Is Livestock Cloning Another Form of Genetic Engineering?

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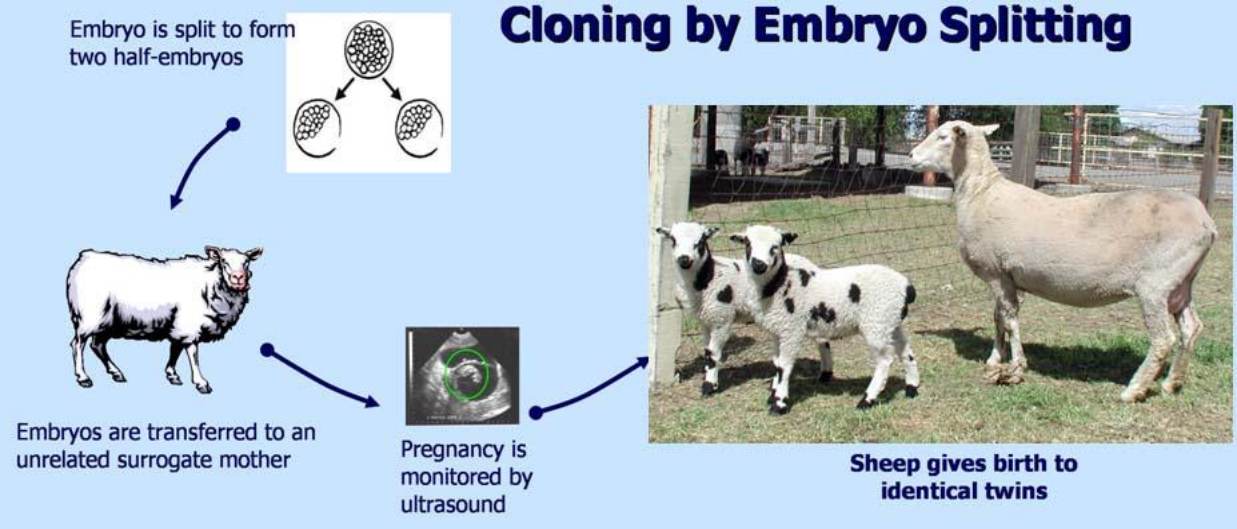
The birth of Dolly in 1996, the first animal cloned from an adult cell, was not universally celebrated. Critics of biotechnology worried that genetically modified livestock would be next, filling the supermarket with identical copies of someone's idea of unnatural perfection. In fact, cloning does not alter the genetic makeup of an animal. Quite the contrary, cloning involves making genetically identical copies of a plant or animal, using asexual reproduction. Many common fruits and vegetables (e.g., pears, apples, oranges and potatoes) are clones, and cloned livestock have already been have been a part of animal agriculture for over 20 years. There is, however, a logical connection between cloning and genetic engineering, and that is actually the reason that scientists were working to develop livestock cloning methods in the first place.

Question: What is a clone?

Answer: A clone is an organism that is descended from — and is genetically identical to — a single common ancestor. Animals can be cloned by two different methods: mechanical embryo splitting or nuclear transfer.

Embryo splitting involves bisecting the multi-cellular embryo at an early stage of development to generate clones or "twins." A 32-cell embryo, for example, might be bisected into two 16-cell twins. This type of cloning occurs naturally (human identical twins result from this process, but fraternal twins do not), but it can also be performed in a laboratory where it has been successfully used to produce clones from a number of different animal species. This technique was first used in agriculture to replicate valuable dairy breeding animals in the 1980s. The Holstein Association USA registered their first embryo split clone in 1982, and more than 2300 had been registered by October 2002[1]. This method has a practical limitation in cattle[2] and sheep[3], in that a maximum of four clones can be produced from each embryo.

Cloning can also be done by nuclear transfer, where the genetic material from one cell is placed into a "recipient" unfertilized egg that has had its genetic material removed by a process called enucleation. In order to begin the development process, the donor nucleus must be fused with the egg through the administration of a brief electrical pulse or a chemical fusion process, after which the embryo starts to divide as if it had been fertilized. In the case of mammals, the embryo is then placed into a surrogate mother where it will develop until birth, where it will be delivered just as with any newborn.

