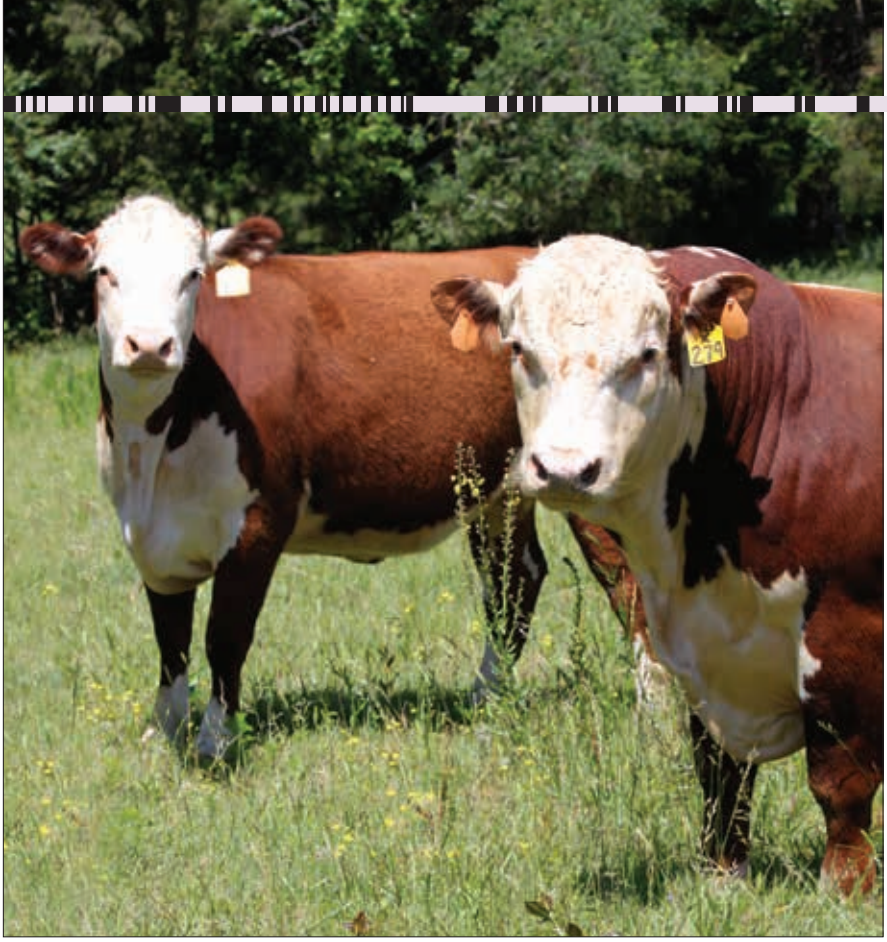
Genetic Factors that Affect Fertility



Identifying genetic markers in seedstock may increase fertility and profitability.

by ***Heather Smith Thomas***

With genomic sequencing technology, it is now possible to find genetic markers for various traits including those that affect fertility in cattle, such as lethal recessive conditions. Alison Van Eenennaam, University of California-Davis (UC-Davis) cooperative extension specialist in animal genomics and biotechnology, is part of a research project focused on sequencing the genomes of bulls to identify genetic markers linked to fertility. The project is in its third year of a five-year grant funded by the U.S. Department of Agriculture National Institute of Food and Agriculture.

"We started in January 2013 with Dave Patterson at University of Missouri (MU) as the leader for this integrated research project," Van Eenennaam says. "The team at Missouri is genome sequencing a number of key bulls in various beef breeds and looking for what we call missing homozygotes in their descendants in the population."

Jared Decker, assistant professor of beef genetic extension and computational genomics, is part of this research team at MU. "So far we have sequenced more than 150 animals," Decker says. "Our purpose in sequencing these is to find genes in which there is a DNA variant that breaks the gene. In other words, the gene no longer codes for a functioning protein. The protein that should be there is no longer being made correctly. Either that protein is too short or has the wrong amino acid in an important spot on the protein; there is a change to that protein that makes it not work properly anymore," he says.

Finding the genes

Decker says that most of these animals are perfectly normal if they are simply carriers of that gene with a protein defect. "If they have one normal copy and one broken copy they can use the protein from the normal copy," he explains. "But if an embryo inherits two copies of the broken gene, one from each parent, this situation is incompatible with life and that embryo is aborted usually early on, before it becomes a fetus."

A rancher may purchase a new bull and wonders why that bull does not sire as many offspring as expected during a short breeding season or why the cows take several cycles to become pregnant. Usually the cows get the bad rap if they conceive late in the season when in reality the problem may be a lethal allele inherited from both parents.

The industry has known about these lethal recessives, embryonic lethals, for a long time.

"Embryos don't always 'take' for a number of different reasons, but some years back people began to wonder about a genetic component," Van Eenennaam says. "If a bull is heterozygous for a given gene (inheriting two different alleles of a certain gene from each parent such as Aa rather than AA or aa), we would expect half of his offspring to inherit the big 'A' allele and the other half to inherit the little 'a' allele," she says.

