An Update On The Science And Commercialization Of Cloning Cattle

By Brad Stroud, DVM

History

Dolly. That's a powerful one word sentence that needs little description. However, Dolly wasn't the world's first engineered cloned offspring. In 1885, Hans Dreisch vigorously shook sea urchin embryos at the two cell stage and separated them into individual cells that each went on to produce two genetically identical offspring (clones). Seventeen years later Hans Speeman innovatively split two-cell salamander embryos with baby hair by forming a loop and tightening the loop between the two cells to split them. Each of the individual cells went on to produce identical twins. Both the sea urchin and the salamander clones were products of embryo splitting or twinning. From a commercial cattle perspective this method of cloning was attempted by Granada Land & Cattle Company back in the late 1980's. A fair number of cloned offspring was produced, but the science was the most appealing part of the process. Unfortunately, and predictably, some of the embryos being cloned were genetically inferior giving rise to extremely expensive culls. Dolly, on the other hand, was the first offspring in the world cloned from an adult animal cell. Keith Campbell and Ian Wilmut, the scientists who lead the team to produce Dolly, knew what the cloned offspring would look like before she was born, unlike the scientists who preceded them who were cloning from embryonic cells. She was produced by using a mammary cell nucleus from an adult sheep instead of cells from an embryo. She opened the eyes of the world to the possibility of duplicating the world's greatest beef and dairy cattle via cloning adult animals. That's why she created so much attention.

Technique

The cloning process is logistically simple. Basically, three things are needed; an egg from any cow, instruments to remove the nucleus or DNA from that egg, and a cell nucleus that contains the DNA from the superior animal that is to be cloned.

Enucleation Procedure

Beginning with the egg, it literally can come from any old cow regardless of her genetic background. Commercial cloning companies use eggs, or oocytes, acquired from ovaries derived from commercially slaughtered cows. Once an egg has been removed via syringe aspiration from the ovary it is placed in an incubator and allowed to mature for about 24 hours. At that time its nucleus, which contains the DNA of the slaughtered cow, is removed using a micromanipulator. This process is called enucleation. It's important to note that only the egg's nucleus is removed by enucleation, and that the egg's cytoplasm, which contains no functional DNA, remains intact. The cytoplasm does, however, contain important micro-organelles that help orchestrate early embryonic development (cell division) once the clone has been constructed.

Skin Biopsy For Gene Banking

Once the slaughtered cow's nucleus has been removed it must receive a replacement nucleus from the superior donor animal. Typically, the cells from the donor are acquired by taking a skin biopsy. The location of choice for the bovine is the ear or the skin under the tail head (Figure 4). Theoretically, any cell like liver, muscle, or kidney cells would qualify because all cells of a particular animal, except the sperm or egg, contain the same DNA or genetic code in their nucleus. Each cell nucleus is genetically identical regardless of cell type or function, so it doesn't really matter which one is used. However, skin cells are easy to acquire via a punch biopsy procedure. That's a relatively non-invasive way to acquire the genetic code of the superior donor animal. Once the biopsy is sent to the lab it is processed and the cells are placed in culture medium and then into incubators where the cells proliferate and grow by the thousands. After a few days the cells are divided into aliquots, frozen, and stored at two or more locations for safety and biosecurity reasons. The processing and freezing of cells for future cloning is called gene banking. Once a client orders a clone or group of cloned calves, the cells are thawed out and processed to be transferred into the enucleated slaughterhouse derived eggs.

Nuclear Transfer

The same micromanipulator that

performed the enucleation process of the original egg is also used to transfer the nucleus of the donor cell into the cytoplasm of the enucleated egg. That process is called nuclear transfer (NT). Once the cell has been injected the cloning process is essentially complete. The nucleus of the slaughterhouse egg has been removed, and has been replaced with the nucleus of the superior donor animal.

The newly constructed embryo now has the genetic code of the donor animal. It will be placed in an incubator and allowed to grow for about six days, and then non-surgically transferred directly into the uterus of a surrogate or recipient female. Once in the uterus, it's back to nature as the embryo will hatch from its shell (zona pellucida) in a day or two post transfer and then begin to elongate. A few days later the embryo derived placenta will begin attaching itself to the uterine wall of the recipient so the embryo can eventually begin to feed off the blood flow from the recipient cow. If all goes well, the clone will be born about nine months after embryo transfer.

Cloning is a Reproductive Tool

As a society we tend to fear the unknown. That's human nature. Six decades ago we were intimidated by AI. Many thought the offspring from frozen semen would be normal as would the protein derived from their meat and milk. Some speculated monsters would result and others thought the technology was immoral for both humans and animals. Embryo transfer and IVF were no different. Naysayers just couldn't imagine how fertilization outside the womb could possibly produce normal healthy offspring. We giggle now as we ponder the past, and we smile as we look at the benefits these reproductive technologies have contributed to society. What's really scary is to think where the beef and dairy industries would be without them. Genetic selection and controlled breeding has had a significant impact on livestock production in the last thirty years. Imagine what the price of meat and milk would be in the US today if not for AI alone.

