

PREGNANCY RATE AND SURVIVAL IN CULTURE OF BOVINE IVF
EMBRYOS FROZEN IN VARIOUS CRYOPROTECTANTS
AND THAWED IN A ONE STEP SYSTEM

T. Suzuki, M. Takagi, M. Yamamoto, S. Saha,
A. Boediono and M. Oe

United Graduate School of Veterinary Sciences, Yamaguchi
University, 753 Yamaguchi, Japan.

Bovine oocytes surrounded with compact cumulus cells were cultured for 20-22 h (38.5°C, 5 % CO₂) in modified TCM-199 supplemented with 5 % superovulated cow serum (SCS) and inseminated by in vitro capacitated sperm. Day 7-8 embryos were equilibrated 10 min in 1.3 M methyl cellosolve (MC), 1.1 M diethylene glycol (DEG), 1.8 M ethylene glycol (EG), 1.6 M propylene glycol (PG) and 1.1 M 1, 3-butylene glycol (BG) solutions, loaded into 0.25ml straws, placed into an alcohol bath freezer at 0°C, cooled from 0°C to -6°C at -1°C/min, seeded, held for 10 min, cooled at -0.3°C/min or -0.5°C/min to -30°C. Straws were then plunged and stored in liquid nitrogen. After thawing in 30°C water, embryos were rehydrated directly in TCM-199 and then cultured for 48 hours in TCM-199 plus 5% SCS. Embryos were considered viable if they progressed to later developmental stages with good morphology. Some of the embryos frozen in each cryoprotectant were thawed and transferred non-surgically without removing the cryoprotectant. Hatched embryos survived freezing and one step dilution as follows: EG (50.0%), MC (53.6%), DEG (56.9%), PG (58.0%) and BG (11.5%). The survival rate of embryos cooled at 0.3°C/min versus 0.5°C/min was not significantly different (P>0.05). However, blastocysts hatched most often (P< 0.01) in vitro when cooled at a rate of 0.3°C/min [64.6 % (31/48)] compared with 0.5°C/min [22.6 % (12 /53)]. Embryo survival in TCM-199 culture relative to cryoprotectant is shown in the table below. Pregnancy rates resulting from embryos frozen in different cryoprotectants were as follows: MC(48%, 10/21), DEG(30%, 3/10), EG(87%, 20/23), and PG(40%, 4/10).

Cryoprotectants	No. of culture	No. of survival (%)	No. of hatched blastocysts (%)
1.3 M methyl cellosolve	69	64(92.8) ^a	37(53.6) ^c
1.1 M diethylene glycol	51	47(92.2) ^a	29(56.9) ^c
1.8 M ethylene glycol	88	79(89.8) ^a	44(50.0) ^c
1.6 M propylene glycol	50	43(86.0) ^a	29(58.0) ^c
1.1 M butylene glycol	52	34(65.4) ^b	6(11.5) ^d

Values within columns with different superscripts are significantly different: a,b:p<0.05; c,d:p<0.01

These results indicate that MC, DEG, EG and PG have utility as a cryoprotectant for IVF bovine embryos with direct rehydration by placing thawed embryos into holding medium.