When a bull is mated with a heterozygous dam, ¼ of his offspring would be expected to be homozygous aa. "However, there are situations where we never find any of his offspring with aa," Van Eenennaam says. "We only see ⅔ of his progeny with the AA genotype and ⅓ with the Aa genotype. If we never see progeny with the aa genotype, this suggests that this particular combination is a lethal defect and the embryo does not survive. If we never find these aa animals in populations where we would

expect to see some, based on allele frequencies, then this combination is what we call a recessive lethal," Van Eenennaam explains.

What cattlemen would see is a cow that didn't get pregnant until the second or third breeding because the conceptus from the first breeding(s) happened to inherit the aa genotype and did not survive. "We are hoping that if we can identify markers for these recessive lethal genes, this will give us information for mating selection strategies, to avoid mating two carriers of the same genetic condition," she says. "This would improve the chances that a cow could get pregnant on the first heat cycle.

"We've seen this phenomenon of missing homozygous genotypes in the large volumes of genetic data collected by the dairy industry. There were certain genes in which you never saw the homozygous genotypes even though you would expect to see them in a certain percentage of the calves. Even if the recessive allele is really rare, perhaps occurring in 1% of the population (one out of 100 animals), then you might expect to see it in the homozygous condition once in 10,000 animals. In the large dairy databases with hundreds of thousands of records, such homozygotes never appeared, even though they might have been expected to show up 8 or 9 times," she explains.

Dairy cattle researchers have used that information to pinpoint those particular genes and then sequenced the DNA at those genes.

"They found there were mutations in those particular genes that caused them to stop functioning," she says. "In the absence of those gene products, the embryo died."

Any conceptus that inherits two copies of a mutant recessive allele for those genes would not survive.

"This occurs very soon after conception," Decker says. "If you examine cows with ultrasound seven days after they have been bred, more than 90% of those matings result in an embryo. But if you look at those cows 45 days later, that percentage drops to about 60 to 66%. During those first 45 days we are losing a lot of pregnancies, and there is evidence suggesting that many of these early pregnancy losses are due to embryos inheriting two copies of a broken gene," he says.

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"Research in Holsteins identified a couple pieces of DNA that carry these broken genes. These pieces are called haplotypes and are strings of variants on the same strand of DNA. If an embryo inherits two copies of these lethal haplotypes, the embryo is aborted," Decker says.

The cow would return to heat and her brief pregnancy would probably go unnoticed. That cow is likely to be culled because she calves late and maybe comes up open the next year. If the rancher has a short breeding season and takes the bulls out after a certain time, those cows don't have a chance to rebreed.

"The cows with lethal recessives usually have longer time intervals between calving and rebreeding," Van Eenennaam says. "The dairy industry has an EPD for number of days from calving until the next pregnancy, and an increased interval can be seen in the EPDs when cows carry these recessive lethals." Dairymen have started managing some of these problems in Holsteins.

Improving fertility

"We are now looking for genetic markers linked to fertility in beef cattle," she says. For cow-calf producers, fertility is an important factor for profitability.

The current research project is sequencing many prominent beef sires in different breeds that have many offspring. "We have tried to sequence the most commonly used bulls such as the most popular artificial insemination (AI) sires, so that we will be more likely to pick up the damaged genes that are floating around in those breeds," Van Eenennaam says. This effort is being led by Jerry Taylor, MU professor of genetics and animal sciences, who is looking for any mutations in those bulls that would be predicted to either damage or totally turn off a gene. The researchers will then cross-compare these with essential genes (certain genes that are known to be required for life) and see if there is any overlap. Mutations in these essential genes might be the ones predicted to be problematic.

"This grant project will develop a research chip in which we can check for all the different predicted damaged/mutated genes," Van Eenennaam says. "We will genotype 10,000 animals, using this chip. Some genes will be apparently non-functional but we still have live homozygous animals. But if there are any missing homozygotes of the identical mutations, this would be very strong evidence that it actually is a lethal allele."

Dave Patterson, MU animal science professor, is in charge of getting the 10,000 animals together as part of the Show-Me Select heifer development program. The proposed outcome is a chip that includes all the recessive alleles that result in embryonic lethality. Animals carrying these alleles would be candidates for more careful mating selection to avoid producing affected homozygous offspring.

Researchers at UC-Davis in collaboration with Brian Kinghorn at the University of New England are simultaneously developing a computer software program to assign mate allocations, determining which bulls to mate to which cows, to avoid double heterozygous matings.

