JNJ-7706621 Treatment during the Post-activation Period Enhances the Developmental Competence of Mouse Somatic Cell Nuclear Transfer Embryos

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Somatic cell nuclear transfer has undergone extensive investigation for animal cloning, but embryos produced through this method display reduced developmental potential compared to naturally reproduced counterparts. During the post-activation period, cytochalasin B is conventionally used in somatic cell nuclear transfer to maintain the successful activation and formation of diploid nuclei in somatic cell nuclear transfer embryos. Despite its benefits, cytochalasin B has associated toxic effects, prompting exploration into alternative compounds. This study aimed to enhance animal cloning efficiency by optimizing post-activation through the substitution of cytochalasin B with JNJ-7706621, a specific inhibitor of cyclin-dependent kinase 1. Parthenogenetic embryos were cultured with cytochalasin B (5 μg/mL) and various concentrations of JNJ-7706621 (1, 10, and 50 μM). The 10 μM JNJ-7706621 and cytochalasin B groups exhibited significantly higher rates of parthenogenetic embryo development than the 1 and 50 μM JNJ-7706621 groups. The 10 μM JNJ-7706621 treatment increased trophectoderm and total cell numbers while decreasing apoptotic cells in parthenogenetic blastocysts compared with the cytochalasin B group. Similarly, in somatic cell nuclear transfer embryonic development, the 10 μM JNJ-7706621 treatment improved blastocyst formation, trophectoderm, inner cell mass, and total cell numbers while decreasing apoptotic cells in blastocysts compared with cytochalasin B treatment. Moreover, the 10 μM JNJ-7706621 treatment resulted in a significantly higher intensity of F-actin in somatic cell nuclear transfer embryos compared to cytochalasin B treatment. It

also reduced the incidence of abnormally distributed cortical actin, uneven division, and blastomere fragmentation compared to cytochalasin B treatment. Furthermore, the 10 μM JNJ-7706621 treatment led to a significantly higher intensity of α-tubulin than cytochalasin B treatment at the one-cell and two-cell stages of somatic cell nuclear transfer embryos. The 10 μM JNJ-7706621 treatment reduced the incidence of chromosome misalignment and abnormal spindle organization during metaphase in somatic cell nuclear transfer embryos compared to cytochalasin B treatment. Additionally, it was confirmed that JNJ-7706621 treatment significantly increased efficiency in cloned mouse production compared to cytochalasin B. Moreover, JNJ-7706621 treatment significantly decreased placenta-fetal weight ratio in cloned mice compared to cytochalasin B. These results suggest that post-activation treatment with JNJ-7706621 enhances somatic cell nuclear transfer embryo full-term development, highlighting potential progress in cloning technology through improved distribution and organization of the cytoskeleton. This research was supported by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program (KGM4252432, KGM1012412), Republic of Korea.