

## **A.I. Technology is Changing Rapidly!! (Molecular Genetics and Sexed Semen)**

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### **New Tools Coming In Bovine Genetic Development**

The United States has been a leader in the implementation and development of new genetic tools to advance dairy and beef populations. Today, more dairy genetics are exported to countries from the U.S. than from any other major dairy producing country in the world. I would like to give you a brief history of the advances in genetic improvement and introduce to you the new technology in molecular genetics. Then, I would like to explain some of the work currently underway and close by providing you insight into what tools you can expect to improve the health and production of your herd.

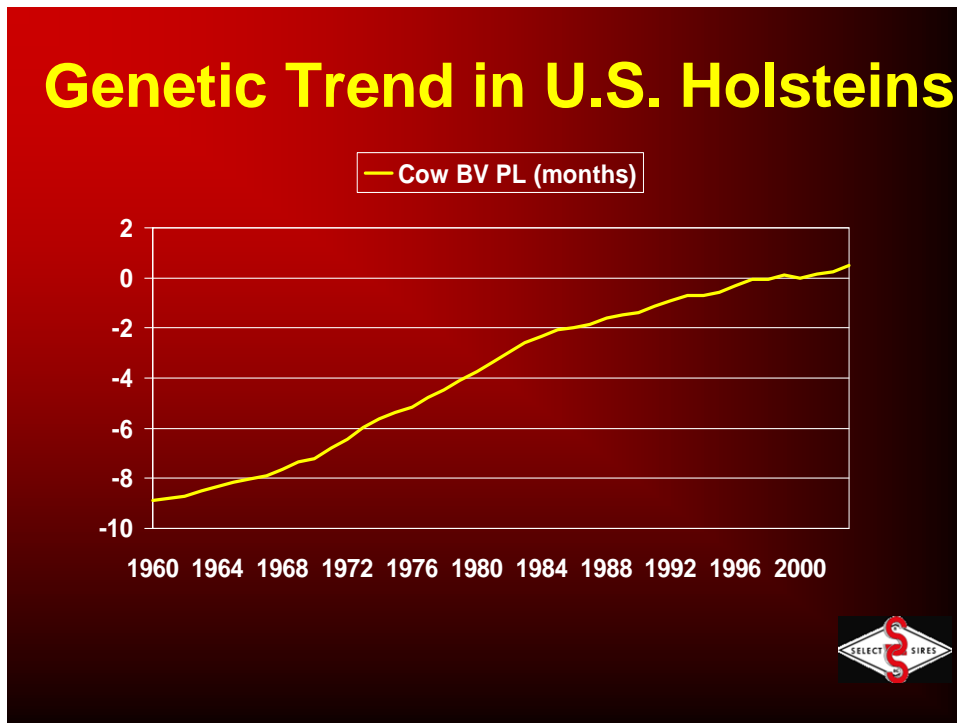
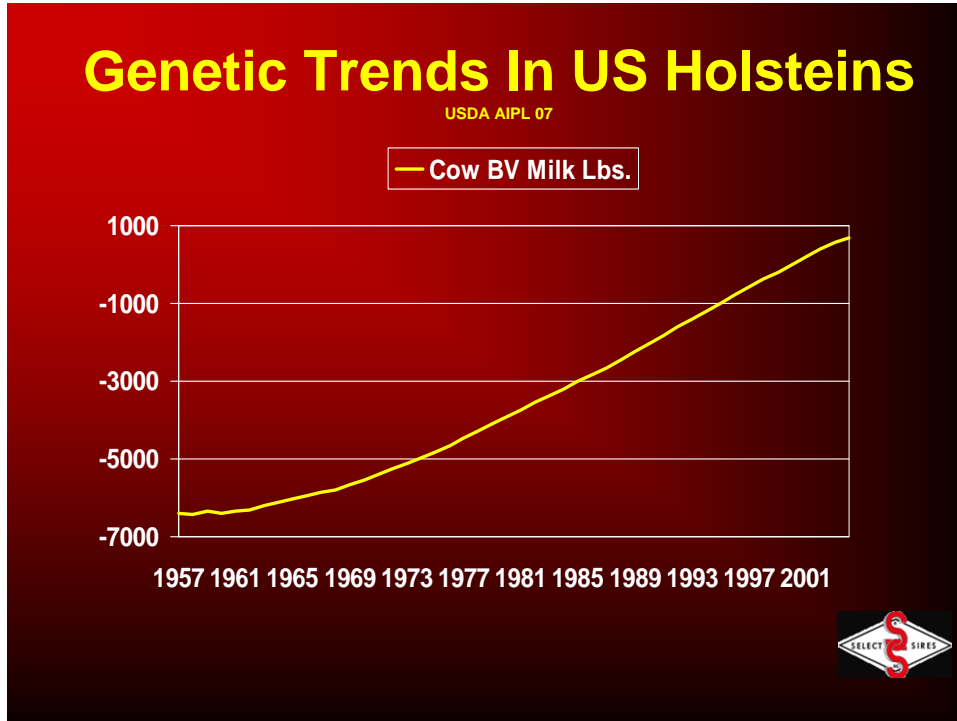
Genetic improvement started in the mid-1800s when European breed societies were formed to track parentage and record characteristics in various dairy and beef breeds. The U.S. followed suit a few years later. In addition, during that time late 1800's and early 1900's bull rings (syndicates) were formed in Europe and the U.S. to allow dairymen the opportunity to afford and share top sires that had traits they wanted to add to their herds.

