Russia's first cloned calf opens door to gene-edited cattle

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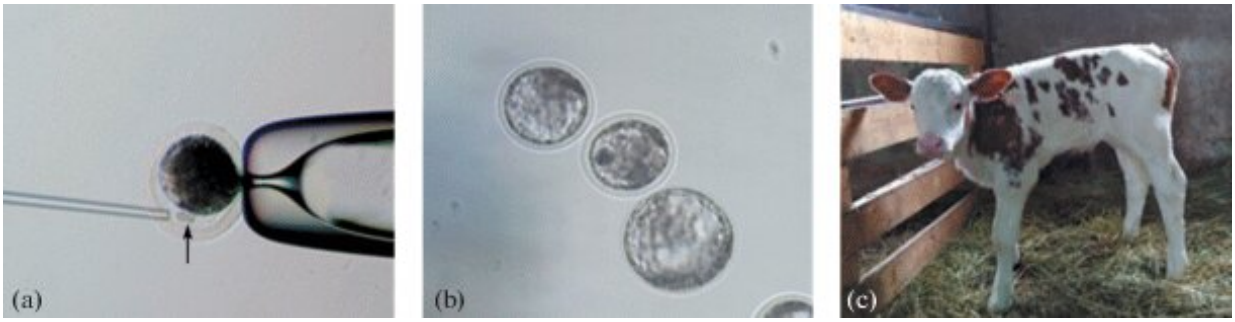

Photographs of (a) the procedure of somatic cell transfer (shown with the arrow) into the perivitelline space of an enucleated oocyte, (b) cloned bovine embryos used for transplantation to recipient animals, and (c) cloned calf (obtained in Russia for the first time). Fig. 1. From: Production of a Cloned Offspring and CRISPR/Cas9 Genome Editing of Embryonic Fibroblasts in Cattle

Researchers from Ernst Federal Science Center for Animal Husbandry, Skoltech, Moscow State University and their colleagues have produced the first viable cloned calf in Russia—and she recently turned one. In a related experiment, the team was able to knock out the genes responsible for beta-lactoglobulin, a protein causing milk allergy in humans, in the hopes of creating gene-edited cows with hypoallergenic milk. The paper outlining the results of the experiment was recently published in the journal *Doklady Biochemistry and Biophysics*.

A team led by Galina Singina of the Ernst Federal Science Center for Animal Husbandry managed to clone the calf using somatic cell nuclear transfer (SCNT), with embryonic fibroblasts as donors of nuclei. Somatic cell nuclear transfer means that a nucleus from a regular cell of a donor animal is transferred into an egg with its nucleus removed, and the resulting embryo is then implanted into the uterus of a cow and carried to term.

While genetically modified mice became a routine worldwide, although still regrettably rare in Russia, gene editing in other animal species remains a challenge, Petr Sergiev, a member of the research team, says, mostly due to high costs and difficulties in breeding and husbandry—mice are very convenient from this perspective with, for instance, a 3-week pregnancy, but other species are not. There is also a lot of accumulated experience in dealing with mice simply because so many labs around the world have been working with them for decades.

"Thus, a methodology leading to cattle with hypoallergenic milk is not only a necessity for agriculture of the future, but also a cool project," Sergiev, who is an Associate Professor at Skoltech, notes.

"The cloned calf was born on April 10, 2020, with a birth weight of 63 kilograms. Now, as she is over a year old, she is an adult animal weighing over 410 kilograms with a regular reproductive cycle. Until she turned one, we kept her in a separate room with her mother, but since May, she has been on daily pasture with the other cows of the Institute. It required some adaptation, but that happened quickly," Galina Singina says.

Cloning a cow is essentially a test run for producing a gene-edited animal, Sergiev explains, as scientists need to make sure all their methodological ducks are in a row before implanting the edited embryos. The researchers already used the Nobel Prize-winning

CRISPR/Cas9 technology to knock out PAEP and LOC100848610, two genes representing beta-lactoglobulin in the bovine genome, and obtain a line of genetically edited embryonic fibroblasts. Their nuclei will then be used for SCNT.

Beta-lactoglobulin, the main allergen in cow's milk, is not an easy target as there are in fact four copies of the genes in a cow's genome (two of each gene) you need to inactivate. So far the best result the team was able to get is three out of four, which Sergiev says is enough to proceed because the "perfect" animal can then be produced via traditional breeding technologies.

The researchers are gearing up to try their hand at the next stage of their experiment by creating a herd of several dozen cows that will have to carry the edited pregnancies to term. "Since it is not a 100% certain process, you have to roll the dice a lot, and it's quite expensive," Petr Sergiev says.

"I think this work will lay the methodological foundation for gene editing in cattle in Russia, which will lead to more complex challenges. For instance, we can make cows produce certain proteins they normally don't for biotechnological purposes," he concludes.

More information: G. N. Singina et al, Production of a Cloned Offspring and CRISPR/Cas9 Genome Editing of Embryonic Fibroblasts in Cattle, *Doklady Biochemistry and Biophysics* (2021). DOI: 10.1134/S1607672921010099

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