

Manipulation of the Pentose Phosphate Pathway in Porcine Oocytes: Effects on Nuclear and Cytoplasmic Maturation

Jason R. Herrick, Amber M. Brad, and Rebecca L. Krisher
Department of Animal Sciences

Oocyte metabolism, particularly the pentose phosphate pathway (PPP), is a critical component of porcine oocyte maturation. The objective of this study was to determine the effects of manipulating the PPP on nuclear (completion of meiosis) and cytoplasmic (glycolytic and PPP activity, intracellular glutathione (GSH) content, and developmental competence) maturation of porcine oocytes. Cumulus-oocyte complexes were recovered from abattoir ovaries and matured for 42-44 h in the presence of 0.01 IU/ml FSH and LH, 10 ng/ml EGF, and 0.6 mM cysteine with or without 0.1 μ M of diphenyleneiodonium (DPI; PPP inhibitor) or pyrroline-5-carboxylic acid (PC; PPP stimulator). Following maturation, a portion of the oocytes were incubated with 5- 3 H]-glucose and 1- 14 C]-glucose to determine the activity of glycolysis and the PPP, respectively. At the conclusion of metabolic measurements, oocytes were stained to evaluate meiotic progression. Remaining oocytes were either frozen for subsequent GSH analysis (DTNB-GSSG reductase recycling assay) or inseminated (5×10^5 sperm/ml; 5 h) and cultured to determine developmental competence.

Exposure to DPI increased ($P < 0.05$) the proportion of oocytes arrested at the germinal vesicle or germinal vesicle breakdown stage (GV, 72%), and decreased ($P < 0.05$) the proportion of oocytes reaching metaphase II (MII, 7%) compared to both control (12% GV, 75% MII) and PC (3% GV, 68% MII) groups. Similarly, treatment with DPI reduced ($P < 0.05$) glycolytic (0.7 pmol/oocyte/3 h) and PPP (0.3 pmol/oocyte/3 h) activity compared to control (1.6 and 0.6 pmol/oocyte/3 h, respectively) and PC (2.0 and 0.7 pmol/oocyte/3 h, respectively) oocytes. Intracellular GSH content was not affected ($P > 0.05$) by exposure to PC (7.4 pmol/oocyte vs. control, 7.4 pmol/oocyte), but was reduced ($P < 0.05$) following treatment with DPI (5.4 pmol/oocyte). Rates of cleavage (day 3) and blastocyst formation (day 6) were not different ($P > 0.05$) between control (77% and 7%, respectively) and PC-treated (78% and 13%, respectively) oocytes, but were decreased ($P < 0.05$) following exposure to DPI (10% and 1%, respectively). In summary, reduced glucose metabolism (glycolysis and PPP) following DPI exposure was associated with decreased nuclear and cytoplasmic maturation of porcine oocytes.