During World Wars I and II, European genetic improvement was disrupted. Some records were lost and destroyed and the rebuilding efforts disrupted progress while the U.S. started to move ahead with implementation of A.I. The advent and implementation of A.I. in the U.S. by the cooperative extension service provided the vehicle to distribute great sires. The first Artificial Insemination station using fresh semen was started in New Jersey in 1938. This allowed many farmers to breed more offspring through semen from top pedigreed bulls. Frozen semen was developed by Dr. Christopher Polled in England in 1951. This allowed for the storage and transportation of semen to greater distances and for greater amounts of time.

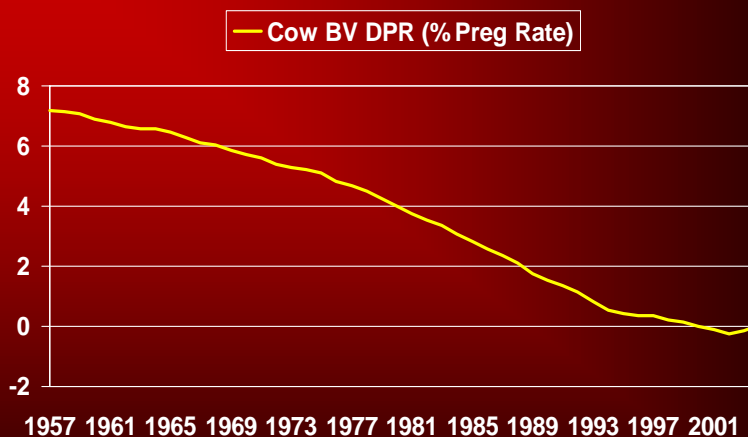
The major improvement was finally to be realized with the development of herdmate comparisons using Dr. Lush's idea and implemented into progeny test programs. In 1962 herdmate comparisons were implemented. The first bull and cow indexes were published in 1964 by R.H. Miller. The concepts of linear type was developed by Dr. Paul Miller at Cornell in 1971 and adopted by the Jersey and Guernsey breed associations in 1980. This replaced the previous true-type model that was previously used by most of the associations.

Below you will see the trends in cow breeding values from 1957 through 2001. You can see that progress was slow until the advent of progeny testing that was implemented in

the 1960's. Since that time, improvements have been made in the area of evaluation models and increased number of sires to be selected and have allowed the continual increase in the genetic trend for milk production in the U.S.



## Genetic Trend in U.S. Holsteins



With the ability to breed for high production, more and more dairymen were requesting animals that could withstand the stress of high production, thus type traits were developed by breed associations to improve udders, feet, legs and total conformation. Low heritability of health traits made those unpopular as a breeding goal but was developed in the late 1990s by USDA. Today's goal for Select Sires and many organizations around the industry is to develop a higher producing animal that is resistant to the many reasons that it would be culled from the herd. The United States Department of Agriculture and the breed associations are measuring over 29 different traits that are available for producers to use to improve the productivity and longevity of US dairy cattle.

