MEET COSMO

The bull calf designed to produce 75% male offspring

Scientists use CRISPR Technology to Insert Sex-Determining Gene

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Genome editing offers an opportunity to introduce useful genetic traits into livestock breeding programs. However, it has proven difficult to insert large DNA fragments into the genomes of livestock embryos using the CRISPR-Cas9 system.

Alison Van Eenennaam, Ph.D., and her doctorate student Joseph Owen from the Department of Animal Science at the University of California, Davis, employed their knowledge of bovine embryogenesis and DNA repair pathways to create new options to improve livestock genetic trails. With the help of colleagues from the School of Veterinary Medicine, they generated a CRISPR calf named Cosmo who carries extra copies of the SRY gene, the sex-determining gene.

In mammals, sex determination is typically dependent on the inheritance of the sex chromosomes, X and Y. Individuals with two X chromosomes are genetically female and individuals with one X chromosome and one Y chromosome are genetically male. Dairy farmers often use "X-sorted" semen in artificial insemination as it contains only sperm carrying an X chromosome and will result in all female calves.

Joey Owen, on the left, and Alison Van Eenennaam on the right, with newborn Cosmo and veterinarians Bret McNabb, DVM, and Tara Urbano, DVM, who delivered the calf.

It is actually only a single gene on the Y chromosome that determines whether an embryo develops as a male or female. This gene is known as the sex-determining region of the Y chromosome or "SRY" for short. SRY expresses a protein in early embryogenesis that initiates male sexual differentiation by triggering a cascade of factors necessary for male gonadal development and shutting down formation of the female gonad.

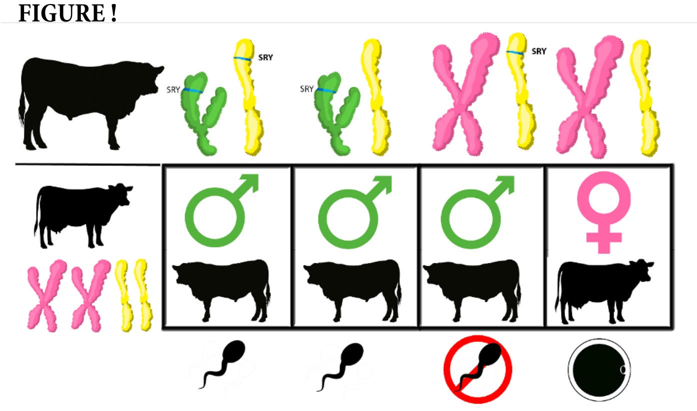
In the case of Cosmo, the SRY gene sequence was copied from the Y chromosome and pasted into Chromosome 17 using the CRISPR-Cas9 genome editing system.

Cosmo is expected to produce 75 percent male offspring: 50 percent of which will be XY males; 25 percent of which will be XX females; and 25 percent of which are expected to be XX individuals that appear male due to the inheritance of the chromosome 17 carrying the SRY gene. These XX males are not expected to produce viable sperm as that requires the expression products of additional genes located on the Y chromosome.

One of the aims of this research was to edit one-cell bovine embryos, known as zygotes, immediately after fertilization to avoid a condition called genetic mosaicism. Mosaicism occurs when two or more groups of cells with different genotypes are present within an individual that has developed from a single fertilized egg. This usually happens when editing takes place during or after the embryo begins replicating its own DNA just before the first cell division. To help avoid mosaicism in this research, the editing regents were introduced into bovine embryos by microinjection just six hours after insemination and before the beginning of DNA synthesis.

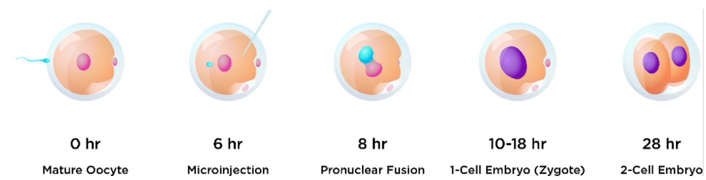
One of the challenges with this timing for researchers hoping to insert a gene into the embryo's genome is

FIGURE !



