RELATIONSHIP BETWEEN OCT-4 GENE EXPRESSION AND BOVINE EMBRYO PRODUCED BY NUCLEAR TRANSFER

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Oct-4 is a transcript expressed in pre-implantation embryos and is associated to totipotency, being used as a gene marker for stem cells. An appropriated reprogramming of somatic nucleus seems to be important to embryos achieve totipotency and develop to further stages. The aim of this study was to evaluate the relationship between levels of Oct-4 gene expression in 8-cell stage embryos, produced by *in vitro* fertilization (IVF) or somatic cell nuclear transfer (SCNT), with blastocyst embryo production. Eight-cell embryos were produced by *in vitro* fertilization or by SCTN with fetal muscle cell. IVF and SCNT embryos were cultured in G1.1 medium plus BSA at 5% CO_2, 5% O_2 and 90% N_2 for 72h and then transferred to G1.2 medium and cultured until eight day post-fertilization at the same atmospheric conditions. Some 8-cell stage embryos were frozen and stored until RNA extraction to evaluate expression of Oct-4 gene. mRNA was extracted from single embryos and cDNA quantified by real time PCR and normalized against expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), used as internal reference gene. Calculations of relative quantification were performed by $2^{-\Delta\Delta C}{}_T$ method, using the lowest value found in IVF embryo as calibrator. Relative quantification was performed in 8-cell stage embryos collected from six batches of *in vitro* fertilization (n=22 embryos) and SCNT (n=24 embryos). Blastocyst rate was calculated based on number of 8-cell embryos. Blastocyst rate and relative quantification of Oct-4 (mean±SEM) was analyzed by analysis of variance and relationship between Oct-4 expression in 8-cell stage embryos (mean per embryo in each batch) with blastocyst rate was performed by Pearson correlation. Despite showing lower values, the expression of Oct-4 in IVF 8-cell embryos did not differ ($P>0.05$) from SCNT embryos (9.87±2.43 vs. 13.02±2.89, respectively) as well blastocyst rate (51.4±8.9% vs. 40.5±2.7%, respectively). Nevertheless, Oct-4 expression in SCNT 8-cell embryos showed high correlation ($R=0.90$; $P=0.012$) with blastocyst rate, whereas for IVF embryos the correlation was lower ($R=0.32$) and not significant ($P>0.05$). These data suggest that, although there is no difference on relative expression of Oct-4 gene between IVF and SCNT 8-cell embryos, the expression of this gene at this stage may be more critical for development of SCNT embryo until blastocysts stage, and may be a consequence of the reprogramming and activation of Oct-4 before maternal-zygotic transition.