However, today there are many challenges to the traditional progeny testing methods in providing genetic evaluations for elite males and females to make improvement and provide elite genetics. The first is the 5-year generation interval which takes a long time to create progress. Progeny testing is costly as to produce a good bull, only one out of 13 bulls progeny tested is selected for active service at Select Sires. In addition, there is slow progress due to low accuracy in low heritability traits that are usually associated with fitness. The new tools of molecular genetics show great promise to create some solutions to improve the speed and success of genetic development for AI companies and US herds to increase the rate of genetic progress by identifying those bulls who received a favorable sample of genes from their parents. So, let me take you through a review of the definitions of molecular genetics and discuss the possibilities.

The present practice of traditional progeny testing is to create a group of progeny (usually around 100 progeny of an animal), measure that progeny's performance and

compare it against its contemporary herd mates. This information is to estimate the parents' contribution and create breeding values for the parents of those individuals. To select the animals to test, it is assumed that the genes of that individual are an equal distribution of half from the sire and half from the dam.

Molecular genetics is where we actually investigate, once the embryo is developed, the actual genes that were transmitted from the sires and dams of these individuals. Today, single nucleotide polymorphisms (SNP's) have been mapped in the bovine by the U.S. Department of Agriculture through partnerships with many universities and countries around the world. These nucleotides make up the two strands of DNA that make up a chromosome. Chromosomes come in pairs and there are 30 pairs of chromosomes in each bovine. Where we find a single nucleotide on one strand of DNA that is different from normal, it is called a polymorphism. Polymorphism in its simplest term means different. These differences are then mapped and appear on various spots across the chromosome. The ability to track these differences and correlate their effects with known genetic levels will allow statisticians to develop prediction models to forecast the performance of an animal using these SNPs as they occur across the 30 chromosomes. Thus, the differences between full siblings can theoretically be identified as soon as the animal is created. Therefore, you will be able to predict the differences among various individuals in terms of their estimated transmitting abilities just from these molecular predictions. This information, when combined with the pedigree information, can be used to better predict the transmitting ability of that individual.

In 2001, a Dutch researcher named Meuwissen published a theoretical research study identifying the improvements that can be made using molecular markers to enhance the accuracy of prediction. As you will see in the chart below, the improved accuracy will move a high heritability trait from a pedigree of 40% to an estimated 85% with the additional marker information and a low heritability trait that is .25 to .30 will increase the accuracy of the estimated transmitted ability to .65 to .70.

	High Heritability	Low Heritability
Pedigree	0.40	0.25 - 0.30
Ped + Markers	0.80 - 0.85	0.65 - 0.70

Meuwissen, et al., 2001 (Genetics 157: 1819-1829)

In 2007, Dr. Paul VanRaden did a theoretical simulation using 50,000 SNPs equally distributed across the 30 chromosomes. He identified that the parent average reliability in the pedigree of 36% would increase to 58% to 71% in accuracy making major improvements in the accuracy of that estimate. Improved statistical methodologies such as a Bayesian method could increase the reliability of these predictions as these statistical techniques are refined.

Under the direction of Dr. Curt VanTassel, the USDA's Animal Improvement Laboratory is presently undergoing a study in cooperation with NAAB members who participate in

the Cooperative Dairy DNA Repository (CDDR). In this research study, 3500 proven animals will be evaluated based on approximately 54,000 different SNPs in Holstein, Jersey and Brown Swiss. Each of these animals will be used to create a haplotype map. The scientists at USDA will develop statistical methodologies to compare their proven information with these SNPs and develop an estimation model for use in these breeds. This information could become part of the national genetic evaluations in early 2009. The contributors to the cooperative dairy DNA repository are Select Sires, CRI, ABS, Accelerated Genetics and Semex.

So, what should we expect to receive from this new marker information in regards to PTA's? First, the PTA's that would be presented would look just like those presented today in either the form of a progeny proof or a parent average PTA. The difference is that the reliability will be higher when the molecular evaluations have been preformed. Conservative initial estimates of Increases in accuracy of production and type estimations of male and female PTA's will go from 30 to 40% in reliability based on the trait to an average of 45 to 60% reliability for most production and type traits. In addition, major improvements in lower reliability traits such as productive life and DPR will move accuracies of a parent average from 15-30% to 40-55% reliability based on the trait. Thus, with the new tools we will know more about a virgin heifer who has had a molecular test, than we will about her mother with two calves and two lactations with no molecular testing. This information will be available in both Jerseys and Holsteins. In addition, as researchers have more time to develop more sophisticated statistical models, we can expect the accuracies of each of these traits to improve over time.

