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Commercial cryopreservation of bovine embryos has relied mainly on slow-cooling techniques. We have developed a simple, two step vitrification technique with in-straw dilution so that embryos can be transferred in the field. We studied factorial combinations of two equilibration concentrations of ethylene glycol (EG) (3.5 and 5 M), two thawing temperatures (20 and 37°C) and two post-thaw waiting times until culture (5 and 15 min). A total of 486 blastocysts sired by three bulls were obtained in vitro in six replicates. Briefly, oocytes were aspirated from 2-8 mm follicles from slaughterhouse ovaries, and matured, fertilized, and cultured in vitro with standard procedures. Vitrification was with 0.25-ml straws preloaded with a 1 cm column of D-HCDM (0.5 M galactose in Hepes-buffered holding medium (HCDM2)), then 0.5 cm air, and then 7 cm of D-HCDM. Embryos were transferred to 1 ml of V1-CDM (3.5 M or 5M ethylene glycol in HCDM2) for 3 min at 24°C. Next, embryos were moved in 1 µl into a 7 µl droplet of V2-CDM (7 M ethylene glycol, 0.5 M galactose, 18% w/v Ficoll 70 in HCDM2) at 24°C. In less than 1 min, the droplet containing the embryo was loaded, followed by 0.5 cm air and 1 cm of D-HCDM. Straws were heat-sealed and plunged vertically, sealed end first, into liquid nitrogen, covering the embryo; then the rest of the straw was slowly immersed. Straws were thawed in air (24°C) for 10 sec and then in water horizontally at 20 or 37°C until ice disappeared. Straws were gently shaken to mix the columns; then, after 5 or 15 min at 24 or 37°C, embryos were expelled and cultured in CDM2 + 5% FCS. Re-expansion and hatching rates were evaluated 48 h post thaw. Data (Table 1) were calculated as a percentage of non-vitrified controls for respective replicates (control means: re-expansion 86%; hatching 54%) and analyzed by ANOVA.

TABLE 1. Mean responses (LSM ± SE) of vitrified embryos (% of non-vitrified controls).

Post-thawing waiting time (min)	Thawing Temp. (°C)	Re-expansion (%) Equilibration concentration of EG (M)		Hatching (%) Equilibration concentration of EG (M)	
		5	3.5	5	3.5
5	20	84±10	86±6	49±12	79±16
	37	96±9	78±8	103±22	79±12
15	20	71±10	64±4	48±17	42±6
	37	96±8	84±4	89±26	73±24

Main effect means for re-expansion were higher (P=0.08) for 5 M (87 %) than 3.5 M (78%) EG for equilibration; also, re-expansion (88 and 76% of controls) and hatching (86 and 54 % of controls) were higher for 37 than 20°C (P<0.05). There was no main effect for post-thaw waiting times for either response (P>0.1), but there was an interaction between time and temperature for re-expansion (P<0.05), indicating poor survival when embryos were thawed at 20°C and kept for 15 min. Equilibration with 5 M EG and thawing at 37 °C were optimal procedures, and embryos did not degrade when kept up to 15 min in straws thawed at 37 °C. Further studies including transfer of embryos to recipients are necessary to corroborate these results.

Key words: embryo, cryopreservation, vitrification, direct transfer.