When Handling Frozen Semen and Embryos, COWBOY LOGIC Ain't Good Enough

by ***Troy Smith***

Singer-songwriter Michael Martin Murphey celebrates the Westerner's thought process in a song titled "Cowboy Logic." Its humorous verse is packed with axioms meant to explain the cowboy way of handling a situation, tackling a piece of work or avoiding it. Like the witty adages of Benjamin Franklin, bits of cowboy logic have become fodder for poets and pundits and are often offered as examples of common sense.

Some samples, however, suggest a penchant for taking shortcuts that can lead to trouble.

Eighteenth-century poet Alexander Pope wasn't much of a cowboy. While he probably couldn't make a hand in cattle country, Pope is credited with coining the phrase, "A little knowledge is a dangerous thing."

Brad Stroud, Weatherford, Texas, veterinarian and cattle embryologist, fears that is a statement that applies to the handling of frozen semen and embryos. He's convinced that the level of knowledge is dangerously low among many people who routinely handle frozen reproductive cells. Stroud says that group probably includes many veterinarians.

In nearly 30 years of embryo transfer work, Stroud has experienced plenty of "anger, frustration and embarrassment" over unexplained breeding failures. The disappointments spurred his search for reasons why too many ova (eggs) recovered from artificially inseminated donor cows were found to be unfertilized. He's now convinced that more attention must be paid to the handling of frozen semen.

"We know how to freeze semen, but do we know how to handle it afterward? Is there an adequate curriculum for training the people that handle semen up until it is thawed and placed in a cow? To my knowledge, none exists," states Stroud. "We have a problem that should be addressed through formal training. The challenge is educating people that don't think they need it."

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Handling frozen semen

Among the evidence that a problem exists are data compiled by the American Embryo Transfer Association. Stroud points to records from 2006 showing that of some 600,000 beef and dairy ova collected during the year, roughly 48% were not viable.

"That seems like a lot of waste, doesn't it?" asks Stroud. "It's certain that unfertilized ova don't make us any money."

Stroud says research involving evaluation of frozen semen prior to use has shown that fertilization and embryo production were correlated with the quality of frozen semen used to breed donor cows. Documentation of the origin of frozen semen shipped to Stroud Veterinary Embryo Service shows that semen samples shipped directly from bull studs, where it was collected, processed, and stored, had an "unacceptable" evaluation rate of two per 100 batches evaluated. Semen samples personally delivered or shipped by animal breeders had an "unacceptable" evaluation rate of eight per 100 batches. This information suggests that semen that had been in the hands of animal breeders and other handlers was four times more likely to be unacceptable than semen coming directly from a bull stud.

"It tells us that bull studs do a good job," Stroud says, "and the uneducated (handlers) too often don't."

There is ample opportunity for mishandling – mistakes that expose frozen semen or embryos to temperatures at which cell damage or death occurs. This can happen when semen samples are received and transferred from a shipping vessel to a storage tank. It can happen when someone is preparing a shipment or taking inventory. Most common, perhaps, are mistakes made when a technician searches among a tank's contents for semen from a certain sire.

In Stroud's opinion these kinds of mistakes are made repeatedly in the field by people who try to be conscientious. They really believe they are doing a good job. Unfortunately, their cowboy logic is flawed.

"If a frozen straw of semen remains solidified during and after an exposure, the perpetrator thinks no harm has been done. Since the ice structure has remained intact, at least to the naked eye, he thinks the sperm should be fine," explains Stroud. "Unfortunately, that's not the case."

The process

When a person understands low temperature biology, witnessing those common handling mistakes should send shivers up his back. It will, in Stroud's opinion, when that person thinks about what happens to reproductive cells during the freezing and thawing processes.

The first step in freezing either semen or embryos is exposure, at room temperature, to cryoprotectants, such as glycerol or ethylene glycol. The purpose, says Stroud, is to remove water from within the reproductive cells. The second step is to slowly cool reproductive cells to minus 30 degrees Centigrade

(C), which causes any remaining intercellular water to move into the fluid surrounding the cells. Then the reproductive cells are ready to be plunged into liquid nitrogen, which is minus 196 degrees C.

According to Stroud, the reason for removing intracellular water is to keep formation of ice within the cells to an absolute minimum. Intracellular ice damages cell membranes, cellular organelles and even chromosomes. It is believed that semen and embryos frozen and stored properly in liquid nitrogen could remain viable for a thousand years or longer. Cellular metabolism essentially ceases and the cells don't age.

Care in handling

Stroud emphasizes that once sperm cells or embryos are cooled to a temperature below minus 130 degrees C, neither can be raised to temperatures above that mark and then re-exposed to lower temperatures, or cell damage can occur. Damage is caused by "recrystallization" — the reorganization of very small ice crystals in the extracellular fluid into much larger crystals that physically invade the reproductive cells. Damage severity depends on the temperature to which frozen sperm or embryos were raised (how much above minus 130 degrees C) and the length of exposure time.

What most people don't think about, says Stroud, is how temperature in the necks of most farm and ranch storage tanks varies in a gradient manner, ranging from minus 75 degrees C to nearly room temperature. For a tank that's three-quarters full of liquid nitrogen, the temperature of vapor just above the liquid is usually about minus 190 degrees, but the temperature one inch from the top of the same tank is only a few degrees cooler than the surrounding environment. Since many technicians routinely raise canisters and canes of semen too high in the tank neck for too long, samples may be repeatedly exposed to potentially damaging temperatures.

Stroud says cane tabs are usually marked with a code, rather than a bull's name. If a technician doesn't have cane codes recorded, the cane is lifted and hung above the tank frost line while a semen straw is removed, frost is wiped away and printed information on the straw is read. If it is the correct straw, it is placed into a water bath to thaw. If not, it is returned to the cane and the search for the desired straw continues. But every time a canister containing canes of frozen semen is raised high enough in the neck of the tank and long enough to allow the straws' internal temperature to exceed minus 130 degrees, a certain amount of cell damage occurs.

Stroud recommends application of the "eight second rule" to provide a safe working time for most handling events.

"If the mistake is repeated over and over, each exposure causes damage that is additive. So, poor handling habits can result in cumulative damage that can decrease sperm fertilizing capabilities and, in some cases, lead to total infertility," adds Stroud. "More often than not, nutritional insufficiencies and poor heat detection inappropriately get blamed for the problems."

According to Stroud, it can take as little as 30 seconds of exposure to the upper third of a tank's neck for the internal temperature of a 0.5 milliliter straw to rise sufficiently for post-thaw sperm motility to be negatively and irreversibly affected. For a 0.25 French straw, even less exposure time is required. Consequently, Stroud recommends application of the "eight second rule" to provide a safe working time for most handling events. However, some samples may still be jeopardized when canisters or canes are raised four or more inches above the frost line or into ambient air.

Cowboy logic isn't good enough when frozen reproductive cells are being handled. Damage to sperm and embryos can begin long before they are thawed, and there is ample opportunity for thermal stress when a person is preparing or receiving semen shipments, taking inventory and transferring semen from vessel to vessel. And while it may be difficult to admit that bad habits could be at fault, the consequences of semen handling methods should be considered when unexplained breeding failures occur. Stroud believes better training in proper semen handling technique could go a long way toward improving the overall success of artificial insemination and embryo transfer. Even for experienced technicians, periodic refresher courses may be well advised. **HW**