Center for Experimental Medicine and Systems Biology

Laboratory of Reproductive Systems Biology
生殖システム研究分野

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In the "post-genome project era," genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Our laboratory studies the mechanisms underlying the mammalian reproductive system in gene-manipulated mice. We are the first group in the world to generate transgenic mice expressing GFP throughout the body (Green mice). We also established the ES cells that give green fluorescent spermatozoa to trace their movement and acrosome reaction during fertilization. Another tool invented in our laboratory is the placenta-specific gene manipulation system using lentiviral (LV) vectors. Using these techniques, we are trying to elucidate the mechanism underlying gametogenesis, fertilization, implantation, and placentation. Our recent interest is using the CRISPR/Cas9 system as a genome-editing tool. The combination of GWAS studies with genome editing will pave the way to understand and control human fertility problems.

1. Sperm and offspring production in a nonobstructive azoospermia mouse model via testicular mRNA delivery using lipid nanoparticles

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Microsurgical testicular sperm extraction (micro-TESE) with intracytoplasmic sperm injection (ICSI) represents the current standard treatment for nonobstructive azoospermia (NOA). However, cures remain unavailable for NOA patients lacking retrievable haploid cells. mRNA supplementation could be a potential treatment for genetic defects leading to impaired spermatogenesis. Lipid nanoparticles (LNPs) have emerged as mRNA delivery vehicles with minimal risk of genome integration; however, their ability to selectively deliver mRNA to specific cell types remains limited. To overcome this, microRNA (miRNA) target sequences were incorporated into mRNA constructs to restrict expression specifically to germ cells. Using pyruvate dehydrogenase E1 subunit alpha 2 (PDHA2) knockout mice as an NOA model with meiotic arrest, we demonstrate that LNP-mediated delivery of *Pdha2* mRNA enables the resumption and completion of meiosis, restores sperm production, and facilitates the generation of healthy fertile offspring via ICSI. Whole-genome sequencing of the offspring confirmed the absence of large-scale genomic abnormalities. Our results provide proof of concept for a safe and effective chemically synthesized LNP-based

mRNA therapy with miRNA-regulated germ cell specificity, offering a promising therapeutic approach to treating male infertility caused by spermatogenesis arrest.

2. Formation of a complex between TMEM217 and the sodium-proton exchanger SLC9C1 is crucial for mouse sperm motility and male fertility

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Sperm motility is essential for male fertility and is tightly controlled by signaling events in the flagellum. *Slc9c1* encodes a sperm-specific Na+/H+ exchanger (sNHE/SLC9C1) that localizes to the flagellum and is indispensable for sperm motility and male fertility. SLC9C1 is unique among Na+/H+ exchangers in that it possesses a voltage-sensing domain (VSD), the physiological function of which remains poorly understood in mammals. Here, by analyzing coevolving genes with *Slc9c1*, we identified *Tmem217*, which encodes a transmembrane protein that is localized in the sperm flagellum. Knockout (KO) of *Tmem217* in mice resulted in sperm motility defects and male infertility, phenocopying *Slc9c1* KO mice. Coimmunoprecipitation and structural prediction analyses indicated that TMEM217 binds to SLC9C1 via its VSD. Further analyses indicated that the amounts of SLC9C1 and its associated protein, soluble adenylyl cyclase (sAC), were lost in mature *Tmem217* KO spermatozoa, leading to disrupted 3′,5′-cyclic monophosphate (cAMP) signaling pathways. Remarkably, cAMP analogs restored the impaired motility and fertilizing ability of *Tmem217* KO spermatozoa in vitro, validating the essential role of TMEM217 in regulating cAMP production. Our findings indicate that the association of TMEM217 with SLC9C1 via its VSD is critical for the proper organization and function of the SLC9C1-sAC-cAMP axis in mature spermatozoa.

