NEW ADVANCEMENTS IN REPRODUCTIVE TECHNOLOGY FOR CATTLE

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Introduction

Use of most reproductive technologies, whether new or older, usually entails some costs. Costs may include time spent learning new procedures, and costs of supplies and services. Most newer reproductive technologies are somewhat expensive, and some only make sense as research tools or for application in niche situations. Delivering the newer reproductive technologies usually occurs via older technologies, such as artificial insemination (AI) and embryo transfer. From the farmer/rancher perspective, most reproductive technologies only make financial sense for seedstock producers. Even artificial insemination with frozen semen may be too expensive to be profitable with nursing beef cattle in the majority of cow/calf operations, although AI of heifers and dairy cows probably would be profitable for most operations.

Another issue is defining "new." Most of the technologies that I will discuss are decades old from a conceptual and research perspective. Some are new in the sense of commercial availability, but some will not be available commercially for some years. That does not mean that they are not worth thinking about.

Still another question is defining reproductive technology. While most would agree that a blood test for pregnancy probably falls into this category, we could argue that genotyping, for example for meat tenderness, is really a genetic technology and should not be considered a reproductive technology. Even so, there is a synergism between genetic and reproductive technologies in that the latter are needed to implement the former in most situations. For example, artificial insemination rarely is appropriate unless the semen is from genetically superior bulls.

Selected New Reproductive Technologies

Sexed Semen

While sexed semen has been thought about for centuries, and seriously considered by researchers for about one century, the first scientifically credible demonstration of producing offspring from sexed sperm was reported in 1989 by Dr. Lawrence Johnson at the United States Department of Agriculture. Since then, considerable research has been done to take what was a laboratory technique requiring surgical insemination of small numbers of sexed sperm into the rabbit oviduct, and convert it into a practical technique for AI of cattle.

Sexed semen is now being produced commercially by most large bull studs in the United States, as well as several bull studs in other countries. The positive aspects of this technology are that accuracy of sexing sperm is close to 90% with large sample sizes, and the calves produced appear to be normal. The main negatives are that sexed semen is expensive, fertility of sexed sperm is somewhat lower than unsexed sperm, and this product currently is not available from all sires, especially those from beef breeds. However, it is possible to get semen sexed and frozen on a custom basis.

How expensive? In the United States, sexed semen currently costs about $20 more per straw than unsexed semen from the same bull. This will decline as technology improves, but probably not greatly for the next several years. Getting semen sexed from your own bull is even more expensive, and is not at all practical unless hundreds of doses are produced. There also is a considerable wait time, and currently this requires taking bulls to a company in Texas.

The largest cost of sexed semen is lower fertility. There are two main reasons for lower fertility: (1) fewer sperm are used per straw, typically 2 million compared to 20 million or more sperm for most bulls with unsexed semen, and (2) sperm are slightly damaged by current sexing procedures, so they do not swim quite normally. The extent of lowered fertility is about 10% under ideal conditions (good nutrition, well trained inseminators, excellent heat detection, very careful handling of semen). However, under average conditions, fertility of sexed sperm is about 70% of the fertility of unsexed sperm. For example, if control (unsexed) semen from a particular bull in a particular herd results in 60% pregnant, the pregnancy rate for sexed sperm likely will be 70% x 60% or 42%. Note that these are the results for AI 12 to 24 hours after clear standing estrus; they will still be lower for timed AI protocols. Sexed semen usually leads to disastrously low pregnancy rates under less than average conditions, and is not recommended unless a successful AI program has already been implemented.

In my opinion, the logical place to use sexed semen is breeding replacement heifers to produce more replacement heifers. These heifers often will be bred AI anyway, and there are several added bonuses: (1) there will be less calving difficulty if most have heifer calves instead of the larger bull calves; (2) the replacement heifers have the best genetics in the herd (if they do not, your breeding program is going backwards); and (3) this allows breeding most of the cows to have terminal cross calves since few replacement need come from the cow herd because most can come from first calf heifers.

Cloning
There are many definitions of cloning and many methods of cloning. Cloning of potential practical value occurs when copying an animal that is genetically outstanding. This usually is done by taking a very small biopsy of skin from the animal to be cloned, growing some of the skin cells in plastic dishes in an incubator, and using nuclei of those cells as the genetic material for the clones. Other body cells also can be used, such as roots of hair, somatic cells in milk or semen, etc. Usually the donor cells are frozen in liquid nitrogen, and thawed when the cloning step is done. This method of cloning is

often termed somatic cell nuclear transfer (SCNT) because somatic (body) cells are used instead of germline cells, such as sperm, eggs, and early embryos.

