

Public Abstract

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Title: Transcriptional profiling to improve porcine preimplantation embryo development in vitro

Culture environments can have profound impacts on the developmental program of the preimplantation embryo. Even though significant improvements have been made to embryo culture environments they are still sub-optimal to the in vivo environment. It is our overall goal to understand the metabolism of these embryos so that we can continue to improve our culture system to become more similar to the in vivo environment and thus increasing embryo viability in vitro. Our first aim was to utilize the transcriptional profile of embryos cultured in vitro to mine transcripts involved with glucose metabolism. Cancer cells metabolize glucose in a manner known as the Warburg Effect in which there is high glucose uptake with lactate production even in the presence of oxygen. These in vitro produced embryos have striking similarities to cancer cells in the expression of transcripts involved with glycolysis, pentose phosphate pathway and lactate production. The second aim was to illustrate that by mining a transcriptional profile database of embryos cultured either in vitro or in vivo and assessing the differentially expressed genes we could get an idea of what the in vitro embryos are lacking in our culture system. An arginine transporter, *SLC7A1*, was identified as up-regulated by 63 fold in in vitro cultured embryos compared to in vivo. By adding additional arginine to our culture system, the expression of *SLC7A1* decreased in blastocysts compared to our controls. There was an improvement in the percentage of blastocysts in embryos cultured with additional arginine. Arginine cultured embryos were also able to produce live piglets when transferred to a recipient gilt. Supplementing the medium with additional arginine also increased nitric oxide production in 4-cell stage embryos compared to controls and inhibiting nitric oxide synthase greatly decreased the number of embryos that made it to the blastocyst stage. The final aim was to continue analyzing the differentially expressed genes in the in vitro or in vivo cultured embryo database. A glycine transporter, *SLC6A9*, was up-regulated in in vitro cultured embryos compared to in vivo by about 25 fold. By adding glycine to our culture system, cell number increased by about 1.5 times our control in day 6 blastocysts. We also found decreased *SLC6A9* message in the glycine supplemented embryos compared to controls. Glycine affected genes involved with glycine and serine metabolism as well as thioredoxin 1 and uracil DNA glycosylase. Mitochondrial function was analyzed and glycine does not affect mitochondrial DNA copy number, membrane potential, or mitochondrial related genes. Embryo transfers were not successful in producing piglets. In the future, experiments looking at how glycine is affecting these embryos later on in development should be conducted. Each of these experiments illustrates the benefits of transcriptional profiling to identify ways to affect embryo development and improve our embryo culture system.