RHD GENE KNOCKOUT IN RHD-POSITIVE HUMAN EMBRYOS BY USING THE CRISPR-CAS9 SYSTEM

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RhD-induced hemolytic disease of the fetus and newborn is a severe condition that can lead to fetal death. Thus, RhD-negative women pregnant with an RhD-positive fetus are at high risk of pregnancy loss.To check the feasibility of RhD conversion in human embryos, this study has been conducted under approval of the IRB. 13 oocytes from a 27 years old RhD-negative donor were fertilized by intracytoplasmic sperm injection with sperm of a homozygous RHD-positive donor. 8 oocytes were injected with sperm simultaneously with the CRISPR-Cas9 vector targeting exon 5 of the RHD gene, whereas the rest 5 oocytes were injected with sperm only (control group) resulting in 9 zygotes (8 from the CRISPR-Cas9 group and only 1 from the control group). All 9 zygotes underwent cleavage, but only 5 reached the blastocyst stage (4 from the CRISPR-Cas9 group and 1 from the control group), however, only 2 blastocysts from the CRISPR-Cas9 group were of high morphology grades.Sanger sequencing of exon 5 of the RHD gene confirmed absence of changes in the control embryo and revealed sequence changes within -1…-4 base pairs from the PAM-site in all 8 CRISPR-Cas9-treated embryos: 7 of them had an indel leading to a frameshift that likely could have resulted in RHD gene knockout, and 1 embryo had an in-frame deletion associated with loss of 2 amino acids in a functionally significant domain. Herein we report the first successful attempt to knockout the RHD gene in human embryos by using the CRISPR-Cas9 vector. Further research is needed to: a) consider the potential off-target activity of the CRISPR-Cas9 system; b) identify actual RhD protein expression levels in cultured embryonic stem cells; c) check the feasibility of inducing a predetermined nonsense mutation in the RHD gene by adding an HDR template to the CRISPR-Cas9 system.