The nuclear transfer is done starting with removing the chromosomes from an oocyte (egg) that is ready to be fertilized, and fusing it to a somatic cell using an electric current (see Fig. 1). Causing the very large oocyte to fuse with the very small somatic cell is very similar to what occurs with normal fertilization, in which the sperm fuses with the oocyte. Therefore, SCNT could be thought of as fertilizing an oocyte with a somatic cell rather than a sperm. Somatic cells are diploid (means 2 or double) in their genetic make-up, with half the chromosomes (which contain the genetic material, DNA) derived from the sperm, and half from the fertilized oocyte that resulted in the original animal being cloned. The sperm and oocyte are normally both haploid. Because the chromosomes are removed from the oocyte when cloning, it is zeroploid; combining it with a diploid somatic cell results in a 1-cell embryo with the normal diploid genetic make-up.
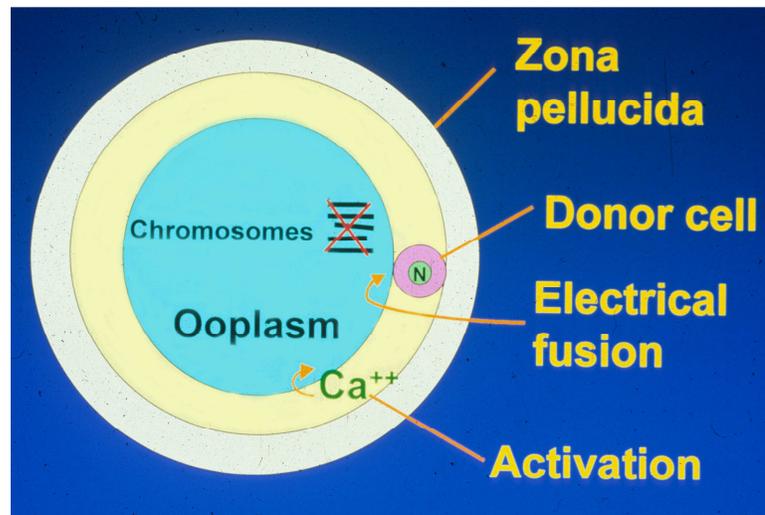

Figure 1. Diagram of cloning by somatic cell nuclear transfer (SCNT).

One other interesting point – at the time of normal fertilization, the oocyte is just sitting there in "neutral," waiting for something to happen; it actually is in the process of dying very slowly. When fertilized by a sperm, this large cell really is turned on by an enzyme that the sperm adds during fertilization. This process, termed activation, causes the oocyte to become active metabolically, and start the process of embryonic development, including duplicating the DNA, dividing to the 2-cell stage, etc. With SCNT, this sperm enzyme is not available, so it is necessary to add an activation step, which is done by giving it an appropriate electrical shock that increases calcium ions (Ca^{++}) in the oocyte.

SCNT is not a very practical procedure for many reasons. First, success rates are very low, making it very expensive. In addition to the expensive equipment needed, plus the services of skilled personnel, many embryos must be transferred, using many recipients to produce few pregnancies. Oocytes must be obtained from slaughterhouse ovaries or via transvaginal aspiration from ovaries of follicle stimulating hormone-treated donor

cows. These expenses add up to well over $10,000 per calf produced, and much more for other species such as horses because success rates are even lower than with cattle.

Current success rates with cattle are around 2% per oocyte fused with a donor cell. From 100 oocytes, only the best 5 to 10 resulting embryos usually are transferred, and these would typically results in 1 or 2 calves, although zero is often the result. Thus, success is highly variable. Abortion rates also are high, and many of the calves die at birth unless expensive intensive care procedures are used. The cause of most abortions and neonatal deaths is a faulty placenta. Many of these fetuses and calves are fairly normal themselves, but a poorly functioning placenta does not prepare them for a good start in life.

Another problem is that the incidence of abnormalities in the calves that do survive after birth is quite high, more than 20%, compared to 1-2% with calves produced by natural mating or AI. One common abnormality is oversize calves, sometimes over 130 lbs, although most of these become fairly normal as adults if taken by caesarian section and given intensive care.

One final consideration is that clones will not be phenotypically identical to the donor or their clone mates. They are almost genetically identical (slightly different due to normally occurring mutations and differing mitochondrial genetics; mitochondria come from the recipient oocyte), but an animal's phenotype (appearance and performance) is only partly due to genetics and can be greatly influenced by environment. The environment of every clone starts out quite differently from the original donor in that each uterus is different, and embryo transfer must be done to make clones. Clones are less alike than identical twins (the gold standard) and identical twins can be quite different in some ways.

