Strategies for the Successful Implementation of Sex Sorted Semen for Beef Females; Essential Advice for your Clients

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Abstract

The ability to predetermine the sex of offspring has tremendous managerial and economic implications in the beef cattle industry. With a world population close to seven billion, there is a growing need to produce more beef via the more muscle laden male. In commercial dairy cattle, the situation is just the opposite: the male is an unwanted by-product of breeding for female replacements.

Rabbit sperm was first stained and sorted in 1989, and within a decade the technology was improved through use of high speed flow cytometric sorting of DNA stained sperm. Sorting of bovine sperm has been available for the bovine industry in the US since 2003. The demand is high, but there are significant differences in how sorted sperm is processed before freezing compared to traditional non-sorted sperm. Also, the sorted sperm is packaged differently than traditional non-sorted sperm, which creates handling issues during shipping and thawing samples in the field. These differences have a significant effect on the conception rates of inseminated females. There are many management considerations on the farm or ranch that must be taken into account before a veterinary practitioner recommends sex sorted frozen semen to his clients.

Résumé

La possibilité de prédéterminer le sexe de la progéniture a d'importantes retombées économiques et de sérieuses implications au niveau de la régie pour l'industrie des bovins de boucherie. Comme la population mondiale approche les sept milliards, il y a un besoin pressant de produire plus de bœuf obtenu par l'intermédiaire des mâles mieux pourvus en muscles. Dans l'industrie des bovins laitiers, la situation est tout le contraire et les mâles sont des sous-produits indésirables de la reproduction pour des femelles de remplacement.

Le sperme des lapins a été coloré et trié pour la première fois en 1989. Dans la décennie qui suivit, la technique s'est améliorée avec le triage cytométrique à grand débit du sperme avec ADN coloré. Le triage du sperme chez les bovins est disponible pour l'industrie bovine'aux États-Unis depuis 2003. La demande est élevée, mais des différences significatives existent dans la manipulation du sperme trié avant congélation et celle du sperme non-trié traditionnel. De plus, le sperme trié est emballé de façon différente du sperme non-trié traditionnel ce qui crée des problèmes de manutention dans l'expédition et la décongélation des échantillons sur le terrain. Ces différences peuvent avoir des conséquences significatives sur le taux de conception des femelles inséminées. Plusieurs considérations au niveau de la régie doivent être prises en ligne de compte à la ferme ou au ranch avant que le praticien vétérinaire suggère à ses clients l'utilisation de semence congelée triée selon le sexe.

Introduction

In 1989 the USDA was successful in staining and sorting rabbit sperm with a high degree of accuracy.[1] A decade later the technology was sped up using high speed flow cytometric sorting of DNA stained sperm while retaining accuracy.[2] This technology was patented by the USDA and the global licensing rights to sort sperm of all species except human by flow cytometry is now owned by XY, Inc.[a] A Texas based company, Sexing Technologies[b] is now sub licensed in the US to sort bovine sperm and has been supplying service commercially for the bovine industry since 2003.

The flow cytometry process used to sort male and female bearing sperm is based on a volumetric difference in DNA between the X and Y-bearing-chromosomes. In the bovine the X chromosome contains approximately 4% more total DNA than a Y-chromosome bearing sperm. Although the human eye can't distinguish that difference a computer can. Freshly ejaculated sperm from a bull is processed and stained with a DNA specific fluorescent stain (Hoechst 33342)[c]. The sperm are then sent single-file, each in their own water droplet, through an elongated tube after being oriented with the edge side of the sperm facing a laser beam. A laser excites the fluorescent DNA stain and the intensity of the fluores-

cence is quantified by a photomultiplier and an optical detector. The more brilliant fluorescent sperm are X-chromosome-bearing sperm (more DNA). The female sperm's water droplet is assigned a positive electric charge as it moves down the tube. Conversely, the male sperm, containing less DNA, produces a weaker fluorescence and is given a negative electrostatic charge. At the end of the tube are two electromagnetic plates, one positive and the other negative, that repel their respective charged water droplet/sperm into conical collecting tubes. There is a third collection tube that receives the non-charged droplets, which contain dead sperm, droplets with two or more sperm, and improperly oriented sperm entering the tube. The process occurs at a rate of about 20,000 total sperm per second. Approximately half of the sperm is unsortable, and the other half is split equally into two populations of X and Y-bearing sperm (5,000 sperm of each sex per second). At this rate, a steady fine stream of what appears to be water, barely visible to the human eye, flows into the three test tubes. The process produces a product of 90% purity based on progeny testing in the field. The degree of purity can be increased by slowing down the rate of speed of the sperm through the cell sorter, or it can be decreased by speeding up the process.