"If we find multiple lethal alleles, like 20 or so, it's not the end of the world," Van Eenennaam explains. "It just means that breeders need to know

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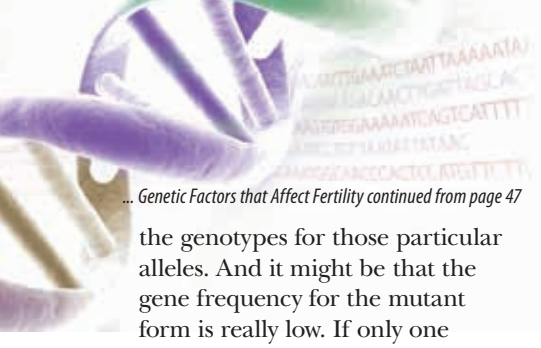

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the genotypes for those particular alleles. And it might be that the gene frequency for the mutant form is really low. If only one animal in 100 carries it, this wouldn't have a huge impact in terms of embryo loss because you'd have to mate it with another animal that carries that allele. And even then there's only a 25% chance that the embryo would inherit both copies. In a random mating population that would be one embryo loss in 40,000 matings," she says.

"We are looking at whether certain lethal recessives appear in just one breed or in several. If it's just in one breed, crossbreeding would take care of the problem because it would never be doubled up. We know that crossbreeding enhances fertility, and this could be one of the underlying contributing factors to that increase in fertility," she says.

Even though lethal recessives do not show up as obvious problems like curly calf, fawn calf, dwarfism, hairlessness, calves with no legs, calves with spinal defects, hydrocephalic calves, etc., purebred breeders should be aware that they do affect reproduction rates.

"It is more likely that animals within a breed will share some co-ancestry and are thus more likely, just by chance, to have some of these alleles at the same gene," Van Eenennaam says. "There is a reason why hybrid vigor exists."

There are also more genes to draw from when crossbreeding and less chance for double recessive traits and lethals.

Mutations occur in all animals all the time. Most of these mutations don't have any adverse effects — the animal is healthy and normal. "In nature, these recessive mutations are not doubled up very often," Van Eenennaam says. "Cattle are outbreeding species under natural conditions. If one parent carries a mutant form of a particular allele, we usually inherit the normal form from the other parent. Offspring rarely inherit the mutant form from both parents. That would only occur when both parents are carriers," she says

The situation changes when populations are limited in their gene pool. "If there is some co-ancestry in the pedigree, and if the mutant form of the allele comes from both the sire and the dam because they shared a great grandsire, for instance, this can result in the inheritance of a recessive lethal allele from both parents," she explains. "These situations are very common in purebred animals because there are prominent founding sires or popular bloodlines that get doubled up. This is the same thing that results with genetic mutations that create abnormal phenotypes (curly calf, dwarfism, etc). But you don't see lethal recessives because the embryo's genetic makeup is incompatible with life."

Making decisions

Van Eenennaam says cattlemen need to be sensible about how they manage breeding decisions. "We know these lethals exist, so we need to know which individuals are carrying which alleles," she says. "Then we can more intelligently manage them. Ideally we'd prefer to not mate two heterozygotes for that particular loci. When you just have one mutation to deal with, it's relatively easy if the mutant allele frequency is not very high. But when there are 20 or more, every animal will probably be carrying one or two mutant alleles. If you have some knowledge about which ones they have, or which pedigrees these are coming from, you can make better selections about which animals to mate those animals to."

At the very least, Van Eenennaam says producers can get an idea about the genetic load of a certain animal, and some will have more than others. "There is some value in having fewer of the deleterious recessives," she says. Breeders do not need to avoid carriers entirely as this might hinder genetic progress by putting too much weight on certain genetic conditions and not enough on the remaining genetic merit of the animal.

"People often think they don't want any carriers in their herd. If you made that decision about these 20-plus alleles, however, you might not have any animals left in your herd," she explains. "Recessive lethals should always be expected, but they are usually at low frequency and can be managed to avoid affected offspring."

Identifying carrier animals, however, would be a way to enable breeders to avoid having embryonic losses.

"More cows and heifers would likely settle on the first breeding which has many benefits," Van Eenennaam says. "Given the value of fertility in every cattle operation, even a 2% increase in conception rates on the first breeding is worth a lot."

If breeders are not strict in selecting for fertility, this problem can quietly build up in a herd such as when cows calve late and are not culled from the herd.

"This allows these variants to creep up to a higher level in a herd," Decker says. "The good news is that after we identify hundreds, maybe thousands, of variants this will enable us to create an EPD for embryonic loss. Now we will have a tool that can help us make progress in controlling this aspect of infertility and improve pregnancy rates."

As more is learned, cattle can be tested for these mutations. Genetic tests provide more tools for making good mating selections when breeding cattle.

"This data can eventually include matching a female up with the right bull that doesn't carry the same set of broken genes," Decker says. "We will have an EPD based on a genomic test. Compared to the other genomic predictions such as for weaning weight, ribeye area, marbling, etc., this EPD will be more specific. The DNA variants used in most genomic predictions are like mile markers along the chromosome. They are not actually the DNA variants that cause the differences between animals. But with this genomic prediction for embryonic mortality, instead of the mile markers we have the points of interest. This test will be unique because we will be testing the variant that is responsible for the phenotype. It's not just a nearby marker; it will be the variant responsible for the trait we are interested in. That's one of the unique things about this project and about the genomic predictions that will come out of it." **HW**