Arginine Supplementation In Vitro Increases Porcine Embryo Development and Affects mRNA Transcript Expression

B.K Bauer*, L.D. Spate, C.N. Murphy, and R.S. Prather
Division of Animal Sciences, University of Missouri, Columbia, MO, USA

Introduction
In vitro culture systems provide the tools to better our understanding of the biology of early embryo development, allowing us to optimize current biotechnology techniques and have vast effects on human assisted reproductive technologies. In vitro culture systems promote development at rates lower than in vivo. Therefore, a previous next-generation sequencing analysis of in vivo fertilized and in vitro cultured (IVC) or in vivo cultured (IVV) porcine blastocyst stage embryos was completed to identify differentially expressed transcripts. This study identified an arginine transporter (SLC7A1) expressed 63 fold higher in IVC compared to IVV blastocysts. After supplementing arginine at 0.36 mM concentrations in the culture medium we could not detect a difference in the SLC7A1 mRNA levels of embryos cultured in 0.36 mM arginine and IVV embryos (Bauer et al., 2010). The overall goal of this study was to determine the effect arginine has on embryo development and SLC7A1 mRNA levels of embryos fertilized in vitro.

Research Design and Methods

\textbf{In Vitro Maturation, Fertilization, and Culture}
Cumulus-oocyte complexes were matured for 44 hours in M199 supplemented with EGF, FSH, and LH. Oocytes with a visible polar body were selected and fertilized in modified Tris Buffered Medium for 5 hours and then placed into one of five treatment groups (Porcine Zygote Medium 3 (PZM3) with 0.0 mM, 0.12 mM (normal concentration of arginine in PZM3), 0.36 mM, 0.72 mM, or 1.69 mM arginine). Twenty-eight hours post-fertilization, cleaved embryos were selected and moved into 25 μl drops of respective culture media and cultured to day 6 in 5% CO₂, 5% O₂, 90% N₂ at 38.5°C. To determine the effect arginine had on development the percent of embryos that made it to the blastocyst stage for each treatment group was analyzed using PROC GLM in SAS (SAS Institute, Cary, NC). A least significant difference post test comparison was completed to determine if significant differences existed between treatment groups (\(\text{**P}<0.05\)).

\textbf{Nuclear and Differential Staining}
For both nuclear and differential staining techniques, all zona pellucidae were removed using a physiological saline solution with a pH of 1.8. For total cell counts, embryos were stained with bisbenzimide (Hoechst 33342; Sigma) for 30 minutes then viewed under UV light. For the second experiment in which a new set of embryos was collected for differential staining, zona pellucidae were removed and differential staining was completed similar to Bauer et al., 2010.

\textbf{Real-Time PCR}
Real-time PCR was completed to assess the effect arginine supplementation had on SLC7A1 mRNA levels. Three biological replicates, each containing 10 blastocyst pools were collected for each treatment group. Total RNA was isolated from each sample and 5 μl was amplified (NuGEN Ovation Pico WTA System) and then purified by using Bio-Rad MicroSpin P-30 Columns. The comparative threshold cycle method was used to calculate expression levels relative to the reference sample and the housekeeping gene, YWHAG. The \(\Delta\Delta^{C_{T}}\) values were log transformed and analyzed by using PROC GLM in SAS.

\textbf{Ilumina Sequencing}
Oocytes were collected, matured and in vitro fertilized as mentioned above. They were then cultured in 25 μl drops of either PZM3 (0.12 mM Arginine) or PZM3 (1.69 mM Arginine). Three biological replicates each containing 10 blastocysts were collected for each treatment group. Total RNA was extracted from pools of 10 embryos and then first and second strand cDNA was synthesized. The samples were purified and then sent to the University of Missouri DNA Core where they generated libraries for each sample. Each sample was loaded and run on a Genome Analyzer II flow cell. The Illumina sequencing resulted in 80 bp reads from each of the samples which were then tiled to a custom transcriptome.

\textbf{Results}

Conclusions

- Supplementing PZM3 with arginine
  - Increases blastocyst development compared to current PZM3
  - Does not affect total cell number compared to current PZM3
  - Increases the number of trophectoderm cells and TE:ICM ratio
  - Decreases SLC7A1 mRNA levels
- Next-generation sequencing identified potential transcripts to investigate further to better understand metabolic pathways and improve culture systems for embryos.
- Evaluating the transcriptional profile is a good method of letting the embryo tell us what it needs for development, and in this case arginine.

References


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