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Genetic Analysis of Bovine Embryonic Biopsies as a Tool to Identify Genes Related to the Establishment of Pregnancy after Embryo Transfer

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Abstract

The purpose of this work is to address the relationship between transcriptional profile of embryos and the pregnancy success based on blastocyst biopsies taken prior to transfer to recipients. Biopsies (30–40 % of the intact embryo) were taken from IVP day 7 blastocysts (n=98) and 60–70 % part were transferred to recipients after re-expansion. Based on the success of pregnancy, biopsies were pooled in three groups: those resulted in no pregnancy (G1), resorption (G2) and those resulted in calf delivery (G3). Gene expression analysis of these groups of biopsies was performed using home made bovine preimplantation specific array (219 clones) and cDNA array (BlueChip) (2000 clones). Three independent pools (10 in each) of biopsies from the three groups were used for mRNA isolation and subsequent RNA amplification. Approximately 2 μg of amplified RNA was used from each group to perform an indirect dye labelling. Images were analysed using GenePix Pro Version 4.0 software. Data analysis performed using Significant Analysis for Microarray (SAM) software. Real-time PCR was used to confirm the resut of microarray experiments. A total of 52 genes were differentially regulated between G1 and G3 and 58 genes differentially regulated between G2 and G3. Biopsies resulted in calf delivery are enriched with genes necessary for implantation like (Cox2 and Cdx2), carbohydrate metabolism (ALOX15), growth factor (BMP15), signal transduction (PLAU) and placenta-specific 8 (PLAC8). Biopsies from blastocyst ended with resorption are enriched with transcripts involved protein phosphorylation (Cytokeratin A) Plasma membrane (Occludin) and glucose metabolism (PGK and aldose reductase). Biopsies from blastocyst resulted in no pregnancy are enriched with transcripts involved inflammatory cytokines (TNF1a), protein amino acid binding (EEF1A1), transcription factors (MSX1 and PTTG1), glucose metabolism (PGK, aldose reductase) and CD9 which is inhibitor of implantation. In conclusion, we generated direct candidates of blastocyst specific genes which determine the fate of the embryo after transfer.

Keywords: Blastocyst, cattle, embryo loss, microarray, preimplantation

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