Cosmo will produce sperm carrying either an X (pink) or a Y (green) sex chromosome, and one copy of Chromosome 17. All Y-bearing sperm will produce a male calf, whereas only half of the X-bearing sperm will produce a female. The other half carrying the SRY gene on Chromosome 17 (yellow) are expected to produce a male-appearing XX individual. However, this animal would not be expected to be produce fertile sperm.

FIGURE 2



The timing of the microinjection of the editing regents is key: lower rates of mosaicism were observed when injecting into embryos 6 hours after fertilization, prior to DNA replication and the first cell division.

evading the cell's predominant DNA repair pathway. In the early stages of embryo development, it is difficult the get the repair pathway to insert large DNA fragments into the genome. To overcome this, the researchers employed a strategy that had proven to be effective in early stage one-cell mouse embryos, with an improved gene insertion efficiency. However, insertion using this method is still not 100 percent efficient. In initial experiments, only around 40 percent of the bovine embryos were found to have the SRY insertion.

Bovine embryo transfers, and the subsequent nine-month pregnancy, are expensive. This meant the team needed to confirm whether the gene insertion had been successful before the embryo was transferred to a surrogate cow. The team opted to use a non-invasive screening approach to determine which of embryos carried the SRY insertion. To do this, the green fluorescent protein (GFP) was inserted alongside SRY. The fluorescent protein meant that any embryo with the SRY insert glowed green when exposed to light in the blue to ultraviolet range.

It took two and a half years to perfect the method to insert a gene into the developing embryo, and anothetr two years to successfully establish a pregnancy. In June 2019, nine seven-day embryos that fluoresced green under UV light were transferred to surrogate cows. A month later one of those cows was confirmed pregnant, and an ultrasound scan revealed she was carrying a male bull calf. And finally in April of 2020, Cosmo, a healthy 110-pound bull calf was born.

The team were able to carry out detailed genetic analyses of the calf. They found that Cosmo carries several copies of the SRY gene on one of the Chromosome 17 pair, and a small insertion on the other. This suggests that, when Cosmo was a newly-formed embryo, the editing resulted in one Chromosome 17 being repaired with a small 26 base pair insertion, and the other was repaired using the GFP:SRY DNA donor fragment to insert multiple GFP:SRY copies on Chromosome 17. He was also confirmed to be XY.

Cosmo has been quite popular with the media, and was featured in stories in Wired magazine and Grist, and appeared in a PBS NOVA documentary special on genome editing called "Gene Editing Reality Check."

Cosmo will reach sexual maturity in early 2021 at which time he will be bred to study if inheriting the SRY gene on Chromosome 17 is sufficient to trigger the male developmental pathway in XX embryos. Such bulls could produce a higher proportion of male market calves. However, at this time the project is still in the research stage and is highly regulated by the Food and Drug Administration, meaning Cosmo and his offspring are not allowed to be marketed, or enter the food supply.

This work was supported in part by grants from Biotechnology Risk Assessment Grant Program competitive grant #2015- 33522-24106 from the U.S. Department of Agriculture; the UC Davis Academic Federation Innovative Development Awards Grant Program; the Russell L. Rustici Rangeland and Cattle Research Endowment of the College of Agricultural and Environmental Sciences; and the California Agricultural Experiment Station of the University of California, Davis. The authors especially thank Randy Perry, Ph.D., and student Ashley Young from Fresno State University, and UCCE Livestock Advisor Rebecca Ozeran, for coordinating the collection of ovaries from the Cargill plant in Fresno for this project.

For more information, visit: animalbiotech.ucdavis.edu.

Cosmo: a targeted knock-in calf developed using the CRISPR-Cas9 system in bovine zygotes.