The benefits of this technology will be a better selection of bull dams and young bulls for progeny testing. This will increase the rate of genetic progress especially in improving low heritability traits. Dairy herds will be able to use young bulls with more confidence and less disaster where animals have serious negative assortment of genes. Additionally, dairymen will be able to better select dams from which to flush or save bulls for breeding as well. Faster progress in health traits will be important in all of the major dairy breeds as herd sizes and production levels continue to increase.

One area that has been talked about in major publications is inbreeding. Understanding the sources of genes will give us a better understanding of the level of inbreeding. This tool will allow us to slow or reduce the levels of inbreeding in production herds. The end result is that we should have better tools to make faster progress, higher producing, healthier, long-lived cows, and continue to expand the US influence in genetics both in the US and around the world.

*Sources for this presentation: 100 Years of Research and Inquiry by the American Dairy Science Association; Meuwissen, et al., 2001 Genetics 157; 1819-1829; Paul VanRaden, PhD, USDA 2007, Dr. Kent Weigel, PhD, University of Wisconsin – Madison; Mr. Chuck Sattler MS, Vice President Genetic Programs, Select Sires, Inc.*

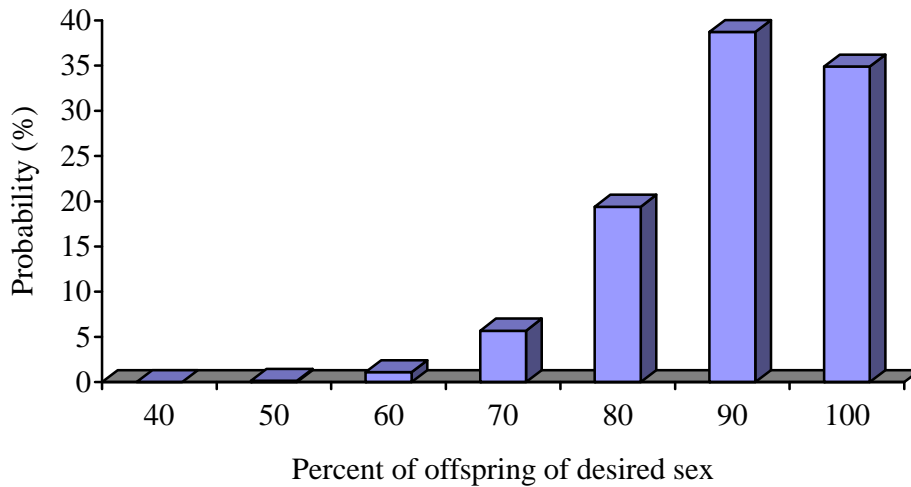
## **Sexed Semen: Is It Finally a Reality?**

Most of us have heard the rumor that sexed semen is “just around the corner” for as long as we have been aware of A.I. Through the years, countless numbers of techniques have been investigated with no potential application in the real world. However, in the 1980’s a breakthrough in semen sexing technology was made by USDA researchers in the Lawrence Livermore Laboratory in California. The patents for this technology were licensed to a company named XY, Inc of Fort Collins, CO, which performed extensive research during the 1990’s to optimize efficiency of these sorting procedures. In 2001, Select Sires partnered with XY, Inc. to set up field tests on the flow-cytometer processed semen.

