CORRECTING A MUTATION IN THE GERM LINE BY CRISPR/CAS9 TO OVERCOME TRANSMISSION OF HEREDITARY INFERTILITY IN MICE

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Aims: Failed Fertilization after ICSI can be caused by mutations in the sperm-related oocyte factor phospholipase C (PLCζ) gene. This can be overcome by artificial oocyte activation (AOA). We aimed to overcome mutation transmission by gene correction through CRISPR/Cas9 delivery during ICSI in a knock-out mouse model. Methods: We employed a previously described PLCζ knock-out mouse model, harboring a homozygous 22 base-pair mutation (Plcz1Δ22bp), causing failed fertilization after ICSI. Two different gRNA molecules were designed to specifically target the mutant allele. Together with the knock-out sperm, the gRNA/Cas9 complex and a repair template, harboring the wild-type Plcz1 sequence and an additional synonymous mutation to track the use of the template, were injected. AOA was performed following ICSI to obtain normal fertilization rates. Results: The different alleles obtained after editing were categorized as follows: unedited Plcz1Δ22bp allele, Plcz1Δ22bp allele harboring additional indel mutations, Plcz1Δ22bp allele corrected with a repair template, and Plcz1Δ22bp allele altered/corrected by alternative mechanisms. An efficient correction rate of 45% of the embryos was observed. Our sequencing data suggest that 25% did use the template during repair attempts and 35% did not. In 40% the template was occasionally used. Mosaicism (=multiple alleles per embryo) was observed in 45% of the embryos. For all parameters, no significant ($p>0.05$) difference was seen between the two gRNA designs and CRISPR/Cas9 editing did not significantly ($p>0.05$) affect the activation and embryonic developmental rates. Embryos that were capable to develop into blastocysts had a normal karyotype. Conclusion: CRISPR/Cas9 can be efficiently used for the correction of a 22 base-pair deletion.