Generation of Porcine Fetuses with Monoallelic *HNF1A* Mutation by CRISPR/Cas9 Electroporation in Oocytes

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Genetically modified pigs are a promising model for advancing biomedical applications and for studying human diseases. Frequently, haploinsufficiency in hepatocyte nuclear factor-1 alpha (HNF1A) is widely recognized as a significant contributor to the human monogenic diabetes form, maturity onset diabetes of the young (HNF1A-MODY). Creating HNF1A heterozygous knockout pig models would serve as a valuable tool for studying human HNF1A-MODY. Although CRISPR/Cas9 microinjection into porcine zygotes has proven successful in generation of gene knockout (KO) piglets, the process requires individual manipulation of each embryo. Hence, the microinjection process is quite time consuming, resulting in low throughput. Delivery of CRISPR/Cas9 by electroporation has been developed to circumvent the drawbacks of microinjection. Past experiments with electroporation of porcine embryos [30V, 1ms pulse, 100ms pulse interval, and 5 pulses (bipolar)] were successful for efficiently generating biallelic edits with CRISPR/Cas9 introduction into zygotes. However, creation of monoallelic edits requires adjustments of the procedure. To generate potential monoallelic mutation on the *HNF1A* gene, matured porcine oocytes were electroporated with CRISPR/Cas9 (1:2 ratio) using 2 pulses [25V, 1ms pulse, 100ms pulse interval] immediately before insemination. Cleaved embryos were transferred to a recipient approximately 44 hours later. A total of 7 conceptuses were recovered on day 19. DNAs from these conceptuses were isolated and sequenced, and the sequence results were analyzed by ICE and TIDE analysis tools. Three of the seven conceptuses had monoallelic edits, retaining wild type allele (proportions ranging from 44% to 60%).