Still another reason that clones (and identical twins) differ is due to epigenetic processes. One example of these is coat color patterns in spotted breeds; these patterns differ among clones.

The one place that cloning already fits in cattle breeding is copying a genetically superior animal for breeding purposes. The best examples of genetically superior cattle are bulls used heavily with AI with hundreds of offspring in herds that keep appropriate records. Such bulls have high repeatabilities for traits of offspring. Cloning such a bull will result in a bull that will produce calves with similar qualities in their offspring. One can think of many applications of this, including insurance on valuable bulls. Instead of a million dollar insurance payment, the company just produces another copy.

Note that cloning merely copies the best available, whereas animals better than the best sometimes occur with normal reproduction. Relying only on cloning the best cattle 10 or 20 years ago would have stopped genetic progress.

One obvious final issue is consumer acceptance of food from cloned animals. The Food and Drug Administration has conducted extensive studies and concluded after many

years that milk and meat from healthy cloned animals is no different from that of non-clones, and is therefore safe to eat. This does not necessarily mean that consumers will buy such food. It is controversial whether such food should be labeled as coming from clones or not.

Sperm Injection
Have you ever had a liquid nitrogen tank fail that warmed up enough to ruin the semen, but it was still fairly cold? Such sperm no longer have sufficiently strong motility to fertilize oocytes, or no motility at all, which ruins the sperm for AI, even though the genetic material is not necessarily damaged. A method that can often rescue these genetics is what I call "fertilization by brute force," injecting the sperm directly into the oocyte. The jargon term for this process is ICSI (intracytoplasmic sperm injection), and it is now used routinely for human infertility cases in which the husband's sperm are fairly normal except for motility characteristics. At Colorado State University, we also use the procedure routinely for stallions whose sperm are otherwise infertile.

The equipment used for ICSI is similar to what is used for cloning, although various details differ. The oocytes for ICSI would be collected from genetically valuable cows by transvaginal aspiration from ovarian follicles. After the sperm is injected, the 1-cell embryos are cultured in vitro as they develop to 2, 4, etc. cell stages. After about 1 week, they have over 100 cells and can be transferred to the uterus nonsurgically.

A number of experimental pregnancies have been produced with these procedures in cattle, but ICSI is not available commercially on a routine basis. Costs would be thousands of dollars per calf produced, which currently makes it impractical except for a few special cases. One application that is receiving considerable study is to use freeze-dried sperm. A fair number of lab animals have been produced by combining freeze drying and ICSI, in some cases from freeze dried sperm that have been stored for months. Storage at room temperature for more than a week seems to be a problem, but there has been some success with storage of freeze-dried sperm for many months in a typical household freezer at -20°C.

Early Pregnancy Tests
Although it is possible to detect some bovine pregnancies by ultrasound as early as 18-20 days after estrus, this procedure is not really reliable until about day 27 in heifers and day 29 in cows, and even this requires very skilled personnel. After day 30, this becomes easier.

There is ongoing research concerning a promising blood test that may become sufficiently accurate for useful pregnancy testing 17-18 days after breeding. A test available for women can be purchased in grocery stores and is accurate even 10 days after conception. Such a test might cost less than $5 for cattle and give an almost instantaneous answer. Because of routine embryonic death, over 10% of cows that are pregnant on day 18 will lose the pregnancy before term, so those positive with such a test would need to be rechecked later. The main value would be in identifying the

nonpregnant cows, because these then could be induced to ovulate and come into heat so that they could be bred again quickly. Such a test may be available in 2-3 years.

Transgenic Animals
Each animal is different genetically from every other animal except for clones and identical twins, triplets, etc. On the other hand, within a species, animals are much more similar to each other genetically than between species. The genetic blueprint of each animal is coded in its DNA, which has a 4-letter "alphabet" consisting of 4 different chemicals, adenine, thymidine, guanine, and cytosine, usually abbreviated A, T, G, and C. The DNA in each of our body cells, and those of other mammals contains about 12 billion of these chemicals (termed bases), 6 billion received from the father's sperm and 6 billion received from the mother's oocyte. In recent years, it has become possible to determine the entire sequence of these bases in an animal, and you likely have heard about sequencing the human genome or the bovine genome. The 6 billion bases always occur as base pairs, with each T connected to an A and each G to a C. While there are some stretches of the 3 billion base pair haploid genome (such as occurs in one sperm) that have not yet been sequenced perfectly, the most important parts are fairly well documented. An example would be the part of the genome that codes for hair color. It may have the sequence . . .AAGTGCAT . . . for black color and . . . AAGTCCAT . . . for red color, in other words one G changed to a C.