Publication

1. Wang H, Pham AH, Mengjiao Luo, Ikawa M, Miyata H. Microtubule Inner Protein CFAP77 Contributes to Sperm Motility and Male Fertility in Mice. ***Androlgy*** 2025 Nov 29;41316927 doi: 10.1111/andr.70152.
2. Mashiko D, Tonai S, Miyata H, Matzuk MM, Ikawa M. SLC35G3 is a UDP-N-acetylglucosamine transporter for sperm glycoprotein formation and underpins male fertility in mice. ***Elife***. 2025 Nov;14:RP107494. doi: 10.7554/eLife.107494.
3. Qiu Y, Shimada K, Ikawa M. CRISPR/Cas9-mediated Genome-editing Reveals 10 Testis-enriched Genes and One Non-testis-enriched Gene are Dispensable for Male Fecundity in Mice. ***Andrology***. 2025 Nov 10;doi: 10.1111/andr.70144.
4. Noda T, Uriu R, Mashiko D , Shinohara H, Qu Y, Taira A, Matzuk RM, Tahala D, Nakano M, Araki K, Yu Z, Zhang Y, Matzuk MM, M Ikawa. GALNTL5 binds GalNAc and is required for migration through the uterotubal junction and sperm-zona pellucida binding. ***Nat Commun***. 2025 Sep 17;16(1):8264. doi: 10.1038/s41467-025-63805-4.
5. Wang H, Shimada K, Pham AH, Oyama Y, Kamoshita M, Kobayashi H, Oura S, Yabuta N, Ikawa M, Miyata H,.Proximity labeling of axonemal protein CFAP91 identifies EFCAB5 that regulates sperm motility. ***Nat Commun***. 2025 Sep 10; 16(1): 8238. doi: 10.1038/s41467-025-63705-7.
6. Shimada K, Chang HY, Wang H, Ikawa M. Compensation for X-linked Pdha1 silencing by Pdha2 is essential for meiotic double-strand break repair in spermatogenesis. ***Development***. 2025 Jul 18; dev.204683. doi: 10.1242/dev.204683
7. Hiraoka T, Aikawa S, Mashiko D, Nakagawa T, Shirai H, Hirota Y, Kimura H & Ikawa. An ex vivo uterine system captures implantation, embryogenesis, and trophoblast invasion via maternal-embryonic signaling. ***Nat Commun***. 2025 Jul 1; 16(1):5755. doi: 10.1038/s41467-025-60610-x
8. Ogawa Y, Lu Y, Kiyozumi D, Chang HY, Ikawa M. CRISPR/Cas9-mediated genome editing reveals seven testis-enriched transmembrane glycoproteins dispensable for male fertility in mice. ***Andrology***. 2025 Jul;13(5):1251-1260. doi: 10.1111/andr.13564
9. Chang H, Lu Y, Yamamoto K, Sun J, Shimada K, Hiradate Y, Fujihara Y, Ikawa M Mouse genome engineering uncovers 18 genes dispensable for male reproduction. ***Andrology***. 2025 Jun 26; doi: 10.1111/andr.70088.
10. Katsuma K, Shimada K, Tonai S, Mashiko D, Iida-Norita R, Kaneda Y, Miyata H, Ikawa M. The absence of both RIBC1 and RIBC2 induces decreased sperm motility and litter size in male mice. ***Andrology***. 2025 Apr 23; doi: 10.1111/andr.70045.

11. Kaneda Y, Lu Y, Sun J, Shimada K, Emori C, Noda T, Koyano T, Matsuyama M, Miyata H, Ikawa M. TEX38 localizes ZDHHC19 to the plasma membrane and regulates sperm head morphogenesis in mice. ***Proc Natl Acad Sci U S A***. 2025 Mar11; 122(10):e2425952122. doi: 10.1073/pnas.2425952122

12. Qiu Y, Shimada K, Yamamoto K, Ikawa M. Loss of CCDC188 causes male infertility with defects in the sperm head-neck connection in mice. ***Biol Reprod***. 2025 Jan 14; ;112(1):169-178. doi: 10.1093/biolre/ioae137.