



Effect of sex on gene expression in equine blastocysts

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Male mammalian embryos grow faster than female; but in equine, embryo size varies greatly and the relationship to sex and gene expression has not been reported. This study aimed to evaluate gene expression according to sex in equine blastocysts. Nonlactating, Saddlebred mares located on two experimental farms were inseminated with the semen of one stallion. At 8 days post ovulation, 17 and 11 embryos from each farm and each from a different dam, were recovered and bisected to obtain samples of pure trophoblast (TE) or inner cell mass enriched trophoblast (TE-ICM). RNA expression was analyzed separately in the TE-ICM and the TE of 16 female and 12 male embryos using paired end, non-oriented RNA sequencing (Illumina, NextSeq500). To discriminate gene expression in the ICM from that in the TE, deconvolution (DeMixT) was used on the TE-ICM data. Differential expression was analyzed (DESeq2) with farm and embryo size as cofactors using a false discovery rate (FDR) <0.05 cutoff. Gene Set Enrichment Analysis was performed using the KEGG and GOBP databases. Female and male embryo size was comparable (respectively, 968±574 and 1087±519µm). Of the 13,786 and 13,210 genes expressed in ICM and TE, respectively, only 184 (51% on X chromosome) in ICM and 415 (39% on X chromosome) in TE were differentially expressed,

with 111 genes in common. Overall, 145 genes in ICM and 312 in TE were significantly upregulated in females including glucose-6-phosphate dehydrogenase (G6PD, log2 Fold Change=0.96 in ICM and 0.83 in TE; FDR <0.001). G6PD is required for detoxification of oxygen radicals, with both cellular damage and growth-stimulant effects. Reduced levels of oxygen radicals have been hypothesized to be responsible for the growth delay in female bovine embryos. In ICM cells, 16 and 1 gene sets were enriched in male embryos in GOBP and KEGG, respectively while 3 KEGG pathways were enriched in female ICM. In TE, 27 and 6 gene sets were enriched in male embryos in GOBP and KEGG, respectively, while no pathway was enriched in female embryos. Enriched pathways in males were mostly common between ICM and TE and were involved in translation and spliceosome, ribosome biogenesis, mitochondrial function, cytoskeleton organization, and amino acid metabolism. Pathways that were only enriched in male ICM related to potassium ion homeostasis; while in TE, they were related to protein export. In females, ICM enriched pathways were related to Krebs cycle, glycosaminoglycan degradation and lysosome. Although no size difference was observed, sexual dimorphism exists and includes genes unrelated to the sex chromosomes.