In addition to being able to sequence DNA reliably, it now is possible to change the sequence by adding, deleting, modifying, or changing the order of these bases. This is becoming relatively easy to do for cells growing in vitro, such as the skin cells discussed earlier for cloning. However, it also is possible to modify the sequence in sperm, oocytes, or early embryos. When such a change is made, it is said to be transgenic, and all cells of a transgenic animal have the change if the sperm or oocyte used to produce them was changed. This is because whenever cells divide, they faithfully copy the DNA sequence, so any change at the 1-cell stage is copied in the 2-cell embryo, again in the 4-cell embryo, etc.

Transgenics represent the ultimate tool for animal breeding. One could theoretically specify the DNA sequence in any portion of the 3 billion base pairs, and this could be done in a homozygous way (same change in the DNA inherited via the sperm as that inherited via the oocyte) or heterozygous (change in DNA from one parent but not the other). The advantage of a homozygous change is that the resulting animal will breed "true," so the change will be present in all offspring. The advantage of the heterozygous state for some genes is hybrid vigor, which is the genetic basis for the benefits of crossbreeding. With heterozygosity, only half of offspring will have the transgenic change.

Transgenic procedures are quite expensive, but have become easier and more reliable with cloning via somatic cells. Before cloning was available, the most common procedure was to inject 1-cell embryos with snippets of transgene DNA, some of which would get inserted into the genome. This was a haphazard procedure with a low success rate. With cloning, the transgenic change is made to somatic cells growing in vitro.

After the change is verified, one simply clones an animal using the genetically modified nucleus of the transgenic cell as a donor.

An example of applying this technology would be to take an outstanding horned Hereford bull, obtain some skin cells, modify the DNA sequence from horned to polled in a homozygous way, clone from the modified cells, and end up with an exact genetic copy of the bull except for being polled; all of his offspring now would be polled. Such changes could be made for any gene, such as hair color, tenderness genes for carcass characteristics, growth genes, etc.

These procedures are getting to be quite reliable, but they are expensive. Costs include the transgenic procedures, cloning costs, embryo transfer, etc. The procedures also are confounded with patent issues, so there are legal and licensing costs as well. For these reasons, plus the low success rates, this technology has been used primarily for research.

One application already being done with cattle is to modify them to produce human pharmaceutical products in bovine blood or milk. Cattle with human blood components are particularly attractive, as cows make good blood donors due to the large volumes that can be obtained. Eventually transgenic cattle also will be produced with useful production traits. One example is making cows resistant to mastitis, which would be particularly valuable for dairy cows.

Societal Issues

There is a fairly strong tendency by consumers to reject animal products produced via some of these technologies, particularly cloning and transgenics. These tendencies are exploited commercially, resulting in being able to charge much higher prices for natural, organic, bST-free, etc. products. Huge investments are required to determine the safety and wholesomeness of food resulting from transgenic or cloned animals. The application of cloning of cattle has been delayed for years due to these concerns. Even offspring of SCNT cattle were not allowed to enter the food chain in the United States until recently, despite no evidence of abnormalities.

The tendency to reject new technologies also occurred when artificial insemination was introduced in the 1940's. There were numerous popular articles about how monsters would be produced, that resulting animals would be unsafe to eat, that it would ruin the animals inseminated, etc.

Essentially all of the technologies described have natural correlates; for example, identical twins are clones and naturally occurring mutations are similar and sometimes identical to transgenic changes. For example, red-colored hair is due to a mutation for black color, and white color due to another mutation. Clearly, safety issues need to be addressed, but where to draw the line is a difficult judgment call, since it never is possible to prove that something does not have some very small detrimental effect.

Conclusions

This brief survey of new reproductive technologies is incomplete, as there are many other new technologies being researched including stem cells. These cells currently have more applications in human medicine than livestock production. The technologies discussed build on others, such as artificial insemination, genetics, and even nutrition. While most of the technologies mentioned are not likely to be used in animal agriculture to any great extent for the next decade, there will be some niche applications, and a few of them may eventually become sufficiently inexpensive and efficacious for widespread use. Meanwhile, it is important to use the powerful and efficacious technologies already available, such as estrus synchronization and artificial insemination. These truly are valuable tools for improving cattle. Perhaps the most difficult task is deciding what traits we really want, so animals do not get too big, give too much milk for the feed available, have too large a ribeye, or have calves that are too small in attempting to prevent dystocia.