ARSBC 2012: The A.I. Technique

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."

Weatherford, Texas, veterinarian and cattle embryologist Brad Stroud said he fears that statement applies to the handling of frozen semen and embryos. He told attendees of the 2012 Applied Reproductive Strategies in Beef Cattle (ARSBC) symposium in Sioux Falls, S.D., that he's convinced the level of knowledge is dangerously low among many people who routinely handle frozen reproductive cells — including many veterinarians.

In nearly 30 years of embryo transfer (ET) work, Stroud said, he 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 said he's now convinced that more attention must be paid to the handling of frozen semen.

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>
> **— *Brad Stroud***

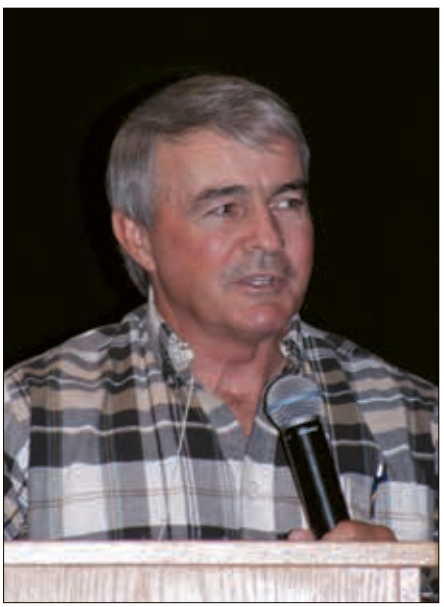
Call for education

"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," stated Stroud. "We have a problem that should be addressed through formal training. The challenge is educating people that don't think they need it."

Among the evidence that a problem exists are data compiled by the American Embryo Transfer Association. Stroud pointed 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?" asked Stroud. "It's certain that unfertilized ova don't make us any money."

Stroud said 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 showed 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 (see Table 1). Semen samples personally delivered or shipped by animal breeders had an "unacceptable" evaluation rate of eight per 100 batches. This 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.



▶ Research involving evaluation of frozen semen prior to use has shown 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, shared veterinarian and cattle embryologist Brad Stroud.

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

There is ample opportunity for mishandling — mistakes that expose frozen semen or embryos to temperatures at which cell damage or death occurs (see Table 2). This can happen when semen samples are received and transferred from a shipping vessel to a storage tank. It can happen when 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.

According to Stroud, 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.

Table 1: Quality assessment, by origin, of beef bull semen shipped to Stroud Veterinary Embryo Services

Origin of shipment	Total no. of shipments	No. classified unacceptable	% unacceptable
Bull studs	426	9	2.1
Owners/others	314	25	8

Note: This is Table 3 in Stroud's proceedings.

Table 2: Opportunities when damaging exposure is most likely to happen

1. Receiving and transferring samples from a dry shipper to a storage Dewar
2. Thawing samples
3. Taking inventory
4. Preparing samples for shipment
5. Breaking a cane (transferring one or more straws from one cane to another)

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," explained Stroud. "Unfortunately, that's not the case."

Freeze-thaw insults

When you understand low-temperature biology, witnessing those common handling mistakes should send shivers up your back. It will, in Stroud's opinion, when you think 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 –30° 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 –196° 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.

However, Stroud emphasized that once sperm cells or embryos are cooled to a temperature below –130° 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 –130° C) and the length of exposure time.

What most people don't think about, said Stroud, is how temperature in the necks of most farm and ranch storage tanks varies in a gradient manner, ranging from –75° 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 –190° C, but the temperature 1 inch (in.) 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.

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 –130° C, a certain amount of cell damage occurs.

Stroud said cane tabs are usually marked with a code, rather than bulls' names. 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 –130° C, a certain amount of cell damage occurs.

"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," added Stroud. "More often than not, nutritional insufficiencies and poor heat detection inappropriately get blamed for the problems."

Table 3: Example of temperatures taken at various depths in a Dewar semen tank

Depth in neck	Temp °C
0 in.	24.1
–1 in.	21.9
–2 in.	11.9
–3 in.	–23.0
–4 in.*	–47.1
–5 in.	–73.6
–6 in.	–110.5
–7 in.	–160.4
–8 in.	–181.2

*Frostline of this Dewar.

Note: This is Table 4 in Stroud's proceedings.

