

[The First Successful Cloning of a Gaur \(2000\), by Advanced Cell Technology](#) ^[1]

By: Appleton, Caroline Keywords: [Interspecies SCNT](#) ^[2] [Somatic cell nuclear transfer](#) ^[3]

[Advanced Cell Technology](#) ^[4] (ACT), a stem cell biotechnology company in Worcester, Massachusetts, showed the potential for [cloning](#) ^[5] to contribute to conservation efforts. In 2000 ACT researchers in the United States cloned a gaur ([Bos gaurus](#) ^[6]), an Asian ox with a then declining wild population. The researchers used cryopreserved gaur skin cells combined with an embryo of a domestic [cow](#) ^[7] ([Bos taurus](#) ^[8]). A domestic [cow](#) ^[7] also served as the [surrogate](#) ^[9] for the developing gaur clone. The successful procedure opened the opportunity to clone individuals from species for which there are few or zero live specimens. The official release of this experiment's data was published in the paper "Cloning of an Endangered Species (*Bos gaurus*) Using Interspecies Nuclear Transfer," in October 2000. In the article, the researchers presented data collected from several cloned fetuses that were aborted before the full term of 283 days. At the time of publication, the gaur bull [fetus](#) ^[10], named Noah at birth, had developed for greater than 180 days. Noah was born on 8 January 2001, but died two days later due to dysentery. The development, birth, and death of Noah became a platform for conservationists and ethicists to critique the role of [cloning](#) ^[5] in society and as a method to conserve species.

In the late 1990s, [Advanced Cell Technology](#) ^[4]'s then Chief Executive Officer and President [Michael West](#) ^[11] recognized a link between the current extinctions and assisted reproduction technologies. He stated that ACT's effort to clone the gaur was the least it could do to help conserve wildlife threatened with anthropogenic extinction. ACT worked under the premise that the gaur was an endangered species although, at the time of the experiment, it was classified by the International Union for Conservation of Nature's Red List of Threatened Species as vulnerable, not endangered. ACT elected to use domestic cows for carrying and birthing the gaur clone because the two species are both members of the Bovidae family. Additionally, ACT had experienced previous success in [cloning](#) ^[5] domestic cows.

[Robert Lanza](#) ^[12], ACT's then Vice President of medical and scientific development, was the experiment's head scientist. Lanza worked with fellow ACT researchers West, [Jose Cibelli](#) ^[13], and Philip Damiani; Francisca Diaz and Carlos Moraes, from the University of Miami, in Miami, Florida; Peter Farin and Charlotte Farin, from North Carolina State University, in Raleigh, North Carolina; and Carolyn Hammer, from Iowa State University, in Ames, Iowa. The researchers worked to fuse a gaur cell with an [egg](#) ^[14] from a domestic [cow](#) ^[7] to produce a gaur clone. This [cloning](#) ^[5] technique is called [somatic cell](#) ^[15] [nuclear transplantation](#) ^[16] (SCNT).

The 1997 announcement of the [cloning](#) ^[5] of Dolly the [sheep](#) ^[17] raised questions surrounding the extent to which clones are genetically identical to their respective donors. Most [mammal](#) ^[18] cells have a [nucleus](#) ^[19] to store DNA, the source for genetic material in [cloning](#) ^[5], in the form of chromosomes. However, those cells also have DNA stored in another cellular structure called the mitochondria. This genetic material is called mitochondrial DNA (mtDNA) and offspring express, or create proteins from, the mtDNA of their mothers in many classes of species, including mammals. For [cloning](#) ^[5], researchers remove the [nucleus](#) ^[19] from an [egg](#) ^[14] cell in a process called enucleation, leaving the mitochondria and the mtDNA in the [egg](#) ^[14] cell. Hence, Dolly was a genetic clone to her donor in a nuclear sense, but not in a mitochondrial sense. If the genetic sources used in SCNT are from the same species, which was the case for Dolly, the result is an intraspecies clone. Alternatively, if the genetic sources used in SCNT are from different species, which was the case for ACT's gaur, Noah, the result is an interspecies clone. At the time of ACT's gaur [cloning](#) ^[5] experiment, it was unclear what ramifications, if any, an interspecies clone would experience due to having nuclear DNA from one species and mtDNA from another species.

