Assessment of a Novel Media System for in Vitro Porcine Embryo Production

K.A. Stroble, J.R. Herrick, M.L. Conover, R.L. Krisher
Department of Animal Sciences

In vitro production of porcine embryos is historically difficult. Culture systems based on the metabolic needs of the oocyte and embryo should result in increased viability. The objective of this study was to compare porcine in vitro embryo production in the standard system (STND) and a newly developed single medium system (Purdue porcine media; PPM) modified for maturation (mat), fertilization (fert), early embryonic cleavage (1) and blastocyst development (2).

Oocytes from abattoir ovaries were matured in either modified TCM-199 (STND) or PPMmat, each supplemented with 0.01 units/mL porcine LH and FSH, 0.5 mM cysteine and 10 ng/mL EGF. PPMmat also contained 5mM glucose, 2 mM lactate, 1 mM pyruvate, 1 mM glutamine, 5 mM taurine, 1X vitamins and 0.5 mg/mL hyaluronate. After maturation, oocytes were denuded and fertilized in mTBM (STND) or PPM fert, both containing 2 mM caffeine, 2 mg/mL BSA and 7.5 mM calcium. Oocytes were co-incubated with sperm for 5 h, then placed into NCSU23 (STND) or PPM1. Embryos were moved from PPM1 to PPM2 after 72 h. In a second experiment, oocytes were matured in PPMmat, fertilized in either PPMfert or mTBM and cultured in PPM1/2 to specifically examine the effects of fertilization conditions. Data were analyzed by Chi-square, significance $P<0.05$.

There was no difference in penetration rate (PPM 93/124, 75%; STND 96/127, 76%), but more oocytes were normally fertilized (2 pronuclei; PN) in PPM (28/124, 23%) than in STND (18/127, 14%). More embryos cleaved in PPM (289/450, 64%) than STND (191/432, 44%). There was no difference in d 6 blastocyst development (PPM 35/442, 8%; STND 52/473, 11%). In the second study, oocytes matured and cultured in PPM but fertilized in mTBM had higher polyspermy (16/40, 40%) and a lower percentage of 2PN per oocytes penetrated (11/27, 41%) than those fertilized in PPMfert (6/40, 15%; 18/24, 75%, respectively). Fertilization in mTBM resulted in lower cleavage (70/110, 64%) and blastocyst development (7/110, 6%) than in PPM (87/110, 79%; 18/110, 16%, respectively). These results demonstrate that a single medium system based upon the metabolic needs of the oocyte and embryo resulted in an increase in normal fertilization and embryonic cleavage, possibly as a result of more correct cytoplasmic maturation and appropriate fertilization conditions.