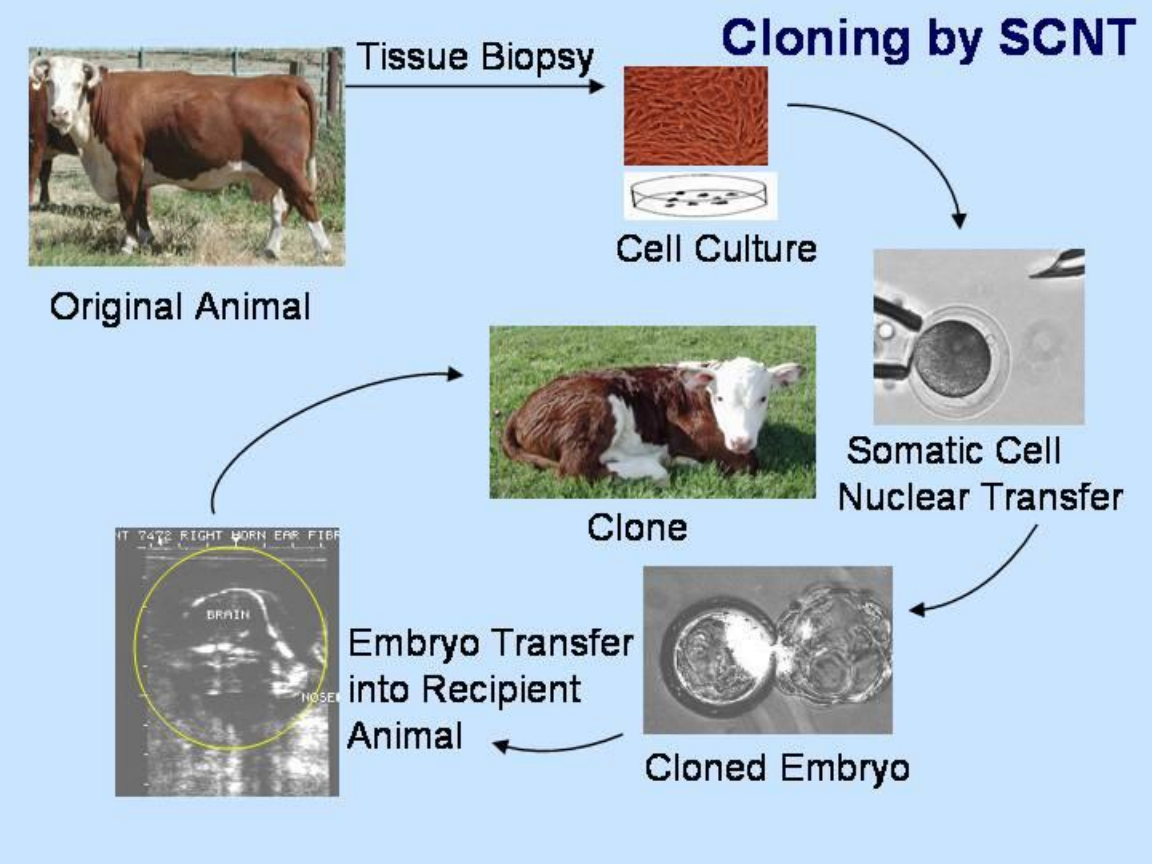


The first mammals were cloned via nuclear transfer during the early 1980s, almost 30 years after the initial successful experiments with frogs [4]. Numerous mammalian clones followed — including mice, rats, rabbits, pigs, goats, sheep[5], cattle[6], and even two rhesus monkeys named Neti and Detto [7] — thanks to nuclear transfer. The Holstein Association USA registered their first embryo nuclear transfer clone in 1989, and approximately 1,200–1,500 cows and bulls were produced by embryonic cell nuclear transfer in North America in the 1980s and 1990s[8]. However all of these clones were produced from the transfer of nuclei derived from early (8–32 cell) embryos, and therefore a theoretical maximum of only 32 clones could be produced from each individual embryo. And then in 1996, along came Dolly.

Q: How did Dolly come about?

A: Dolly the sheep, was the first animal to be cloned via nuclear transfer from a cultured somatic cell derived from an adult [9]. This process, known as SCNT (for somatic cell nuclear transfer) cloning, allows cloning to be performed on a potentially-unlimited number of cells from an adult animal whose performance and traits are well known.

A diverse range of species have now been successfully cloned from adult tissues using SCNT including cattle[10], mice[11], pigs [12], cats [13], rabbits [14], goats [15], dogs[16], rats[17], and zebra fish [18]. It was estimated in October 2007 that there were 500–600 SCNT livestock clones in the United States (Barbara Glenn, Biotechnology Industry Organization, personal communication). Very few of these valuable clones will themselves enter the food supply, rather food products will likely be milk and meat derived from the sexually produced offspring of these SCNT clones.



Q: Why is cloning a hit-or-miss proposition?

