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News & Highlights

Germline Gene-Editing Creates Enhanced Livestock—Technical and Especially Ethical Issues Challenge Its Use in Humans

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Using clustered regularly interspaced short palindromic repeats (CRISPR)-based molecular tools [1], scientists are engineering—as they are also doing with plants [2]—animals with advantageous traits, like disease resistance and improved food yield [3]. While these innovative techniques could one day be applied in humans, technical hurdles and ethical concerns likely place this possibility far in the future.

The enhancements rely on germline gene editing, which alters the genes in a way that passes the changes on to offspring. Germline gene editing differs from the somatic cell gene editing used in the highly promising new treatment recently approved for the human disease sickle cell anemia [4]. The genetic changes made by these treatments in somatic cells—cells that do not become offspring—are not passed on to future generations.

"When you introduce edits in germ cells—the eggs, sperm, or a pre-implantation embryo—these become offspring," said Shoukhrat Mitalipov, director of the Center for Embryonic Cell and Gene Therapy at Oregon Health and Science University in Portland, OR, USA. "Now you have a whole organism with that change, including that organism's future eggs, sperm, and offspring."

Almost all the gene editing being done in animals, especially livestock, is germline gene editing. "As a breeder, I care about traits that get passed on to the next generation," said Alison Van Eenennaam, a cooperative extension specialist in animal genomics and biotechnology at the University of California, Davis, in Davis, CA, USA. "If I have not introduced the edit into the germline, then I have not been successful."

Researchers are using germline gene editing to produce farm animals that are more resistant to infections and ones that more efficiently produce meat and milk [5]. They have also created animals to use as improved models of human disease and to provide organs less likely to induce rejection as xenotransplants in humans [6]. It is also used with the hopes of avoiding painful practices, such as dehorning in cattle, and the production of unwanted offspring, resulting in the mass killing, for instance, of male chicks in egg production systems [7].

Most cattle grow horns, which farmers remove early in life to prevent injuries to themselves, the animal itself, and other cattle in the herd. The removal procedure is invasive and painful. The trait varies naturally among some breeds of cattle, which provides an opportunity for gene editing. "The Angus breed does not grow horns, but the horned allele is present in dairy cattle. I would like to bring that allele in from Angus, but I do not want a half Angus–half Holstein because that would not be optimal for dairy production," said Van Eenennaam. "I want to introduce the naturally occurring hornless allele to get a dehorned animal with elite dairy genetics, and I can do that with gene editing."

Early experiments to create dehorned cattle via gene editing, reported in *Nature Biotechnology* in 2016, resulted in five hornless calves [8]. Van Eenennaam, collaborating with the company Recombinetics (Eagan, MN, USA), then produced six hornless calves from one of these edited bulls (Fig. 1) [9]. Recombinetics shelved the project in the United States, however, in part due to the high cost of meeting regulatory requirements for commercial approval through the US Food and Drug Administration (FDA) [10].

Disease resistance is another use case for gene editing in livestock that has received a substantial amount of research attention. When infectious diseases get into modern farming systems, it can require culling an entire herd. Animals gene edited for resistance

Fig. 1. Researchers have used gene-edited sperm from bulls to create hornless dairy cow offspring. Using an invasive and painful procedure, farmers remove the horns of most breeds of cattle early in life to help prevent injuries to the animal, farmers, or other cattle in the herd. The germline gene editing of the hornless trait into different cattle breeds created these hornless calves, shown here flanking a horned bull from a control group. Credit: Alison Van Eenennaam, with permission.

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may reduce the risk of such devastating losses due to disease outbreaks [7]. For example, outbreaks of porcine reproductive and respiratory syndrome virus (PRRSV) have been estimated to cost the pig industry in the United States and Europe more than 2.5 billion USD annually [11]. To address this problem, scientists at the company Genus plc (Basingstoke, UK) germline gene edited pigs to modify the macrophage surface protein CD163 which can mediate the entry of PRRSV into host cells, creating piglets entirely resistant to North American and European strains of the virus [7]. The company expects to receive US FDA approval to commercialize their gene-edited pigs by 2025 [11].

Similar work has been done in chickens. In a study published in *Nature Communications* in October 2023, researchers led by a team at the Roslin Institute at the University of Edinburgh (Edinburgh, UK) created chickens resistant to avian influenza by editing the gene for the intracellular protein ANP32A, which the virus requires to replicate [12]. The edit changed the shape of the virus binding site on ANP32A, reducing the viruses' ability to replicate in the bird's cells. When challenged with a dose of 1000 infectious particles of bird flu, one of ten gene-edited birds became infected, while all ten unedited animals got sick. The virus-challenged unedited birds also spread the infection to seven of ten unedited chickens housed with them. With the virus-challenged edited birds, the infection did not spread to any of the ten edited chicks housed with them [13].

Viruses can, however, quickly adapt to evade the changes introduced by gene editing. For instance, in the chickens gene edited for bird flu resistance, the researchers found that the virus quickly adapted to use the protein from the edited gene to replicate, and even developed the ability to replicate without using the protein from the edited gene at all [14]. They concluded that multiple genes would have to be edited to stop bird flu completely.

Germline gene editing can also be used to make animals more productive, increasing their economic value. One common target for increasing productivity is the myostatin gene, which increases muscle mass—meaning more meat on each animal [7]. "A mutation in the myostatin gene causes hypertrophied muscle—called the double muscle phenotype in Belgian Blue cattle—which yields about a 30% increase in lean muscle," said Van Eenennaam. By editing this gene to the mutant form, researchers have created a variety of animals with increased muscle mass, including cattle [15], pigs [16], sheep [17], rabbits [18], and goats [18].

