SURVIVAL RATE OF FROZEN-THAWED IVF BOVINE EMBRYOS IN RELATION TO EQUILIBRATION TIME USING VARIOUS CRYOPROTECTANTS

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The objective of this study was to examine the relationship between cryoprotectant and equilibration time on development of IVF derived embryos in culture after thawing. Good to excellent quality day 7 to 8 blastocysts and expanded blastocysts were suspended in 1.6M propylene glycol (PG), 1.8M ethylene glycol (EG), or 1.3M methyl cellosolve (MC). These concentrations were based on previous experiments (Suzuki et al., Therio. 34: 1051-1057, 1990; Voelkel et al., Therio. 37: 687-697, 1992) and/or our pre-experiments. Embryos were equilibrated in each cryoprotectant for either 10, 20, 40, 60, 80, 120 minutes while protected from light. The embryos were then loaded into 0.25 cc straws and placed into a programmable freezer at 0℃ and held for 2 minutes. They were then cooled to -5.5℃ at 1.0℃ at 1℃/minute seeded and held for 10 minutes, then cooled to -30℃ at 0.3℃/minute and plunged into liquid nitrogen. Frozen embryos were thawed in a water bath at 30℃, rehydrated directly in holding medium and cocultured 48 hours with cumulus cells in TCM-199 supplemented with 5% superovulated cow serum (SCS, collected on day 7) and 5μ g/ml insulin. Results are shown in Table 1. as the number and percent of embryos developing to hatched blastocysts. There were no significant differences in development to hatched blastocysts of embryos in each equilibration time with either PG or EG. The number of embryos developing after equilibration in MC was similar to those in PG or EG when equilibration times did not exceed 40 minutes (χ^2-analysis). But the number of embryos developing to hatched blastocysts significantly different among each cryoprotectant (2 way ANOVA analysis).

Table 1. Effect of equilibration time and cryoprotectant on subsequent development of IVF derived bovine morulae to hatched blastocysts.

	Equilibration time (min)					
CP	10	20	40	60	80	120
PG	13/42 (31.0)	17/41 (41.5)	20/50 (40.0)	11/41 (26.8)	15/48 (31.3)	13/44 (29.5)
EG	19/40 (47.5)	28/50 (56.0)	19/45 (42.2)	14/29 (48.3)	13/35 (37.1)	21/43 (48.8)
MC	21/44 (47.7)^a	28/65 (43.1)^a	17/35 (48.6)^a	11/40 (27.5)	8/37 (21.6)^b	6/41 (14.6)^b

CP : Cryoprotectant.
a,b : Values with different superscripts differ significantly (χ^2-analysis, $P<0.05$).

Our results suggest that the toxicity of different cryoprotectants is related to equilibration time and that PG and EG were relatively non-toxic as is exposure to MC for short periods.