### **Flow-Sorting Technology**

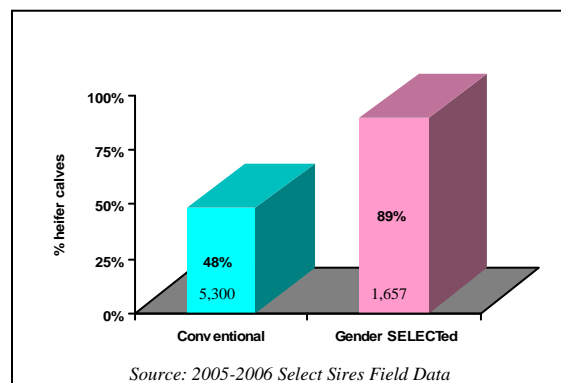
One of only a few repeatable techniques to sex sort sperm at a high level of purity uses a device called a flow-cytometer to detect a 3 to 4% difference in DNA content between male and female sperm and sort them with upwards of 90% purity. The first step in this procedure is to dilute sperm to a very low concentration and stain them with a fluorescent dye. The sample is then sent through the flow-cytometer at 60 mph under 30 to 60 psi of pressure. As sperm pass through the internal laser beam, the fluorescent dye is excited. Because of the larger X chromosome, female sperm emit slightly more light than male sperm, which possess the smaller Y chromosome. Detectors measure the amount of fluorescence and assign positive or negative charges to each droplet containing a single sperm. Charged deflector plates then split the single stream into 3 streams: positively charged particles containing one sex go one way, negatively charged particles containing the other sex are deflected in the opposite direction, while uncharged droplets containing multiple sperm or unidentified sex pass straight through. Confirmed with tens of thousands of offspring born in world-wide research trials, the procedure separates sperm of the two sexes with ~90% purity. However, that still leaves 10% of the undesired sex available to compete for fertilization. The table below illustrates the probabilities of all possible occurrences of offspring generated by 90% pure sexed semen.

Probabilities of all possible occurrences of offspring gender from 10 pregnancies generated by 90% pure sexed-semen.



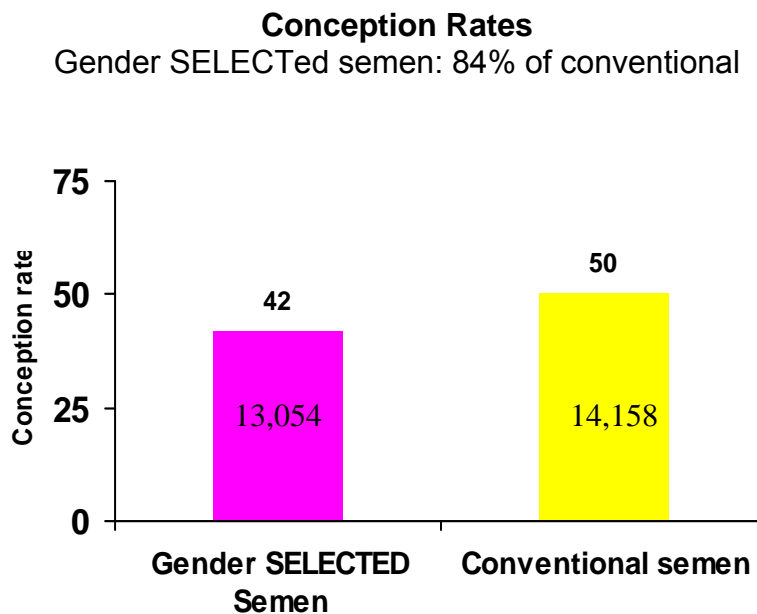
Commercialization of sexed semen in the U.S. was initiated with a 2003 license granted to Genetic Resources International (GRI) in Navasota, TX. In late 2004, Select Sires partnered with GRI and sent four proven sires to Texas to begin collection and processing of sex-sorted semen. In 2005-2006, Select Sires conducted a nationwide field test and collected information on over 27,000 services. These trials were conducted in a random sample of herds with average or better reproductive efficiency in order to accurately assess product performance for the “average producer.” The resulting calvings have a current data set of nearly 7,000 offspring so far with a gender ratio of 89% heifers when using sexed semen.

### Gender Bias of Calves Reported To Date



## Technology Limitations

There are several major limitations that have stifled implementation of sex-sorted semen. Without question, reduced conception rates have been a primary hurdle. As you can imagine from the description above, sex sorting of sperm is a highly invasive procedure that negatively impacts sperm viability and longevity compared to normally cryopreserved semen.



*Source: 2005-2006 Select Sires*

In addition, the procedure is extremely slow and inefficient. To properly sort, sperm must be precisely oriented as they pass through the laser and fluorescence detectors in the flow cytometer. Due to the flat shape of bovine sperm heads, only about 30% are correctly oriented and half of these are female. Thus, only 15% of the sperm going into the machine are recovered as a marketable, sexed product. The high rate of sperm loss precludes use of Select Sires' "most elite" sires for production of sexed semen.

