Aryl hydrocarbon receptor targets are upregulated in porcine blastocyst-stage embryos that were cultured in vitro: a transcriptional analysis.

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INTRODUCTION

In vitro culture of embryos results in developmental delays compared to embryos developing in vivo [1]. Moreover, exposure to the culture environment can cause changes within the embryo that produce gestational and postnatal defects [2,3]. Identifying these changes, whether they are metabolic, transcriptional, or epigenetic, has been a main goal of many embryo culture labs.

A previous transcriptional profiling endeavor revealed numerous differences between in vivo-derived and in vitro-cultured porcine embryos [4]. This dataset provided the basis for different modifications to porcine zygote medium-3 (PZM-3), such as addition of arginine, glutamine, and PS48, an activator of phosphoinositide 3-kinase (PI3K), resulting in our current MU3 medium [5].

Our new dataset, presented here, was acquired to determine if the modifications directed the transcriptional profiles of in vitro-produced porcine embryos towards the in vivo state. However, mining the dataset revealed message for cytochrome P450 family 1 subfamily A member 1 (*CYP1A1*) in the in vitro-produced embryos but complete absence of message in the in vivo-derived embryos. The aryl hydrocarbon receptor (AHR) promotes transcription of *CYP1A1*, which encodes a monooxygenase involved in xenobiotic metabolism. Inhibition of AHR activity in in vitro-produced rat embryos has been shown to improve developmental rates [6].

OBJECTIVES

- To identify differences in the transcriptional profiles of three sources of porcine embryos that may account for reduced competency in in vitro-produced embryos
- To determine if inhibiting AHR activity in in vitro-produced porcine embryos improves developmental parameters

MATERIALS AND METHODS

Embryo Recovery and Production

In vivo-derived (IVV)

- One uterine horn of an artificially-inseminated gilt was flushed on Day 6 to recover blastocyst-stage embryos

In vivo-matured, in vitro-cultured (IVC)

- One oviduct and tip of a uterine horn of an artificially-inseminated gilt were flushed on Day 2 to recover 4-cell embryos that were cultured for 4 days in MU3 medium to obtain blastocyst-stage embryos
- IVV and IVC embryos were collected from the same gilt per replicate

In vitro-matured and –cultured (IVMC)

- Cumulus-oocyte complexes were aspirated from slaughterhouse-derived prepubertal gilt ovaries, matured, and fertilized according to standard procedures in our laboratory
- Presumptive zygotes were cultured for 6 days in MU3 medium, and blastocyst-stage embryos were collected

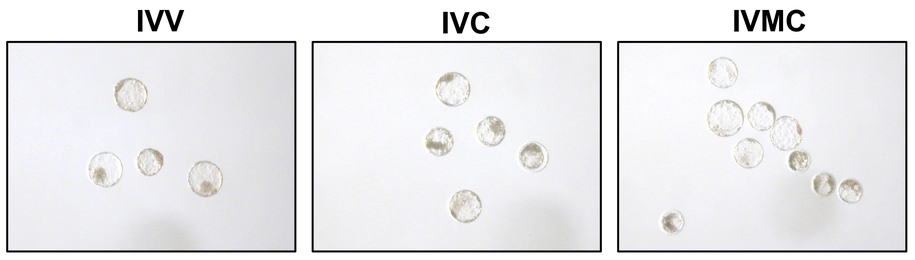

Figure 1. Representative images of blastocyst stage embryos from each group.

RNA-Sequencing

- Total RNA was extracted from pools of 10 blastocysts from each group to synthesize first and second strand cDNA for sequencing by using the Illumina platform
- Reads were mapped to the Sus scrofa genome by using STAR (version 2.7.1a) with default options

Embryo Culture with AHR Agonist and Antagonists

- IVMC presumptive zygotes were cultured in MU3 with dose curves (0.1, 1, or 10 µM) of ITE (agonist), CH-223191 (antagonist), and SR-1 (antagonist) until Day 6
- Development to the blastocyst stage was recorded and embryos were stained with 10 µg/mL Hoechst 33342 to determine total number of nuclei in the embryos

Statistical Analysis

- Three replicates were collected per group for RNA-seq and for culture studies
- For RNA-seq, pairwise comparisons were performed to test for differential expression of genes by using the Bioconductor package DESeq2
- Differentially abundant transcripts were subjected to KEGG pathway analysis by using gProfiler
- Shapiro-Wilk test was used for assessing the normality assumption for each experiment
- Embryo culture studies were analyzed by using a general linear model in SAS 9.4
  - Multiple comparisons were conducted by Tukey's test with $P < 0.05$ considered significant

References

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This research was funded by the United States Department of Agriculture, National Institute of Food and Agriculture (2019-67011-29543).

