P-01

Antioxidant treatment reduce reactive oxygen species induced by electrical activation in porcine parthenogenetic and somatic cell nuclear transfer embryos.

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Introduction : The object of this study were 1) to measure the level of reactive oxygen species (ROS) after various the electrical activation of porcine oocytes and 2) to know the effect of glutathion (GSH) on the generation of ROS in porcine parthenogenetic and somatic cell nuclear transfer (SCNT) embryos.

Material and Method : Ovaries were collected from gilts at a local slaughterhouse and cumulus-oocyte complexes were cultured for 44 hr in the modified tissue culture medium (TCM)-199. Embryos were obtained after in vitro fertilization, parthenogenetic activation (PA) using electric pulse, and SCNT using electric pulse for fusion/activation. At day 2, the relative level of ROS contents within cleaved embryos was measured using dichlorohydrofluorescein diacetate assay.

Result : PA embryos (2 kV/cm electric pulse, 30 sec, 1 mM of Ca^{2+} in a pulsing medium) show significantly higher level of ROS compared to

IVF embryos. (214.9 vs. 58.97 pixels/embryo, $p<0.001$). In a pulsing medium supplemented with the various concentrations of Ca^{2+}(0, 0.05, 0.1 or 1.0 mM) levels of ROS were gradually increased. (74.3*, 124.8, 138.7, 200.0* pixels/embryo, respectively; *means significantly difference, $p<0.05$) Using the various voltages of DC pulse (1, 2, 2.5 or 3 kV/Cm), levels of ROS were gradually increased (151.5*, 200.0, 226.1, 314.8* pixels/embryo, respectively;* means significantly difference, $p<0.05$). To reduce ROS generation, an antioxidant, GSH were supplemented with the pulsing medium. (0.125 mM) GSH-treated group show significantly low level of ROS compared to untreated group. (120.9 vs 200.6 pixels/embryo, $p<0.001$) Similarly, SCNT embryos using a pulsing medium supplemented with GSH show significantly reduced ROS level compared the untreated group. (126.7 vs 198.7 pixels/embryo, $p<0.001$)

Discussion : ROS readily alter the pattern of DNA methylation, accelerates telomere shortening, and associated with karyotypic abnormalities. To improve development potential of electrically activated emryos, especially cloned embryos, electrical activation condition should be optimized to reduce ROS generation. The present study demonstrated that using antioxidant, GSH, during electrical activation could decrease the detrimental effect of ROS in porcine PA and SCNT embryo. This study was supported by grants from the Ministry of Science and Technology (Top Scientist Fellowship) and the Biogreen 21-1000520030100000.