Differences in Sex Sorted Sperm and Non-sorted Sperm

The process of staining, sorting, processing for freezing, and packaging the sorted sperm differs from traditional non-sorted sperm.

Non-sorted sperm are not stained. Sorted sperm are stained with a DNA specific dye that is non-intercalating and binds to the minor groove of the DNA helix. Sperm with Hoechst 33342 dye bound to the DNA are capable of fertilizing ova and producing healthy live young.[3]

The sorting process itself puts a certain amount of stress on the individual sperm. To push sperm through the flow cytometer/cell sorter requires mechanical pressure, which could harm anatomical structures in the sperm head, midpiece, or tail. Consider also that sperm exiting the cell sorter hit the collection tube at about 100 km/hr.[4] Logically, those are unnatural processes. Although there is no scientific evidence that staining and sorting damage the sperm's DNA, post-thaw motility is partially affected as evidenced by sluggish motility during microscopic exam (personal observation). Also, it is very likely that some bull's sperm cannot survive those challenges, which would lead to a decrease in its ability to fertilize ova. Post-sorting semen evaluation is a pretty good indicator of how stressful the process is on individual samples. However, practitioners microscopically evaluating thawed samples of sex sorted semen in the field should be careful to note that some samples appear sluggish immediately post-thaw, but "wake up" or increase speed of motility in five to ten minutes (personal observation). Also, IVF trials at Colorado State University with sorted and non-sorted controls shows about a 30% lower blastocyst rate with sorted sperm.[4] Recent advances show a closer correlation between IVF blastocyst rates of sorted and controlled sperm (Sexing Technologies, personal communication).

The biggest difference between sorted and non-sorted sperm is the total number of motile sperm that is packaged in each straw. Most bull studs package approximately 15 to 40 x 10^6 motile sperm per straw. However, sex sorted sperm is packaged in units of 2.1 x 10^6 motile sperm per straw. This is obviously a function of time and economics. Generally, a bull owner only wants either male of female sorted sperm and not both. Mathematically, at 5000 sperm sorted per second, it takes seven minutes to sort 2.1 million sperm, which is enough for one straw. Ten straws will take over an hour. Considering that the cell sorter alone costs over a quarter of million dollars, it's easy to see why the sorting companies must package fewer sperm per straw.

Another big difference between sorted and traditional frozen semen is how they are packaged. In the US most bull studs traditionally freeze bull semen in 0.5 ml plastic straws. Conversely, sex sorted sperm is frozen in 0.25 ml straws. This poses some real challenges in the field for cattle breeders, AI technicians, and otherwise anyone who is responsible for receiving shipments of frozen semen, thawing samples, taking inventory, or preparing samples for shipping. The 0.5 ml straw can stand more abuse relative to heat exposure during routine handling than can a 0.25 ml straw. In other words, handling exposure is a real threat to damaging or even destroying frozen samples in the field by those who don't understand the pathophysiology of exposure damage, and under what conditions damage begins to occur.

Management Considerations for Sex Sorted Sperm

Considering the differences between sorted and non-sorted frozen semen, management plays a big role in the ultimate outcome of sorted sperm. Sexing Technologies currently recommends the use of its sorted product for nulliparous females only. This recommendation is based on field trials done in the years preceding the commercialization of the science.[7] Heifers have a much smaller uterus than do mature cows. The smaller physical size of the tract allows a lower dose of sorted sperm to complete the journey from the body of the uterus to the sight of fertilization in the oviduct. The uterus of a mature cow is two to five times larger than that of a heifer, which makes a low dose insemination into the

cow less efficient. Therefore, recommendation number one is to inseminate single ovulating virgin heifers only.