RESULTS

Differentially Abundant Transcripts Between the Three Sources of Embryos

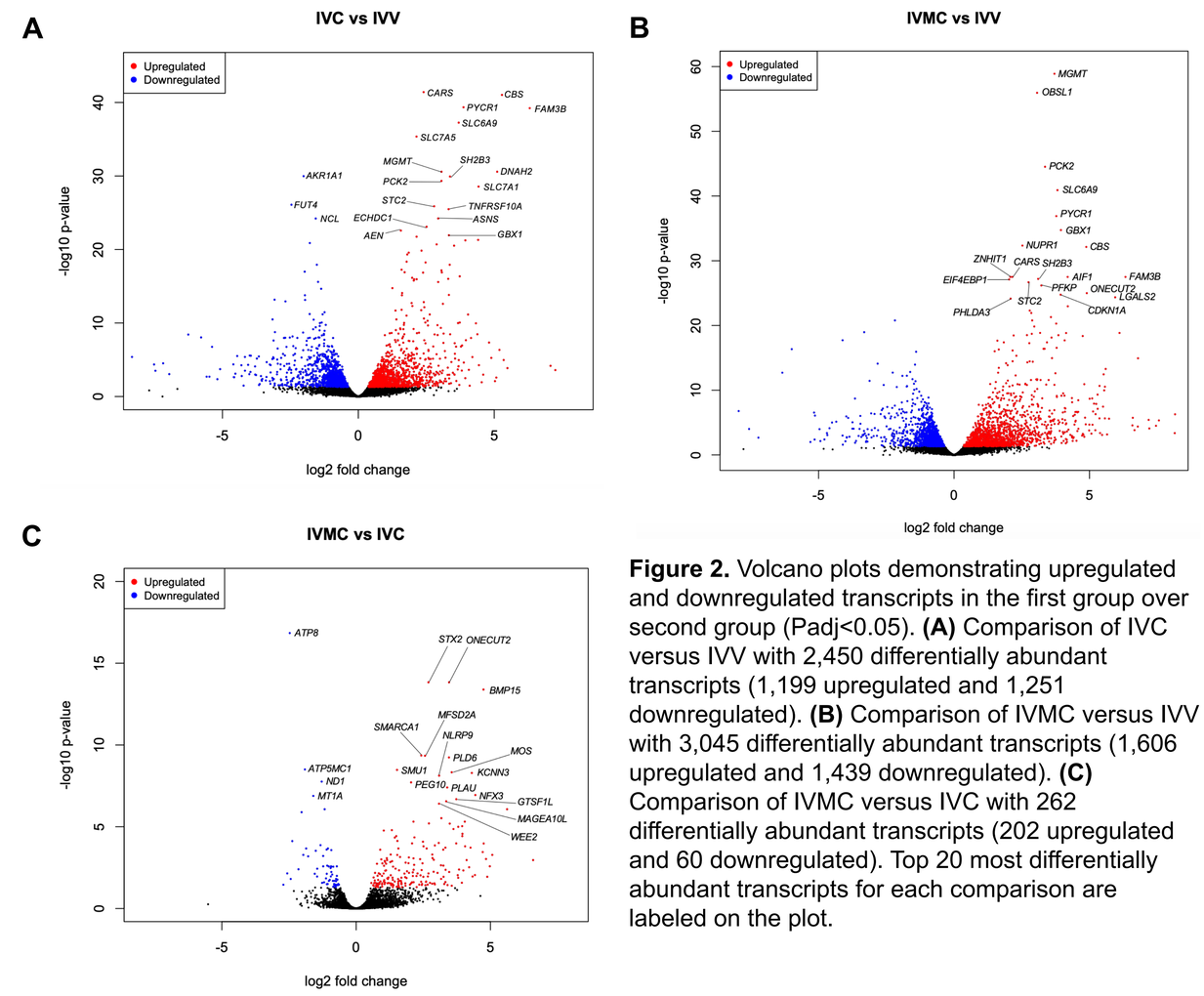

Figure 2. Volcano plots demonstrating upregulated and downregulated transcripts in the first group over second group (Padj<0.05). **(A)** Comparison of IVC versus IVV with 2,450 differentially abundant transcripts (1,199 upregulated and 1,251 downregulated). **(B)** Comparison of IVMC versus IVV with 3,045 differentially abundant transcripts (1,606 upregulated and 1,439 downregulated). **(C)** Comparison of IVMC versus IVC with 262 differentially abundant transcripts (202 upregulated and 60 downregulated). Top 20 most differentially abundant transcripts for each comparison are labeled on the plot.

