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Improved in vitro developmental competence of porcine embryos with citrate supplement in culture medium.

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Citrate has played a role as an energy substrate and stimulates fatty acid synthesis and is a chelator of metal ions (e.g., Ca²⁺), a feature that may be of importance for maintaining junctional integrity and thus of importance for compaction and blastocoel formation. The objective of our study was to investigate the effect of citrate supplementation, and the effects of a two-step culture system, which involves the use of potential culture condition for both early cleavage and later stage embryos, on the developmental competence of porcine embryo derived from in vitro fertilized (IVF). Ovaries were collected from gilts at a local slaughterhouse and cumulus-oocyte complexes were matured for 44 hr in the modified tissue culture medium (TCM)-199 and fertilized in mTBM for 6 h. In Experiment 1, the IVF embryos were cultured in modified North Carolina State University (mNCSU)-23 medium supplemented with various concentrations (0, 0.5, 2.5 or 5 mM) of citrate. In Experiment 2, embryos were cultured for 6 days in mNCSU-23 medium supplemented with 0.05% PVA, 0.4% BSA plus citrate or



not. In Experiment 3, using two-step culture, embryos were cultured in mNCSU-23 medium supplemented with 0.5mM citrate and 0.4% BSA for 6 days, citrate from Days 0 to 2 and then without citrate from Days 2 to 6. alternatively, citrate was not supplemented from Days 0 to 2 and then applied for another 4 days. The supplementation of 0.5mM citrate during IVC increased the rate of embryo cleavage and the frequency of blastocyst development. In addition, The developmental rates of blastocysts in the citrate, BSA containing group were significantly higher than no citrate, BSA containing group. More blastocysts in containing citrate from Days 0 to 2 were obtained. Our results demonstrated that supplementing citrate containing mNCSU-23 medium with 0.4% BSA for 2 days and without citrate for another 4 days improved porcine IVF embryo development. This study was supported by grants from the Ministry of Science and Technology (Top Scientist Fellowship) and the Biogreen 21-1000520030100000.