The second recommendation is to use only very experienced AI technicians. Total time from thaw to actual deposition of semen is more critical with sorted sperm since they have been stressed more than non-sorted before freezing. Quarter-cc straws require different equipment than half-cc straws, which could be confusing to an inexperienced inseminator. Also, virgin heifers tend to have much smaller cervixes than mature cows. Experience counts when it comes to passing a Cassou[d] gun through a tight cervix that may have a kink or two.

Heat detection is also a critical factor in achieving high conception rates with sexed sperm. Although there is no scientific evidence to support it, sorted sperm likely has less longevity post-insemination than non-sorted sperm. Based on insemination trials in purebred beef cattle operations using electronic heat detection systems[e], the most effective time to inseminate beef heifers with sorted sperm is 16 to 20 hours post-first-mount. Cattle operations that use heat detection aids as their only means of heat detection will get lower conception rates than those who use visual observation or some electronic form of heat detection. For farms using manual heat detection two to three sessions per day the recommendation is to estimate first mount time and inseminate 16 to 20 hours later. When in doubt, breed later (16 to 20 hrs), not earlier (8 to 12 hrs) after an observed estrus for best results.

Synchronization protocols for timed AI (without heat detection) are certainly not recommended for sex sorted sperm. The assumed prime window for insemination is smaller for sorted sperm than non-sorted. Timed breeding without heat detection, especially when a fair percentage of prepuberal heifers are in the mix, can lead to very poor conception rates. If timed breeding schemes are the only choice, two units of semen per heifer are recommended. The synchronization protocol would determine when the two inseminations would be performed. Consideration must be given to the added expense of a second unit of semen and the labor to sort and inseminate females. Visible or electronic heat detection is the recommended protocol.

Selection of the heifers to be inseminated is an important consideration as well. Depending on the breed, the heifers should be the proper age and weight to be mature and healthy enough to cycle. Selecting heifers too young or too small (pre-puberal) can lead to poor results when synchronized with approved progesterone devices.[8] CIDRs[e] can trigger an estrus response in non-cycling heifers, but conception rates on those females will be lower compared to heifers that have already begun cycling naturally before synchronization.

Often an AI technician will thaw ten or more straws of frozen semen simultaneously to save time between inseminating females. Depending on the circumstances, the last few units could be thawed for 30 minutes of more before entering the uterus. For sex sorted sperm, it is recommended to minimize the time from thaw bath to heifer; less than five minutes is ideal.

Facilities are also a management concern. Gathering and sorting groups of females to single out one or more for insemination can be stressful to both man and beast. Well designed pens and chutes will minimize time and stress from sorting until insemination. Good breeding chutes with adequate individual animal restraint help minimize time from thaw until semen deposition into the uterus. Poorly designed facilities can ad significant amounts of time to thawed sorted semen, thus producing lower conception rates.

Handling frozen semen, especially sorted sperm packaged in quarter-ml straws, is a major concern. Watching cattle breeders and their employees handle frozen semen packaged in half-ml straws over the years has been an enlightening experience. Not a single client at Stroud Veterinary Embryo Services (> 1500) has demonstrated an understanding of how to handle frozen semen or embryos in a satisfactory manner. In almost all instances proper handling protocol has not been followed. Exposing frozen semen to damaging temperatures is more the norm than not for clients in my practice. Furthermore, our extensive data set shows that owner handled frozen semen is eight times more likely to be rejected for use after microscopic evaluation than semen shipped directly from a bull stud to my embryo transfer facility.[9] In other words if owners or their agents hand deliver frozen semen for donor breeding to our facility, it has likely been mishandled by any number of people after it left the bull stud where it was collected, processed and stored. There is no formal training for animal owners on this subject other than what they get at AI school. They are not covering the subject thoroughly enough.

