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Generation of porcine parthenogenetic blastocysts under the protein-free defined culture conditions from oocyte maturation to preimplantational embryonic development *in vitro*

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A serum or bovine serum albumin (BSA) free (chemically defined) medium that is able to consistently support the development of a reasonable number of oocytes to the blastocyst stage is required to elucidate factors essential to porcine parthenogenetic or nuclear transfer embryo development. In the present study, attempts were made to develop a defined culture system for pig oocytes and parthenogenetic embryos after maturation, activation and culture *in vitro*. Tissue culture medium 199 with epidermal growth factor, gonadotropin and polyvinyl alcohol (PVA) was used as maturation medium. Porcine immature oocytes were recovered from slaughterhouse ovaries and cultured for 44 hours in maturation medium. Oocytes were activated by electrostimulation and cytochalasin B treatment, and cultured in North Carolina State University 23 medium (NCSU23) with 4mg/ml BSA or 1mg/ml PVA for 7 days. Proportions of activated oocytes that cleaved and that reached to the blastocyst stage in NCSU23-BSA were 71.4% and 15.8%, respectively. Supplementation of NCSU23-BSA with amino acids (AAs; essential and non-essential) enabled 79.5% cleavage and 58.9% blastocyst development of oocytes after activation. When BSA was replaced with PVA in NCSU23 and NCSU23-AAs, 69.8% and 75.7% of oocytes cleaved and 1.9% and 42.6% developed to the blastocyst stage, respectively. Regardless of culture media (NCSU23-BSA versus

NCSU23-PVA), AAs supplementation supported blastocyst development significantly ($p<0.01$). Our results indicate that porcine oocytes can be successfully developed to the blastocyst stage under the chemically defined culture condition after parthenogenetic activation when amino acids are supplemented in the medium.

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