86. REDOX REGULATION OF HYPOXIA-INDUCIBLE FACTORS IN BOVINE BLASTOCYSTS

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Addition of the OXPHOS uncoupler 2,4-dinitrophenol (DNP) to culture media, to favour the production of ATP via glycolysis during compaction and blastulation, has a beneficial effect on bovine embryo development (1), most likely through a change in REDOX state. Hypoxia-inducible factors (HIFs) are transcription factors that mediate O2-dependent regulation of numerous genes thought to be important for further embryo development, including glucose transporters (GLUTs), glycolytic enzymes and angiogenic growth factors. HIF DNA-binding is regulated by protein stabilisation and nuclear translocation in response to hypoxia or the Fe-chelator desferrioxamine (DFO). REDOX regulation of HIF activity is also postulated to occur. We have demonstrated O2-regulated gene expression in bovine blastocysts (2). Here we test our hypothesis that addition of DNP or DFO post compaction alters the expression of HIFs and HIF-regulated genes. Bovine IVP embryos were cultured to Day 5 under 7% O2. Morula were further cultured in ±10 µM DNP, or ±1 µM DFO, for 72 h. Resulting blastocysts were pooled and total RNA isolated. Real time RT-PCR was performed for quantification of reaction products, normalised by measuring 18S rRNA for each sample. Statistical analyses were performed by ANOVA, with significance determined at α = 0.05. Real time RT-PCR analysis of HIF1α and HIF2α mRNA revealed that both subunits were altered by addition of either DNP or DFO. Following DNP treatment, HIF1α and HIF2α mRNA were significantly increased (P<0.05 respectively), HIF1α mRNA was increased following DFO treatment, although not significantly. In contrast, DFO decreased HIF2α mRNA levels (P<0.05). These findings have not been previously reported in either embryonic or somatic cells. GLUT1 expression was significantly increased by the addition of DNP or DFO (P<0.05), and was non-significantly increased following DNP treatment. VEGF mRNA expression was not altered by either treatment. These results suggest that alterations in intracellular REDOX, associated with altered metabolic preference or induced expression of HIFs, regulates specific genes in bovine blastocysts.

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