**World of Reproductive Biology**

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**Precise Gene Engineering Achieved in Monkeys**

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Researchers have created monkeys with a precise gene modification, excising two specific genes simultaneously. The technique used to make the modifications, the CRISPR/Cas9 system, could lead to the generation of monkey models of various human diseases.

Until now, gene modification in primates has been unpredictable, relying on the introduction of new genes via engineered viruses. These methods have resulted in genetically engineered monkeys, but researchers have not been able to control the site of gene introduction or the number of copies of the gene. Techniques commonly used in mice rely on recombination events to incorporate introduced genes into embryonic stem cells. This is not feasible for use in primates due, in part, to the animals’ long generation times, which make screening for offspring with incorporated genes impractical.

Jiahao Sha et al. [1] bypassed these limitations by using the CRISPR/Cas9 system, a new technique that has been successfully deployed to genetically engineer several species, including mice and rats (see [2]). The system targets specific DNA sequences in the genome using Cas9 proteins in combination with what are known as single-guide RNAs. The Cas9 proteins generate single-stranded DNA breaks at the location specified by the RNAs.

The researchers injected Cas9 mRNA and single-guide RNAs designed to target three specific genes into one-cell stage embryos of cynomolgus monkeys (*Macaca fascicularis*). They analyzed the genome of 15 of the resulting embryos, and showed that eight of them had evidence of knockout in two genes simultaneously. The researchers also implanted embryos into female monkeys and examined the first infants born: a pair of twins (the rest of the females are still gestating). They found that two genes, PPARG and RAG1, were deleted in both twins.

The researchers further showed that the CRISPR/Cas9 system did not cause gene loss at sites other than the intended gene target. Such off-target effects have been observed with the system in other animals, and are one potential barrier to using CRISPR/Cas9 in humans.

**References**


**Progesterone Promotes Vascular Leakage Prior to Implantation**

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Implantation of the mammalian embryo into the uterus is a major regulator of vascular permeability in a variety of tissues. But it does not seem to be a regulator of this form of vascular leakage early in the implantation stages. Instead, the researchers pinpointed a role for Nur77/TR3, which has recently been shown to destabilize the junctions between endothelial cells, causing leakage. The researchers showed that Nur77/TR3 regulates expression of proteins at the cell junctions in blood vessels of the endometrium, including PECAM-1, VE-cadherin, and claudin-5. VEGF activity kicks in during later events of implantation, where it is known to be involved in functions such as vascular remodeling during embryo attachment.

Progesterone is known to regulate many of the genes necessary for uterine receptivity and implantation. The new findings add another trick to its repertoire, showing that it induces a transcriptional program via Nur77/TR3 that affects the expression of adhesion proteins at cell junctions in the blood vessels. The result is increased vascular permeability. The findings may explain why users of long-term, progestin-only contraceptives can experience abnormal endometrial bleeding.

**X-Inactivation Is Essential for Differentiation in the Embryo**

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The cells of female embryos wait to differentiate until one of their X chromosomes is inactivated, according to new findings in mouse embryos and embryonic stem cells. X-chromosome inactivation allows the cells to exit the pluripotent state and continue development. X-chromosome inactivation is known to occur through up-regulation of the noncoding RNA Xist on one X chromosome, which is then silenced. Previous studies have also uncovered a role for pluripotency transcription factors, such as Oct4 and Nanog, which are