CRISPR/Cas9 genome editing and its application for study of fertilization

Masahito Ikawa (ikawa@biken.osaka-u.ac.jp)

Research Institute for Microbial Diseases, Osaka University, Osaka, 565-0871, JAPAN
The Institute of Medical Science, The University of Tokyo, Tokyo, 108-0071, JAPAN

CRISPR/Cas9 system has opened the new era for reverse genetics. In 2013, we developed the efficient gene knockout system in mice by injecting the plasmids expressing humanized Cas9 (hCas9) and single guide RNA (sgRNA) into zygotes (1). Now it is replaced by electroporation of oocytes with CAS9/crRNA/tracrRNA ribonucleoprotein complex for simple genome editing (knockout, point mutation, tag insertion, etc). Altogether, to date, we have knocked out 272 testis abundant genes and analyzed the phenotypes in vivo. Whereas 168 of the KO mouse lines were fertile and did not show any drastic phenotypes, 9 KO mouse lines showed lethality. The remaining 103 KO mouse lines showed infertility or subfertility and propelled our research. I would like to introduce our record of CRISPR/Cas9 mediated genome editing in mice and recent findings in mammalian fertilization.

1. Mashiko D. et al., Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. ***Sci Rep.*** 2013 Nov 27;3:3355.
2. Miyata H et al., Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. ***PNAS.*** 2016 Jul 12;113(28):7704-10.
3. Satouh Y and Ikawa M, New Insights into the Molecular Events of Mammalian Fertilization. ***Trends Biochem Sci.*** 2018 Oct;43(10):818-828.