Although the 3,000 to 5,000 sperm of each sex sorted per second sounds like a lot, this translates into ~1.3 hours of sorting to process enough semen for a standard 20 million sperm/straw dosage. Thus, due to the slow sorting speed, commercialization is only possible with very low sperm numbers per dose (~2 million). If these limitations were not enough, the high cost of flow cytometry equipment (~\$250,000 per machine) and intensive amounts of highly skilled labor required to sort sperm dictates that sexed semen will not be inexpensive. Because of the low sperm numbers per dose and compromised sperm viability, Select Sires only recommends its use in well-managed, highly-fertile, virgin heifers. While many research herds have realized very acceptable conception rates, averages indicate well-managed herds that achieve 60 to 65%



conception rates in virgin heifers with normal semen can expect 45 to 55% conception rates with sexed semen.

Based on the favorable field results, Select Sires began marketing sexed semen in the fall of 2005. In early 2006, four sorting machines were installed at Select Sires headquarters in Ohio to expand the sexed semen lineup. In the fall of 2006, two additional sorting machines were added to bring the annual production capacity to 350,000 straws. Orders for sexed semen have exceeded our expectations.

### **What is the return on investment for sexed semen?**

The return on investment for the dairy producer depends on a complex interaction between the initial conception rate with non-sexed semen, the percent reduction in conception (if any) due to use of sexed semen, the price differential between sexed and conventional semen, expected gender ratio for sexed vs. conventional, and the value differential between bull and heifer calves. Most of these factors will change considerably from herd to herd, which differentially affects the breakeven value of sexed semen to each respective producer.

To calculate a return on investment (ROI), Select Sires has recently developed a sexed semen calculator in Microsoft Excel format which incorporates more than 20 other variables which will vary from herd to herd. It will assist you in determining how this new opportunity can best be utilized in your individual operation.

Based on the product available today, the best return on investment will be achieved by limiting this product to virgin heifers only and following the “Keys to Success” (see below) to insure optimum probability for conception.

### **Keys to success**

Use of sexed semen will require a breeding gun designed to accommodate the smaller diameter ¼ cc straws. Straws are to be thawed and handled identical to their ½ cc counterparts. However, the smaller diameter and compromised semen quality will make them much more sensitive to cold-shock and errors in semen handling. To maximize potential for success:

- Thaw straws in 95° F water bath for 45 seconds.
- Semen thawing and handling environments should be warm and draft free.
- Warm all semen handling equipment including guns, sheaths, and paper towels prior to contacting straws.
- Only highly experienced technicians should use this product.
- Use only in well-managed, virgin heifers that have achieved greater than 60% of their mature weight by 14 months and in moderate or better body condition.
- Inseminate heifers 8 to 12 hours after observed estrus (AM/PM Rule).
- Use of estrus synchronization and breeding to observed estrus is encouraged, but use of timed-AI in the absence of observed estrus is discouraged.

## **Other methods of sorting semen**

A number of new sex-sorting technologies and companies have recently appeared. Other methods include gender specific antibodies, centrifugation, and free flow electrophoresis. As you evaluate other technologies, please take the time to be wary, and ask numerous questions in order to make informed decisions. If the sex-sorting technology is not based on flow-cytometry and the patents developed by USDA, you should ask for scientific evidence that the procedure can, in fact, sort sperm. Accept nothing short of hundreds of births to assess whether the procedure can effectively produce offspring of the desired sex. Similarly, conception data should be based on thousands of services and should be based on palpated pregnancy data, as simple non-return data may mask results and distort the success that can actually be achieved. To date, only flow cytometry provides the best combination of sorting purity and commercial adaptation.

## **Summary**

There is no question sex-sorted sperm for gender selection is now a reality. The product currently offered by Select Sires is backed by over 5 years of extensive field research and has been rapidly accepted by the U.S. marketplace. Currently, 18 of the 23 proven Holsteins and 2 of the 3 Jersey sires in the Select Sires sexed semen lineup have demand that exceeds the supply.

Because of continuing research, there will likely be improvements to the product and there will be the potential to utilize sex-sorted semen on lactating cows but current fertility results indicate its best fit right now is on virgin heifers.

*Sources and Credit for Program and Research: Mel DeJarnette MS, Select Sires Senior Reproductive Physiologist, Clifton Marshall MS, Select Sires Vice President of Research and Quality Control; Dr. Ray Nebel PhD, Select Sires Senior Reproductive Physiologist; Dr. Don Monkey DVM, Select Sires Vice President of Operations*

## Notes

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