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Identification of Differentially Regulated Genes in Bovine Blastocysts using an Annealing Control Primer System

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The identification of embryo-specific genes would provide insights into early embryonic development. However, the current methods employed to identify the genes that are expressed at a specific developmental stage are labor intensive and suffer from high rates of false positives. Here we employed a new and accurate reverse transcription-polymerase chain reaction (RT-PCR) technology that involves annealing control primers (ACPs) to identify the genes that are specifically or prominently expressed in bovine early blastocysts and hatched blastocysts produced *in vitro*. Using these techniques, a total of nine expressed sequence tags (ESTs) of genes that were differentially expressed in hatched blastocysts, as compared to blastocyst embryos, were cloned and sequenced. The cloned genes or ESTs (C1-C9) all exhibited significant sequence similarity with known bovine genes (99~100%; FTL, RPS12, LAPTM4a, and RPL12) or ESTs (80~94%; AIBP, CULLIN-1, HDLP, COX5a, and RECS1) of other species. As revealed by real time RT-PCR, these genes were regulated upstream in the hatched blastocyst stage during early implantation. These results suggest that this new, PCR-based differential display RT-PCR technique is a very useful tool for the identification of stage-specific genes of preimplantation embryos.

Key words: ***Bovine embryo, Blastocyst, Hatched blastocyst, ACP system.***