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Gene Editing affects Embryo Quality in Rhesus Macaques

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Because of their close evolutionary relationship to humans, rhesus macaques (*Macaca mulatta*) are an important animal system to create nonhuman models of disease allowing for studying various diseases and developing treatments. Advances in gene editing and Assisted Reproductive Technologies have made it possible to routinely generate embryos with specific mutations in nonhuman primates. Edited blastocysts can be used for embryo transfers with the goal to create infants with specific mutations of interest.

We are invested in learning how gene editing alters embryo development, and we hypothesize that it may have adverse effects on embryo quality. To assess embryo quality, we analyzed 367 blastocysts that were generated in the ART Core at the ONPRC from 2021-2024 including projects that involve gene editing using CRISPR or piggyBac technology (n=219) and non-editing projects that commonly involve IVF between heterozygous carriers of specific gene mutations (n=148). Blastocyst quality was evaluated considering their individual age and morphology. In rhesus, blastocyst development usually occurs from day six to ten, with the older embryos being of lower quality. To query whether gene editing influences the day of blastocyst development, we performed a t-test which revealed a significant difference between the mean days (age) of edited and unedited blastocysts (7.959±0.06 days vs 7.655±0.06 days, $p < 0.001$). By applying the guideline for embryo grading from the Society for Assisted Reproductive Technology, we defined blastocysts into good, fair and poor based on their morphology of inner cell mass and trophectoderm. A chi-square test of independence revealed that the relation between embryo projects (edited/unedited) and embryo grade (good, fair, poor) was significant, X^2 (6, n=384) = 402.172, $p < .001$). Unedited embryos are more likely to be of good quality, while edited embryos are more likely to be of fair or poor quality. Furthermore, to assess if there is a similar potential for blastocysts from edited and non-edited projects to lead to implantation and pregnancy following an embryo transfer, we analyzed 72 embryo transfers and found no statistically significant difference performing a t-test (edited: n=51, 12 pregnancies, unedited: n=21, 6 pregnancies, $p = 0.6588$.

Our analysis provides strong evidence that gene editing affects embryo development and blastocyst morphology. We observed a deceleration in their general development rate as well as an adverse effect on their morphology. Gene editing technology is known to have the potential to cause cytotoxicity and also double stranded DNA breaks, which could be the reason. By providing more detailed insight into the quality of blastocysts and working cooperatively, gene editing technology can focus on improving those adverse effects.