In Japan, scientists have created and commercialized extra-productive fish breeds by also editing the genes for myostatin and other proteins [19]. Regional Fish Institute, Ltd. (Kyoto, Japan) has successfully brought the red sea bream with gene-edited myostatin to market [20,21], gaining approval to sell the fish in 2021. The fish grow 17% more muscle while being fed the same food as their unmodified counterparts. Japanese consumers are also eating tiger puffer fish that grow to about twice their normal size, created by editing four genes responsible for appetite and growth [21].

Although germline gene editing could improve animal welfare and the economics of raising livestock, the research has generated some controversy. The ethics of modern industrial farming systems are already decried by many, and some critics charge that gene editing may worsen animal welfare by promoting further crowding of animals already subject to poor living conditions. "Gene editing might encourage industrial forms of agriculture," said R. Alta Charo, emerita professor of law and bioethics at the University of Wisconsin–Madison Law School in Madison, WI, USA. "The objection is not that the editing hurts the animal, it is that it worsens a practice that some people already object to."

The potential use of germline gene editing in humans provokes a more fraught ethical conversation. The clearly appropriate use would be for preventing disease (and not for selecting blue eyes, blond hair, or athletic ability for example), but even that raises difficult questions, said Charo. According to a 2017 US National Academies report from the Committee on Human Gene Editing that Charo co-chaired [22], germline editing to cure human disease must meet very stringent conditions [23]. "The report did not say that germline editing was ethically indefensible," said Charo. "We thought it was defensible under some very specific circumstances. But the report also laid out conditions that would make it extremely difficult to go forward with it."

The disease would need to be compellingly problematic—life-threatening or life-destroying. The disease could not have good treatment alternatives, such as using either donated gametes or embryo selection during *in vitro* fertilization. "There was only one clear example we could come up with," said Charo. "A couple both homozygous for Huntington's disorder."

Even with compelling reasons, germline gene editing technology is not ready for use to cure human disease, Mitalipov said. As detailed in a March 2023 report in *Nature Communications*, recent work from Mitalipov's laboratory studying germline gene edits in human embryos (Fig. 2) highlights one major issue with human germline editing—it can be challenging to determine if an edit was successfully incorporated before embryo implantation (Fig. 3) [24]. This problem also plagues a currently well accepted

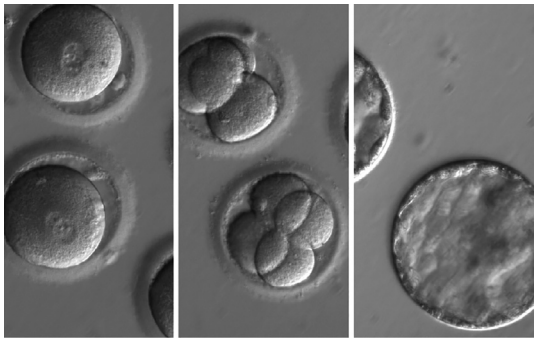

Fig. 2. Researchers have successfully edited human embryos using CRISPR in this experiment to fix a genetic mutation causing hypertrophic cardiomyopathy. These images show the development of embryos after co-injection of CRISPR enzymes and sperm from a donor with the mutation. This experiment repaired the disease-causing mutation from the moment of fertilization, which would prevent it from being passed to future generations. The experimental embryos are destroyed, not implanted. Credit: Oregon Health and Science University, with permission.

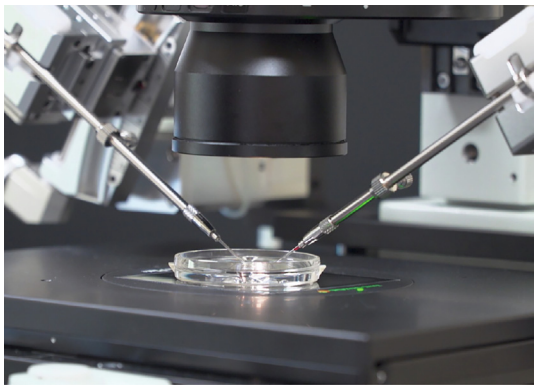

Fig. 3. Germline gene editing in humans using CRISPR to prevent inherited diseases from passing to offspring is challenging. The image shows the special microscope used to separate cells from an embryo for genetic testing. Studies have shown that whole genome sequencing, used to detect genetic changes in early human embryos, fails to accurately reflect gene edits. These and other limitations must be overcome before the implantation of gene-edited embryos to establish a pregnancy can be attempted. Credit: Oregon Health and Science University/Sara Hottman, with permission.

and widely used practice, the pre-implantation genetic testing of embryos created via *in vitro* fertilization. "In humans, we do not want to induce unwanted changes. We do not want to make errors. We want to precisely edit the genes to correct mutations," said Mitalipov. "As it currently stands, we need to wait for better tools to do these types of corrections."

Nevertheless, gene-edited human embryos have already been implanted and born as apparently healthy infants. In 2018, a lone scientist gene edited twin female embryos, followed by a third embryo a year later, to resist human immunodeficiency virus (HIV) infection [25]. The research violated all global norms, said Charo, and the scientist served a three-year prison sentence for the illegal experiments [26].

The near universal alarm over that work led to a September 2020 report, *Heritable Human Genome Editing*, prepared by an 18-member panel of experts jointly convened by the US National Academy of Sciences, the US National Academy of Medicine, and the UK Royal Society [27]. According to a blog post by Francis Collins, then director of the US National Institutes of Health, the report "concluded that heritable human genome editing is too technologically unreliable and unsafe to risk testing it for any clinical application in humans at the present time" [28]. For now, at least, it seems germline gene editing of animals will stay within the barnyard.

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