Theriogenology 40:651-659, 1993

PREGNANCY RATE AND SURVIVAL IN CULTURE OF IN VITRO FERTILIZED BOVINE EMBRYOS FROZEN IN VARIOUS CRYOPROTECTANTS AND THAWED USING A ONE-STEP SYSTEM

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Received for publication: *November 24, 1992*
Accepted: *May 10, 1993*

ABSTRACT

Bovine oocytes surrounded with compact cumulus cells were cultured for 20 to 22 hours (38.5°C, 5% CO_2) in modified TCM-199 medium supplemented with 5% superovulated cow serum (SCS) and inseminated by in vitro capacitated spermatozoa. Day 7 to 8 embryos were equilibrated for 10 minutes 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. They were then loaded into 0.25-ml straws, placed into an alcohol bath freezer at 0°C, cooled from 0°C to -6°C at -1°C/minute, seeded, held for 10 minutes, and cooled again at -0.3°C or -0.5°C/minute to -30°C. Straws were then plunged and stored in liquid nitrogen After thawing in 30°C water, the embryos were rehydrated in TCM-199 medium 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 nonsurgically 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 vs -0.5°C/minute 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 /minute (64.6%, 31/48) than at -0.5°C/minute (22.6%, 12/53). Pregnancy rates resulting from embryos frozen in the different cryoprotectants were as follows: MC (48%, 10/21); DEG (30%, 3/10); EG (74%, 20/27); and PG (40%, 4/10). These results indicate that MC, DEG, EG and PG have utility as cryoprotectants for the freezing and thawing of IVF bovine embryos.

Key words: cryoprotectants, IVF, bovine embryo, direct transfer

INTRODUCTION

A method of cryopreservation permitting direct transfer of bovine embryos to recipients after thawing would be a

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