

VIABILITY OF FROZEN-THAWED BOVINE IVM/IVF EMBRYOS IN
RELATION TO AGING USING VARIOUS CRYOPROTECTANTS

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ABSTRACT

Bovine IVF embryos developed on Days 7, 8 and 9 were equilibrated with 1.6 M propylene glycol (PG), 1.8 M ethylene glycol (EG), 1.1 M diethylene glycol (DEG) or 1.3 M ethylene glycol monomethyl ether (EME) for 10 to 20 min in modified phosphate buffered saline (mPBS) supplemented with 10% superovulated cow serum. The embryos were loaded into 0.25-ml plastic straws and were placed directly into a 0°C alcohol bath chamber and held for 2 min. They were cooled from 0°C to -5.5°C at 1°C/min and then seeded, followed by a 10-min holding period at -5.5°C. The straws were then cooled to -30°C at 0.3°C/min before plunging into liquid nitrogen. Embryos were thawed and placed directly into the culture medium and washed 3 times. The survival rates of the Day-9 embryos based on reappearance of blastocoele, expansion, and hatching after 48 h of post-thaw culture were significantly lower ($P < 0.01$) than those of the Day-7 and 8 embryos, in all of the cryoprotectants tested. On the other hand, while the reappearance of blastocoele and expansion of blastocysts after 48 h of post-thaw culture were not significantly different among each cryoprotectant, the percentage of hatching blastocysts were significantly different between DEG and EME ($P < 0.05$), between DEG and EG ($P < 0.01$) and between PG and EG ($P < 0.05$). These findings demonstrate that the age of the embryo (Day 7 and 8) is very important for the successful freezing of IVF bovine embryos. Also, as to the hatching rates, EME and EG are superior as cryoprotectants than the other 2 cryoprotectants tested.

Key words: bovine, IVF embryo, freezing, cryoprotectants

INTRODUCTION

Recently, advances in embryo transfer technology have enabled us to produce bovine embryos through the fertilization and development of in vitro matured oocytes derived from slaughtered cattle. Blastocysts thus produced have the ability to develop into live calves after transfer into recipient cows (5,6). Therefore,

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