Mosaicism diminishes the value of pre-implantation embryo biopsies for detecting CRISPR/Cas9 induced mutations in sheep.

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Authors: Marcela Vilarino, Fabian Patrik Suchy, Sheikh Tamir Rashid, Helen Lindsay, Juan Reyes, Bret Roberts McNabb, Talitha van der Meulen, Mark O Huising, Hiromitsu Nakauchi, Pablo Juan Ross

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Public Summary:
Generation of a human pancreas in sheep for the purpose of organ transplantation would have important implications for the current shortage in donor organs. For a human pancreas to develop in the host sheep, the development of the host's pancreas must be blocked. Although genetic mutation of PDX1 can disrupt pancreatic development, mutating this gene in sheep embryos has previously been inefficient. Here, we tested embryonic biopsy methods for gene mutation analysis, and found that genetic mosaicism can be a major problem to genome editing sheep embryos.

Scientific Abstract:
The production of knock-out (KO) livestock models is both expensive and time consuming due to their long gestational interval and low number of offspring. One alternative to increase efficiency is performing a genetic screening to select pre-implantation embryos that have incorporated the desired mutation. Here we report the use of sheep embryo biopsies for detecting CRISPR/Cas9-induced mutations targeting the gene PDX1 prior to embryo transfer. PDX1 is a critical gene for pancreas development and the target gene required for the creation of pancreatogenesis-disabled sheep. We evaluated the viability of biopsied embryos in vitro and in vivo, and we determined the mutation efficiency using PCR combined with gel electrophoresis and digital droplet PCR (ddPCR). Next, we determined the presence of mosaicism in ~ 50% of the recovered fetuses employing a clonal sequencing methodology. While the use of biopsies did not compromise embryo viability, the presence of mosaicism diminished the diagnostic value of the technique. If mosaicism could be overcome, pre-implantation embryo biopsies for mutation screening represents a powerful approach that will streamline the creation of KO animals.