Animal Reproduction

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CLONING, TRANSGENESIS, AND STEM CELLS

Homology-directed gene editing using electroporation of bovine zygotes

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Gene editing can occur via two pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). While NHEJ is rapid but prone to errors, HDR employs a homologous DNA template for precise outcomes, albeit being a rare event. Electroporation (EP) offers a straightforward method for delivering gene editing components into the cytoplasm of bovine embryos; although, its efficacy in facilitating knock-ins by HDR remains uncertain. Here we used EP to introduce a stop codon in the prolactin receptor gene, testing two HDR enhancers: SCR7, a NHEJ inhibitor, and RS1, a HDR stimulant. SgRNA, Cas9 and single strand oligodeoxynucleotide (ssODN) containing a stop codon were delivered by EP (Nepa21 electroporator, Nepagene, Chiba, Japan) to *in vitro*-fertilized oocytes, which were randomly allocated to the following groups: A) Control: no EP (n = 77); B) ControlHDR: EP of sgRNA, Cas9, and ssODN performed at 8h post-fertilization (n = 74); C) RS1: as in B followed by 24h incubation with RS1 at 7.5 µM (n = 70); D) SCR7: as in B followed by 24h incubation with SCR7 at 1 µM (n = 74); E) RS1+SCR7: as in B followed by 24h incubation with RS1 at 7.5 µM and SCR7 at 1 µM (n = 72). Embryos were cultured for seven days in three replicates. Single blastocysts underwent DNA extraction. PCR-generated DNA fragments from the target site were sequenced and analyzed by SeqScreener Gene Edit Confirmation (Thermo Fisher, Waltham, USA). Presence of indels (insertions/deletions) and HDR (stop codon insertion) was considered relevant when their rates per embryo exceeded 5%. Data were compared by Fisher's exact test (proportion of embryos with indels or HDR) or ANOVA and shown as mean ± SEM when appropriated. Significance was set at $P < 0.05$. No difference ($P = 0.95$) in blastocyst rates among Control (25.7 ± 9.5%), ControlHDR (19.8 ± 5.6%), RS1 (20.1 ± 2.7%), SCR7 (19.7 ± 7%) and RS1+SCR7 (25.1 ± 8.3%) groups was found. The proportion of embryos with indels (91.6% [11/12], 62.5% [5/8], 85,7% [6/7] and 87,5% [7/8] for ControlHDR, RS1, SCR7 and RS1+SCR7, respectively; $P = 0.25$) and the indel mean rate per embryo (71 ± 8.3%, 51.2 ± 11.5%, 42.2 ± 8.4% and 65.3 ± 14.4% for ControlHDR, RS1, SCR7 and RS1+SCR7, respectively; $P = 0.24$) were similar. Likewise, no difference ($P = 0.12$) in the proportion of embryos with HDR among ControlHDR (41.6% [5/12]), RS1 (12.5% [1/8]), SCR7 (57.1% [4/7]) and RS1+SCR7 (25% [2/8]) groups was found. HDR mean rates per embryo ranged from 5.4% to 70.1%, with no significant difference ($P = 0.21$) among ControlHDR (31.8 ± 10.6%), SCR7 (9.2 ± 2.4%), and RS1+SCR7 (18.5 ± 8.9%) groups. The RS1 group was excluded because only one embryo had HDR. No indels or HDR were found in the Control group. Despite the limited number of embryos, this study demonstrates EP's ability to deliver gene editing components for HDR in bovine embryos. However, the tested molecules did not enhance HDR efficiency post-EP. Therefore, EP offers a potential method for knock- in insertion in bovine embryo genome, yet further research is needed to enhance HDR rates.