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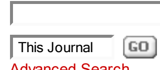

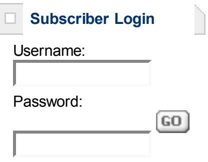

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187 IMPRINTED GENE EXPRESSION IN *IN VIVO*-AND *IN VITRO*-PRODUCED BOVINE FETUSES AND PLACENTAS

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Abstract

Some gestational alterations associated with bovine somatic cell nuclear transfer (SCNT) are presumably consequences of abnormal imprinted gene expression. This work aimed to evaluate the expression patterns of imprinted genes IGF2 and IGF2R in bovine fetuses and chorioallantoic membranes derived from *in vivo*- and *in vitro*-produced embryos. Fetuses were produced by AI (*in vivo* group, $n = 3$), IVF ($n = 3$), parthenogenesis ($n = 3$), or SCNT ($n = 2$). Cows with positive pregnancy diagnosis after ultrasonographic examination were slaughtered between Days 33 and 36 of gestation. The reproductive tract was transported on ice to the laboratory, where fetuses and chorioallantoic fragments were collected and stored in liquid nitrogen. Total RNA extraction was performed using TRIzol, according to manufacturer's instructions, and the reverse transcription reaction was carried out with 1 µg of total RNA, 6.75 µm oligo pd$(T)_{12-18}$, and 50 U of reverse transcriptase (Improm-II, Promega, Madison, WI, USA). The relative quantification of IGF2 and IGF2R transcripts was done using real-time PCR with SYBR Green dye. The average efficiency of PCR amplifications was estimated for each gene using a linear regression on the logarithm of fluorescence per cycle (Ramakers *et al*. 2003 Neurosci. Lett. **339**, 62–66), and the expression ratios were calculated according to the method described previously by Livak and Schmittgen (2001 Methods **25**, 402–408). To verify statistical differences, a pair-wise fixed reallocation randomization test (Pfaffl *et al*. 2002 Nucl. Acids Res. **30**, e36) was used. All expression ratios were normalized by glyceraldehyde 3-phosphate dehydrogenase expression and calibrated by the *in vivo* group (expression assumed as 1.00 for all genes and tissues). The analysis of relative differences on transcript levels of imprinted genes in fetuses revealed IGF2 down-regulation ($P < 0.05$) in the SCNT (0.19) and parthenogenetic (0.02) groups when compared to the *in vivo* group and IVF fetuses (2.02). In chorioallantois, IGF2 was down-regulated ($P < 0.001$) in parthenotes (0.001) when compared to the *in vivo*, IVF (3.13), and SCNT (0.98) groups. IGF2R was down-regulated ($P < 0.001$) in SCNT chorioallantois (0.25) when compared to the *in vivo* group. Low expression of IGF2 in parthenogenetic fetuses and chorioallantois confirms its imprinted status in bovine. Alterations in the relative frequency of IGF2 and IGF2R transcripts were observed in bovine SCNT-derived fetuses and chorioallantoic membranes, respectively, supporting the hypothesis that abnormalities in the expression of imprinted genes are causes for the low efficiency of SCNT procedures in this species. Such alterations suggest modifications in DNA methylation patterns at IGF2 and IGF2R imprinting centers.

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