Once frozen semen or embryos have been processed and stored in liquid nitrogen at a temperature of – 196° Celsius, they will be damaged if warmed up to – 130° Celsius, and then returned to a temperature that is below – 130° C, i.e., liquid nitrogen or vapor. The damage is caused by tiny ice crystals in the extracellular solution coalescing (at – 130° C) and re-forming into large crystals after being re-introduced to below – 130°C. This is called the re-crystallization effect.[5] Cell damage occurs when extracellular crystals enlarge and invade cell membranes of sperm and embryos. In the case of sperm cells, the acrosome is the most affected component. The severity of crystal reformation and consequent cell damage depends upon the exposure above – 130° C and the duration of that exposure. There are many situations during routine handling that contribute to exposure in-

duced cell damage. These damages are cumulative, and over time, can contribute to a noticeable decrease in conception rates. In rare cases, exposure is so severe that semen is completely non-motile (dead) post-thaw. At our in-house embryo transfer facility we see at least one batch of dead sperm post-thaw every year, some of which is due to a Dewar going dry of liquid nitrogen, and some from gross mishandling. There is an obvious difference in the two.

The surface-to-volume ratio of quarter-cc straws is much greater than that of half-cc straws, which means that quarter-cc straws (sexed sorted sperm) will dissipate heat at a much faster rate than traditional semen packaged in half-cc straws. Considering that 0.5 ml straws warm to – 100° C in about 10 to 12 seconds in the neck of a typical farm or ranch Dewar,[6] quarter-cc straws will do so in half that time or less. It becomes very clear that sex sorted sperm stored in quarter-cc straws can be easily exposed/damaged during routine handling, i.e., thawing samples for inseminating. One significant exposure event, or several short cumulative ones, can lead to a significant reduction in conception rates on a batch of frozen sex sorted semen.

Breeding Parous or Superovulated Females

There is great temptation in the field to ignore the recommendation to breed sorted sperm to virgin heifers only. Some of my clients have inseminated multiparous females with the standard 2.1 x 10^6 sperm with mixed results. However, it appears that both the science and art of staining and sorting are steadily improving. In the 2006 spring breeding season, a purebred beef client inseminated 25 mature cows using the standard 2.1 x 10^6 sperm/straw and got 16 cows pregnant through one heat period. I was excited when we palpated the cows, but he later told me that they used two straws on each cow per estrus period. The first insemination was done about 12 hours post-first-mount, and the second at 24 hours. That's pretty expensive semen, but he got a desired result that could prove to be a good investment several years down the road when he reaps the genetic benefits of those extra heifer calves that will be born in 2007.

Recently, Sexing Technologies has started packaging 5 x 10^6 sperm per straw for inseminating superovulated donors for embryo collection. The data that I have so far is strictly anecdotal in nature, but initial results are promising. The first two donors collected were mature Simmental cows that were inseminated with two straws each at exactly 18 hrs post-first-mount. Donor A produced 15 viable embryos out of 25 total ova collected. Donor B produced 20 viable embryos out of 21 total ova collected. A third donor was recently collected at the same farm and we recovered 8 viable embryos from 11 total ova collected. A fourth donor produced only one ova and it was unfertilized. More data is needed before we can make recommendations to use it or not in embryo transfer programs.

Conclusion

Pre-determining the sex of offspring has been one of the most sought after technologies since the beginning of recorded history. We finally have the tiger by the tail, but sorted sperm requires ultimate management conditions in order for it to work efficiently under field conditions. A cattle breeder's ability to properly store, handle and deliver a healthy dose of semen to the estrus female at the appropriate time is essential for conception. A breakdown of any of these components can be catastrophic to herd reproduction, especially when using sex sorted sperm. Many who try it will be unhappy with the outcome. But, those who truly have an understanding of genetic, nutritional and reproductive management will make it work in their favor. Veterinary practitioners with insight into the differences between sorted and non-sorted sperm will be able to advise their clients accordingly. To advise against using it in certain conditions is the responsible thing to do. The client and companies who produce it will both be better off not hearing bad news about the sorted product. Those breeders who have the proper infrastructure, knowledge, management savvy and commitment to implement the technology will benefit greatly from its advantages.

Footnotes

[a] XY Inc., Fort Collins, CO 80524
[b] Sexing Technologies, Navasota, TX 77868
[c] Calbiochem-Behring Corporation, La Jolla, CA USA
[d] Cassou, IMV, Minneapolis, MN
[e] HeatWatch®, Denver, CO 80216
[f] EAZI-BREED™CIDR® Pfizer Animal Health, New York, NY

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