DETERMINATION OF DIFFERENTIALLY DISPLAYED OXYGEN-SENSITIVE GENES IN BOVINE BLASTOCYSTS

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Oxygen-regulated gene expression in the bovine embryo contrasts markedly with that observed in the mouse. Under low (2%) oxygen moderate changes in gene expression are observed in the bovine blastocyst, compared with 3- to 4-fold increases in the mouse. We have determined that these moderate gene expression changes are most likely regulated by Hypoxia-Inducible Factor (HIF)-2 transcription factor activity in the bovine, in the absence of HIF1, although HIF2 target genes are largely unknown. The aim of this study was to screen, by differential display RT-PCR, for putative oxygen-regulated transcripts that might confer developmental competence in blastocysts cultured under varying oxygen atmospheres post compaction.

In vitro-produced bovine blastocysts were generated using standard protocols. Compact morulae were randomly allocated to treatments under either 2%, 7% or 20% oxygen for 72 h from Day 5. Blastocyst RNA was isolated using TriReagent and samples were reverse transcribed using Superscript II. cDNA was amplified using 10-mer primers in reactions containing ^{32}Pα-labelled dCTP. Resulting bands were detected by autoradiography, excised, purified and ligated into pGEMT vectors for transformation and sequencing. Seven clones were identified as having high homology with known sequences in GenBank. Real-time PCR was undertaken to confirm oxygen-regulation using Sybr green master mix.

Myotrophin mRNA was significantly increased following 2% oxygen culture, compared with 20% cultured blastocysts ($P < 0.01$), as was GLUT1 ($P < 0.01$). The expression of anaphase-promoting complex showed a significant association with oxygen, being higher in 2% cultured blastocysts ($P < 0.05$). Acetyl-coA-acetyltransferase I, chronic myelogenous leukemia tumor antigen (CML66), cyclin I, NADH dehydrogenase subunit 2 and ribonucleotide reductase M1, genes identified using differential display, were not altered by post compaction oxygen concentration.

This study has identified potentially HIF2-specific regulated genes, and supports the hypothesis that reduced oxygen concentrations post-compaction may influence bovine embryo development through oxygen-regulated changes in gene expression.

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