【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project : Development of male infertile model mice by genome editing and comprehensive study of fertilization

IKAWA Masahito

(Osaka University, Research Institute for Microbial Diseases, Professor)

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Keyword : CRISPR/Cas9, Experimental Animals, Infertility, Fertilization, Contraceptive

【Purpose and Background of the Research】

The life of mammals, including humans, is passed on continuously through fertilization via sperm and eggs. Until now, biochemical approaches have been used to analyze the formation and function of sperm, which cannot be generated in culture. However, recent analyses using gene knockout (KO) mice have shown that factors previously thought to be essential for fertilization (Acrosin, GalTase, Fertilin, etc.) are not essential while factors thought to be unrelated, such as Calmegin and ACE, have emerged. In this study, we will develop genome-editing tools that facilitates in vivo studies in mice. With these, we will knock out testis-specific genes to investigate the biology of sperm and fertilization in mammals. Further, we will generate male infertility model mice.

【Research Methods】

The main purpose of this study is to generate KO mice for about 400 genes that have not yet been KO'd out of about 1,000 genes that are expressed specifically in the testis, and to clarify their physiological functions in male reproduction. For this purpose, we will design several guide RNAs (gRNAs) for each target gene and introduce them into fertilized eggs together with CAS9 protein to produce KO mice with the protein-coding region largely removed. Fertility of the resulting KO male mice will be determined by mating tests, and the genes that cause male infertility without the ability to produce pups will be selected (Figure 1). Based on our experience, about 30% of the genes are expected to be indispensable for fertility. For infertile male mice, we will investigate spermatogenesis in the testes, sperm maturation in the epididymis, and fertilization ability of mature sperm based on histological anatomy and cell biological analysis to determine the cause of infertility.

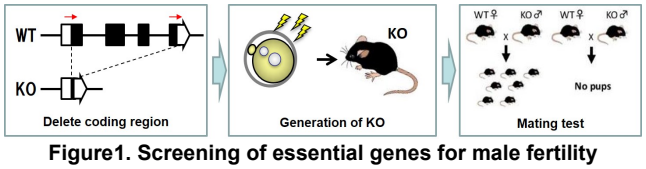

Figure1. Screening of essential genes for male fertility

In particular, in cases where fertilization fails despite apparently normal sperm, we will investigate the cause of the fertilization failure at each step of the fertilization process (Figure 2), using electron microscopy, CASA (Computer Assisted Sperm Analysis), in vitro fertilization, ICSI, and embryo transfer. For proteins essential for fertility, we aim to identify their localization and interacting factors in tissues and cells by producing specific antibodies or labeling with peptide-Tags, and to clarify their physiological functions at the molecular level. In addition, we will replace mouse endogenous genes with human ones (wild type and diseased type), and use them as a model for developing treatments for male infertility.

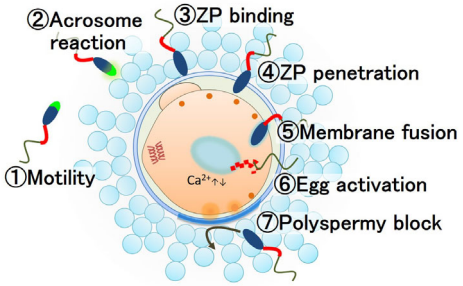

Figure2. Steps of mammalian fertilization

Of note, to achieve the above studies, we will combine CRISPR/Cas9 genome editing with viral vectors, ES cell chimera analysis, and reproductive engineering to constantly update and improve the efficiency of genome editing mouse production and analysis.

【Expected Research Achievements and Scientific Significance】

In most countries, infertility affects about one in six couples with a half attributed to problem on the male side. However, the genetic factors of infertility are not well understood. The mice obtained in this project will serve as a model for human infertility and lead to the development of diagnostic and therapeutic methods. On the other hand, about 40% of all pregnancies are unintended. Until now, the only oral contraceptive available has been the female pill. The gene inhibitors discovered in this project will open up the possibility of male contraceptives.

【Publications Relevant to the Project】

- Miyata H, et al., Sperm calcineurin inhibition prevents mouse fertility with implications for male contraceptive. ***Science*** 350: 442-445 (2015)
- Miyata H, et al., Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. ***PNAS*** 113: 7704-7710 (2016)
- Kiyozumi D, et al., NELL2-mediated lumicrine signaling through OVCH2 is required for male fertility. ***Science*** 368:1132-1135. (2020)

【Homepage Address and Other Contact Information】

https://egr.biken.osaka-u.ac.jp/