

## **Production of cloned goats following serial somatic cell nuclear transfer and its effect on gene expression and methylation patterns of imprinted genes**

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Serial cloning by somatic cell nuclear transfer (SCNT) is an essential tool for expansion of livestock transgenic lines or resetting a lifespan of transgenic cells for additional genetic manipulations. In this study, we successfully produced second-generation cloned goats using donor neonatal fibroblasts from first-generation clones. However, our attempts to produce any third-generation clones failed. SCNT efficiency decreased progressively with the clonal generations. The rates of pregnancy loss were significantly greater in recloning groups ( $P < 0.05$ ). While no pregnancy loss was observed during the first round of SCNT, 4 out of 9 pregnancies aborted in the second round of SCNT and no pregnancies were established in the third round of SCNT. We investigated the expression of 21 developmentally important genes in live cloned (G 1) and recloned (G2) offspring using real-time PCR. The expressions of most of these genes in live clones were found largely comparable to naturally reproduced control goats, but *FGF10*, *MECP2* and *GRB10* were differentially expressed ( $P < 0.05$ ) in G2 goats compared with G 1 and controls. To study effects of serial cloning on DNA methylation, the methylation pattern of differentially methylated regions (DMRs) in imprinted genes *H19* and *IGF2R* were analyzed. Aberrant *H19* DNA methylation patterns were detected in G 1 and G2 clones.

## **Gene expression profiles of bovine leukocyte populations in response to trophoblast extracellular vesicle co-culture**

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Embryonic loss is an important problem in the cattle production industry. In order to increase reproductive efficiency, it is important to understand the mechanisms that regulate placental and embryonic development, and the immune regulatory interactions that exist between placental cells and immune cells during pregnancy. Our hypothesis is that trophoblast cells from functional placentas alter the gene expression profile of maternal immune cells via secretion of extracellular vesicles (EV) in vitro. To test this, bovine placental cells will be isolated and cultured for 48h, culture supernatant will be harvested, and EV s will be isolated by a differential ultracentrifugation method. Different immune cell populations (CD4+ T lymphocytes, CD8+ T lymphocytes, y/o-T cells, and natural killer (NK) cells) will be sorted by flow cytometry. These cells will be either cultured alone (control group) or co-cultured with trophoblast derived EV s for 72 hours. Total RNA will be isolated from these cells to perform gene expression analysis by microfluidic chip real time RT-PCR (96.96 Dynamic Array; Fluidigm). Gene expression of cytokines, transcription factors and CD markers for the following genes will be analyzed: IL1, IL2, IL4, IL5, IL6, IL8, IL10, IL12A, IL12B, IL13, IL15, IL17, IL18, IL23, IFNG, TNF, TGFB, CSF2, FoxP3, TBX21, GATA3, CD25, IL2RA, and CTLA4. We expect to observe a strong effect of trophoblast cell derived EV on the gene expression profile of immune cells and to clarify some of the regulatory interactions between fetal maternal cells.