Germline Topic: Resistance to editing human embryos via CRISPR
Rep. E AlQaffas

Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated endonucleases (e.g. Cas9) can be used as a nicking enzyme that precedes homology-directed repair (HDR, the insertion of an external segment where a specific "cut" is made in a target gene) in human cells (1). CRSPIR and HDR has been used to modify human zygotes tripronuclear (3NP) in which the modification yielded low efficiency of gene editing coupled off-target mutations (2). Using a different technique, however, called non-homologous end-joining (NHEJ) the efficiency of the modification was high in 3NP cells (3). Higher HDR repair rate (33%), however, was shown when the editing occurred at the M cell replication phase (4). In addition, targeting certain genes mutation, G6PD and β-globin, HDR improved up to 100% and 50% for the two respectively when editing human dual pronuclear zygotes using CRISPR-Cas9 system (5). One of the possible gene-resistance to editing is the presence of DNA damage repair mechanism such as Fanconi anemia M gene (6) or that the preferable template for DNA repair comes from the Oocyte (7).

Based on the findings above, I recommend that gene editing via CRSPIR-Cas9 of zygotes should be considered after better understanding of what the different mechanisms are and how to escape them when providing a template for HDR. Until then, I can suggest that this method should only be used with human zygotes when there is absolute need for it.

Citations:

1- Cong, L., Ran, F., Cox, D., Lin, S., Barretto, R., Habib, N., . . . Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science (New York, N.Y.), 339(6121), 819-23.
2- Liang, Puping, Xu, Yanwen, Zhang, Xiya, Ding, Chenhui, Huang, Rui, Zhang, Zhen, . . . Huang, Junjiu. (2015). CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein & Cell, 6(5), 363-372.
3- Kang, X., He, W., Huang, Y., Yu, Q., Chen, Y., Gao, X., . . . Fan, Y. (2016). Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. Journal of Assisted Reproduction and Genetics, 33(5), 581-588.
4- Lin, S., Staahl, B., Alla, R., & Doudna, J. (2014). Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. ELife, 3(2), E04766.
5- Tang, L., Zeng, Y., Du, H., Gong, M., Peng, J., Zhang, B., . . . Liu, J. (2017). CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. Molecular Genetics and Genomics, 292(3), 525-533.
6- Luo, Yunhai, Maizels, Nancy, Hartford, Suzanne A., Zeng, Ruizhu, Southard, Teresa L., Shima, Naoko, & Schimenti, John C. (2014). Hypersensitivity of Primordial Germ Cells to Compromised Replication-Associated DNA Repair Involves ATM-p53-p21 Signaling. PLoS Genetics, 10(7), E1004471.

How did I found this subtopic:

1- After watching: https://www.youtube.com/watch?v=BCO-U1glK14/
2- The presenter mentioned that the cells didn't use the provided sequence alongside CRISPR. Rather, it fixed the double break from the other haploid. If true this suggests that there's a mechanism gamete, during this phase, can fix itself by resisting germline editing.
3- So, I went the cited article:
   Winblad, N., & Lanner, F. (2017). Biotechnology: At the heart of gene edits in human embryos. Nature., 548(7668), 398-400.
4- After reading parts of the article I decided to pick Human gamete resistance to genome editing via CRISPR. I follow this subtopic looking at the following articles (they are be mentioned in "Citations" part of this report.