According to Stroud, it can take as little as 30 seconds of exposure to the upper one-third of a tank's neck for the internal temperature of a 0.5-milliliter (mL) straw to drop sufficiently for post-thaw sperm motility to be negatively and irreversibly affected. For a 0.25-mL 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 4 in. or more above the frost line or into ambient air.

Cowboy logic isn't good enough when handling frozen reproductive cells. Damage to sperm and embryos can begin long before they are thawed, and there is ample opportunity for thermal stress when preparing or receiving semen shipments, taking inventory and transferring semen from vessel to vessel. 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 said he believes better training in proper semen handling technique could go a long way toward improving the overall success of AI and embryo transfer. Even for experienced technicians, periodic refresher courses may be well-advised.

Stroud spoke during Monday's ARSBC session focused on AI technique. Visit *www.appliedreprostrategies.com/2012/SiouxFalls/newsroom.html* to listen to his presentation and to view the accompanying proceedings paper.

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Get Your Plan in Synch

Planner helps choose best protocol, plot schedule and analyze economics of various synchronization options.

*by **Shauna Rose Hermel,** editor*

When talking about synchronization of estrus, we spend a lot of time talking about how to get the cattle ready for a successful artificial insemination (AI) program, Sandy Johnson told attendees of the 2012 Applied Reproductive Strategies in Beef Cattle (ARSBC) symposium in Sioux Falls, S.D. People are part that process, too. Using the "Estrus Synchronization Planner," available as a free download at *www.iowabeefcenter.org/estrus_synch.html,* can help producers get the people involved in the program in synchrony, too, said the associate professor and livestock specialist at Kansas State University's Northwest Research and Extension Center, Colby.

"It's a spreadsheet-based tool that we have developed to help bring things together," Johnson said, describing the planner compiled by the Beef Reproductive Task Force.

Johnson explained that the task force was formed in 2000 when a group of extension specialists with research appointments wanted to join forces in a multi-state effort to effectively communicate to beef producers the latest information related to reproductive technologies. Rapid development of new systems to synchronize estrus and their associated acronyms created confusion in the industry, which resulted in a need to embark on a coordinated effort to provide clarity. Including others from industry, the task force standardized the terminology being used and developed protocol sheets providing recommendations for synchronizing heifers and cows (see pages 90 and 91).

"Those recommendations then represent a joint effort not only of the academic group but also working together with those in the rest of the industry … to create this short list of protocols to make your job easier of trying to select an appropriate protocol for your situation," she explained. The task force reviews and updates the protocols annually and hosts ARSBC symposia to help disseminate the information to improve success with reproductive technologies.

The planner

The "Estrus Synchronization Planner" is a spread-sheet-based decision-making tool that incorporates task-force recommended protocols to help producers choose the right protocol, visualize the schedule and communicate that to others involved in the program's success.

When you download the planner from the Iowa Beef Center (IBC) website, you will be asked to provide your name and email address, Johnson said, noting that that information will allow the task force to send you updates.

The goals of the planner are to direct you to recommended protocols, recognizing slightly different treatments exist for cows vs. heifers, and to generate a barn calendar that tells you what to do on what day and at what time.

Walking symposium attendees through the process of using the planner, Johnson explained that it starts by asking you the date you want to start breeding, the time of day and then how much time you want to spend detecting heat. Based on your responses, the program gives you a list of protocols recommended to fit your situation.

Johnson said the feature cattlemen find-

►Sandy Johnson walked attendees through a spread-sheet-based decision-making tool that helps producers choose the right synchronization protocol, visualize the schedule and communicate that to others involved in the program's success.

most useful is the calendar generated for the protocol selected. That schedule allows you and others involved in your synchronization program to see upfront if there would be a scheduling conflict with any treatment on the schedule. If you don't like the schedule, you can modify it from the front end.

The program also allows comparison of the cost of different synchronization programs, allowing you to input specific costs, such as for heat detection and pharmaceutical products, she explained. The program calculates a cost per AI pregnancy based on projected heat detection and conception rates.

Getting the right thing done on the right day, delivering the right product on the right day and at the specified intervals will help you complete a successful synchronization and AI program, Johnson said.

Johnson spoke during Monday's ARSBC session focused on AI technique. Visit *www.appliedreprostrategies.com/2012/SiouxFalls/newsroom.html* to listen to her presentation and to view the accompanying PowerPoint and two proceedings papers.

Fig. 1: Example of the "Estrus Synchronization Planner"