Considering all the reproductive technologies, cloning is a relatively rudimentary breeding tool. It's the most predictable of the aforementioned technologies in that it only duplicates an animal's genotype, and does not gain or lose genetic progress by producing new matings. In some instances however, genetic gain isn't necessary. On rare occasions, numerous economically valuable genes are expressed in certain individuals. That's a classical situation where cloning becomes an attractive option for purebred cattle breeders. Those animals are the ones that contain the genes of economic interest that need to be distributed in mass until significantly better animals are produced by AI or embryo transfer.

Opportunities For Cloning Purebreds

There are a variety of situations where cloning can be implemented in the purebred cattle industry. Economics is the obvious driving factor. Currently the cost to produce one cloned calf is approximately fifteen thousand dollars. Some animals are certainly viable candidates for cloning based on economics alone. Livestock insurance can protect the monetary investment in an expensive bovine, but once dead the genetic value is lost forever unless the animal has been biopsied and gene banked for possible cloning.

Combining Technologies

Producing ten cloned copies of a valuable female and then flushing all ten genetically identical copies could mathematically produce ten times as many calves compared to the donor alone. That's a situation where combining two reproductive technologies like ET and cloning makes sense. Throw in sex sorted sperm to fertilize the cloned offspring's eggs for the flush and the breeder really begins to take control of his breeding program.

FDA

Cloning commercial livestock is a mute point until the FDA rules on the safety of milk and meat from cloned animals entering the food chain. They have done their research and have found that there is no scientific difference in the meat or milk from cloned and non-cloned beef and dairy animals. The current administration hasn't signed off on their response as of yet, and rightfully so. Beyond the science are social issues that have to be taken into consideration. The public must be educated about the science and the safety of cloned animals before they will accept eating milk or meat from them no matter what the government says. The most important element of the educational process is understanding that cloned animals are not genetically modified as are some plant foods that we currently eat. Clones are simply genetically identical twins removed by time. Their genome or genetic code has not been modified by the process. Historically, there have been hundreds of thousands of cloned animals, in the form of identical twins, in the milking herds and the beef feedlots of the US. Consumers haven't complained a bit because they haven't had reason. Furthermore, if we cloned one of a set of identical twin dairy cows and made five additional copies by cloning, the milk from all seven of the cloned sisters would be virtually indistinguishable from a genetic standpoint. The components i.e., proteins, sugars, fats, etc would also be similar. There certainly would not be any foreign or toxic substance as a result of the cloning process. It's up to the cattle industry to educate their own and the general public before the benefits of cloning reach the supermarket and ultimately the dinner table. Dairy and beef producers who utilize the technology will be able to produce a better quality product for less money than their competitors who don't. Ultimately, the consuming public will be the big benefactors of cloning technology - products they buy will be consistently better, healthier, and less expensive.

New Responsibility for Animal Owners

Before Dolly, once an animal died its genotype or genetic code essentially became extinct unless it had an identical twin wandering around somewhere, which was bound to die also at some point. Owners of extremely valuable domestic livestock now carry a responsibility that their forefathers did not. They can choose to preserve the genotype of a great animal by gene banking its cells before death, or literally, let it become dust. Just like a species can become extinct without intervention, so can a great animal's genotype. Although cells from animals dead for a couple of days have produced live healthy cloned offspring, that's not the best approach. Anyone in possession of a genetically superior animal should gene bank before a debilitating disease sets in or accidental death prevents genetic preservation. To be clear, gene banking cells doesn't necessarily commit the owner to the expense of cloning. Biopsied processed cells can be stored and never cloned if the owner decides not to. In other words, gene banking and cloning are separate business entities, but you can't clone without gene banking first.

The Future Of Cloning

Cloning is currently an inefficient process. Licensing is an important issue as well as equipment and personnel to run it. There are also marketing and administrative costs, but the biggest expense is recipient females. Pregnancy rates on transferred embryos produced by cloning, or nuclear transfer, are not as high as with traditional embryo transfer. Also, there are higher incidences of early embryonic death, mid and late term abortions, and stillbirths as compared to traditional breeding methods. However, cloning will not have as big an impact on the purebred beef and dairy industries. As previously mentioned, cloning does not make genetic progress. Breeding great sires to great dams does, and so it will always be. Purebred breeders should continue to mate for phenotype, or meat and milk EPDs. But every now and then many genes of economic importance will align and a genetically superior animal will result. Cloning that bull or cow would help that breed disseminate those traits to other breeders thereby pushing the industry in a progressive direction. Economics will dictate a breeder's decision about his own cattle, but in the end the consumer is the real winner. ■