KEGG Pathway Analysis for Differentially Abundant Transcripts

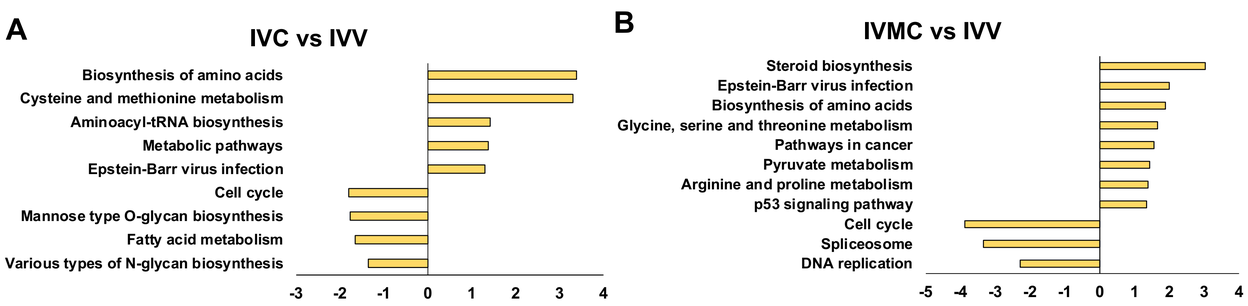

Figure 3. (A) Upregulated (positive) and downregulated (negative) pathways in IVC embryos compared to IVV embryos. **(B)** Upregulated and downregulated pathways in IVMC embryos compared to IVV embryos. Pathway enrichment was not detected between IVC and IVMC embryos.

Increased AHR Transcriptional Targets in In Vitro-Cultured Embryos

Table 2. Average counts of differentially abundant transcripts regulated by AHR.

Gene Symbol	IVV	IVC	IVMC	P_{adj} (IVMC vs IVV)
CYP1A1	0	956	381	7.6E-09
ARNT	338	471	560	0.045
BATF3	47	135	166	0.026
BMF	35	142	148	2.2E-06
DDIT4	1560	5503	6804	2.0E-07
FAM32A	1220	1689	1598	0.090
LMCD1	8	56	92	1.3E-06
NFE2L2	4054	5925	5815	0.012
PITPNM2	6	88	80	4.2E-07
PRPS1	818	1443	1376	0.00037
RND1	40	45	148	0.018
SAT1	1761	2364	3527	0.0018
SLC7A5	3801	16369	13594	4.1E-18
TPCN1	786	1559	1236	0.010
TRAFD1	1497	2791	2413	0.0062

Development of Embryos Cultured with AHR Antagonists or an Agonist

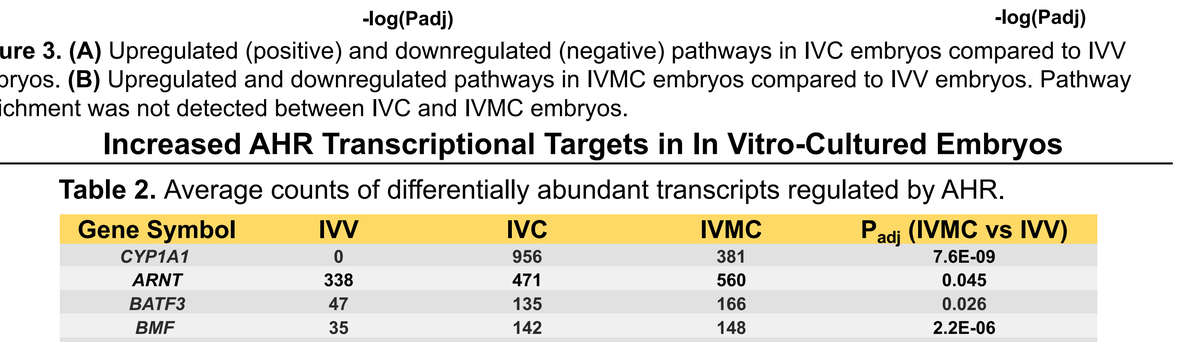

Figure 4. Developmental parameters for embryos cultured with dose curves of CH-223191 (CH), SR-1 (SR), or ITE. **(A)** Percentage of embryos developed to the blastocyst stage on Day 6 (n=40 presumptive zygotes per group per replicate). **(B)** Total number of nuclei in blastocyst stage embryos (n=5-15 embryos per group per replicate). No development to the blastocyst stage for SR-1 at 10 µM; thus, data are not available (NA). Data are presented as means ± SEM. Different superscripts (^{a,b,c}) indicate statistical differences.

SUMMARY

- In vitro culture of porcine embryos results in numerous transcriptional differences compared to embryos developing in vivo.
  - Transcripts related to the cell cycle are decreased in in vitro-produced embryos.
  - Transcripts related to amino acid synthesis and metabolism are increased in in vitro-produced embryos.
- Transcriptional targets of AHR are increased in in vitro-produced porcine embryos.
- Treatment of in vitro-produced embryos with AHR antagonists does not improve development to the blastocyst stage.
- The AHR antagonist, CH-223191, added at 10 µM increased total number of nuclei.