A: The proportion of adult cell nuclei that successfully develop into live offspring, after transfer into an enucleated egg, is very low[19]. High rates of pregnancy loss have been observed after transfer of the eggs containing the adult cell nuclei into recipient animals[20]. This, together with other problems such as 'large offspring syndrome' (where cloned lambs and calves are often large at birth), placental abnormalities, edema, and perinatal deaths have raised some animal welfare concerns. Many of these problems appear to result from incorrect reprogramming of the transferred nuclear DNA as it transitions from directing the cellular activities of a somatic cell to directing the complex developmental pathway required to develop into an entirely new embryo[21]. Scientists are researching ways to decrease the frequency of cloning abnormalities, and it has been found that they are partly associated with the type of tissue that originated the nuclei used to make the clone[22]. The animal health risks associated with the cloning process are not unique to SCNT cloning, and all have been observed in animals derived via other commonly-used assisted reproductive technologies (e.g., embryo transfer, in vitro fertilization), or natural mating[23].

Q: How about milk or meat from clones? Is it the same?

A: Studies examining the composition of food products derived from clones have found that they have the same composition as milk or meat from conventionally-produced animals[1,8,24–31]. Milk and meat from clones produced by embryo splitting and nuclear transfer of embryonic cells have been entering the human food supply for over 20 years with no evidence of problems. The U.S.

Food and Drug Administration (FDA) has broad regulatory jurisdiction over animals and foods, and does not currently regulate either the practice of assisted reproductive technologies in livestock, or provide for specific regulation of foods from animals based on their derivation.

However, in 2001 the Center for Veterinary Medicine at the FDA determined that it should undertake a comprehensive risk assessment to identify hazards and characterize food consumption risks that may result from SCNT animal clones[32] and therefore asked companies not to introduce these cloned animals, their progeny, or their food products (e.g., milk or meat) into the human or animal food supply (http://www.fda.gov/cvm/CVM_Updates/clones.htm). As there is no fundamental reason to suspect that clones will produce novel toxins or allergens, the main underlying food safety concern was whether the SCNT cloning process results in subtle changes in the composition of animal food products [33].

In December 2006, the FDA released a 678-page draft risk assessment which examined all existing data relevant to 1) the health of clones and their progeny, or 2) food consumption risks resulting from their edible products, and found that no unique food safety risks were identified in cloned animals. (http://www.fda.gov/cvm/CloneRiskAssessment.htm). The draft risk assessment therefore concluded that "***food products derived from animal clones and their offspring are likely to be as safe to eat as food from their non-clone counterparts, based on all the evidence available.***"

The release of the Draft Risk Assessment and its associated documents neither lifted the moratorium on food products from SCNT clones and their progeny, nor completed the FDA's consideration of this issue[23]. Therefore, as of December 2007, owners and producers of SCNT livestock continue to observe the voluntary moratorium on the sale of SCNT clones and their progeny into the food chain, while waiting for the completion of the final risk assessment and guidance from the FDA on the marketing of these animals.

Q: Will cloning be used to make genetically engineered animals?

A: Although cloning is not genetic engineering *per se*, there is a logical partnership between the two technologies. Cloning offers the opportunity to make genetically engineered or transgenic animals more efficiently from cultured somatic cells that have undergone precise, characterized modifications of the genome. The first genetically engineered mammalian clones were sheep born in 1997 carrying the coding sequences for human clotting factor IX, which is an important therapeutic for hemophiliacs[34]. Cloning has also be used to generate genetically engineered cows that produce human polyclonal antibodies for use in medicine[35]. It is envisioned that these unique cows will make it possible to create an efficient, safe, and steady supply of human polyclonal antibodies for the treatment of a variety of infectious human diseases and other ailments including organ transplant rejection, cancer and various autoimmune diseases, such as rheumatoid arthritis.

Cloning also offers the possibility of producing animals from cultured cells that have had selected genes removed. This "gene knockout" technique, commonly used in research with mice and the subject of the 2007 Nobel Prize in medicine, enables selective inactivation of specific genes in livestock with applications for both agriculture and biomedicine. For example, cloning

has been successfully used to produce cattle from cells lacking the gene for the prion protein responsible for mad cow disease[36], and pigs have been produced that lack the allergenic proteins that are responsible for the rejection of pig organs when used for transfer into human organ-transplantation patients[37]. Cloning may also have some utility as one approach contributing towards the preservation of rare and endangered species [38].

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More Information on Livestock Cloning from the U.S. Food and Drug Administration

- **Cloning Primer**
  http://www.fda.gov/cvm/CloningRA_Primer.htm

- **Cloning Myths**
  http://www.fda.gov/cvm/CloningRA_Myths.htm

- **Animal Cloning: FAQs about cloning for consumers**
  http://www.fda.gov/cvm/CloningRA_FAQConsumers.htm