Previous attempts at [cloning](#) ^[5] gaurs using gaur-to-domestic-cow ^[7] [embryo transfer](#) ^[20] and gaur [in vitro](#) ^[21] [fertilization](#) ^[22] had been unsuccessful, resulting in calves that were stillborn or that died shortly after birth. The scientists at ACT decided to artificially reproduce the gaur using interspecies [cloning](#) ^[5] in part because other methods had failed and because they wanted to show that mammalian fetuses can develop after an enucleated [egg](#) ^[14] of one species is artificially fertilized with a [nucleus](#) ^[19] from a skin cell of an animal from another species.

The initial stage of the interspecies [cloning](#) ^[5] experiment required healthy gaur cells and enucleated domestic [cow](#) ^[7] eggs. For gaur cells, ACT received cryopreserved gaur skin cells from the Center for the Reproduction of Endangered Species at the San Diego Zoo, in San Diego, California. Once the cryopreserved gaur skin cells thawed, ACT researchers cultured the cells in Petri

dishes to propagate an actively dividing gaur cell line. Bovine eggs were then selected according to Damiani and Cibelli's previously tested method of bovine [egg](#)^[14] evaluation to ensure the domestic [cow](#)^[7] eggs cells were mature and of high quality. The researchers enucleated each selected bovine [egg](#)^[14] and reported confirmation of success by using fluorescent [microscopy](#)^[23], a process of dying and inspecting the eggs under a [microscope](#)^[24].

The researchers transferred gaur genetic material into the enucleated domestic [cow](#)^[7] eggs by fusing and then activating the cells. To fuse the cells, the researchers placed a gaur cell under the protective layer of an enucleated [egg](#)^[14] and applied a single electric pulse to fuse the cells into a cellular complex. The researchers then chemically activated and cultured the artificially fertilized eggs, observing the cells' developments into the [blastocyst](#)^[25] stage. The researchers produced eighty-one blastocysts out of the 692 fused cellular complexes. Forty-four [blastocyst](#)^[25]-stage embryos passed the quality selection criteria and were transferred into one extension of the [uterus](#)^[26] (uterine horn) in thirty-two hormonally prepared recipient domestic cows at Trans Ova Genetics, an [embryo transfer](#)^[20] service facility, in Sioux Center, Iowa. Presumably, some domestic cows received more than one [blastocyst](#)^[25], but these figures were not reported. The researchers used [ultrasound](#)^[27] to validate that eight cows had successfully become pregnant with the gaur clone blastocysts forty days after the transfer.

To test if the gaur clone embryos would develop normally, the team wanted the embryos to develop into fetuses before beginning experimental analysis of the clones. ACT electively removed a single gaur [fetus](#)^[10], at developmental day 46, and a set of gaur twins, at developmental day 54, from their [surrogate](#)^[9] mothers via cesarean section. These three fetuses were analyzed morphologically and genetically. The team reported that all measures of normal development were met, including but not limited to ears, noses, eyes, limb buds or limbs, and presumptive mouths. The morphological data of the developing fetuses provided support for ACT's interspecies nuclear transfer technique as a [viable](#)^[28] way to avoid host rejection and the development issues that scientists observed during previous experiments of artificial reproduction of gaurs.

To evaluate the genetic character of the removed gaur fetuses, the team analyzed the fetuses' DNA and mtDNA. A forelimb was removed from each [fetus](#)^[10], minced, and cultured to produce actively dividing cell lines. These live cells were processed and subjected to chromosomal inspection, called [cytogenetic analysis](#)^[29]. Cells were also collected from eleven tissue types for use in two mtDNA tests.

The cytogenetic test consisted of counting the chromosomes in the [nucleus](#)^[19]. For this test, the gaur cells were halted during [mitosis](#)^[30] at the metaphase stage, when chromosomes align in the middle of the cell before cell division. The researchers then prepared the cells for a microscopic chromosome count. The gaur is the only species in the Bovidae family in which individuals have fifty-eight chromosomes, whereas domestic cattle, like most Bovidea species, have sixty chromosomes. The researchers found that the gaur fetuses had fifty-eight chromosomes, showing that they must have developed from the gaur donor nuclear genetic material. This indicated that the [cloning](#)^[5] methods were successful.

In order to understand the process of both nuclear and mitochondrial DNA transfer in interspecies [cloning](#)^[5], the scientists still needed to know the origin of the mtDNA in the developing embryos. For the first mtDNA analysis, the researchers selected a particular fragment of mtDNA known to differ between the gaur and the domestic [cow](#)^[7]. The researchers amplified these segments of mtDNA in high quantity using a process called polymerase chain reaction (PCR). To analyze these amplified chromosomal segments, the researchers used gel electrophoresis. They placed the PCR products from domestic [cow](#)^[7], gaur, and four gaur fetuses in a gel subjected to an electric current. DNA has an electric charge, making the DNA segments travel toward the opposite charge at the end of the gel. The smaller segments can travel through the gel faster than the larger segments, ending up further down the gel in the same amount of time. Clusters of DNA segments of the same size create rectangular bands on the gel. By looking at the bands in the gel, the researchers compared the mtDNA segments from each species and the fetuses to figure out the origin of the mtDNA segments. The results showed that cells of the gaur fetuses contained domestic [cow](#)^[7] mtDNA.

In the second analysis, the researchers sampled cells from eleven tissues types in the gaur fetuses to perform PCR and gel electrophoresis. Sampled tissues ranged from heart to brain to gonads. Additionally, in the last cycle of PCR, the researchers tagged with radioactive phosphorous a segment of DNA that is conserved in both gaur and domestic [cow](#)^[7] mtDNA, but in different positions in the genetic code. The researchers used phosphor imaging to analyze the tagged mtDNA because the radioactive phosphorous tags glow under certain light conditions, making the analysis visible. The researchers concluded that the fetuses had exclusively replicated the maternal domestic [cow](#)^[7] mtDNA.

The experimenters noted that because they had used fused whole gaur cells, rather than gaur [sperm](#)^[31], to the [cow](#)^[7] oocytes, the gaur mtDNA was inserted into the [egg](#)^[14] when they made the [zygote](#)^[32]. Thus, if the gaur mitochondria replicated at a rate equal to the [cow](#)^[7] mtDNA, then approximately two to five per cent of the mtDNA should have been from the gaur. Yet, it was not. The lack of gaur mtDNA in the gaur fetuses was consistent with what was found in [cloning](#)^[5] experiments with Dolly, as all of the cloned [sheep](#)^[17]'s mtDNA had derived from the [egg](#)^[14]. The researchers concluded that because the gaur and [cow](#)^[7] mtDNA are

functionally equivalent, the fusion product behaved like a [fertilized egg](#)^[33] and eliminated any extra gaur mtDNA before beginning to replicate.

In August 2000 ACT submitted its experimental results to the journal *Cloning*. ACT researchers had impregnated eight domestic cows with interspecies embryos of gaur and domestic cows, and they had shown that the embryos were in fact gaur clones. Out of the eight gestational cows, three were elected for premature [fetus](#)^[10] removal and four had spontaneous abortions, leaving one gaur left to develop. ACT decided to name the gaur clone Noah prior to his birth.

On 8 January 2001 Noah was born by cesarean section and ACT became known for successfully creating a clone of a species of interest to conservationists. The calf was considered one of the healthiest [mammal](#)^[18] clones then born due to his ability to stand on his own quickly, as well as due to his alertness, blood sugar and acid levels, and musculature. Over the next twenty-four hours, however, Noah contracted dysentery, an infection almost always fatal for newborn animals. Within forty-eight hours, Noah died. ACT claimed that the cause of death was unrelated to the experimental procedure. The public's reaction to Noah's death contributed to the discussion of the prospect of [cloning](#)^[5] humans—ACT's admitted ultimate purpose—and the ethical use of reproductive science in conservation.

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Subject

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Arizona State University. School of Life Sciences. Center for Biology and Society. Embryo Project Encyclopedia.

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Last Modified

Wednesday, July 4, 2018 - 04:40

DC Date Accessioned

Saturday, July 27, 2013 - 00:09

DC Date Available

Saturday, July 27, 2013 - 00:09

DC Date Created

2013-07-26

DC Date Created Standard

Friday, July 26, 2013 - 07:00

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