Fertility Factors
Fertility Research: Genetic Factors that Affect Fertility
By Heather Smith-Thomas

With genomic sequencing technology, it is now possible to find genetic markers for various traits (good and bad), including factors that affect fertility in cattle, such as lethal recessive conditions. Alison Van Eenennaam, PhD, Cooperative Extension Specialist, Animal Genomics and Biotechnology, UC-Davis is part of a research project focused on sequencing the genomes of bulls to look for genetic markers linked to fertility. The project is in its second year of a 5 year grant funded by the USDA National Institute of Food and Agriculture.

“We started in January 2013. Dr. Dave Patterson at University of Missouri is the leader for this integrated research project. 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,” she says.

Dr. Jared Decker, Assistant Professor, Beef Genetic Extension and Computational Genomics, is part of this research team. “So far we have sequenced about 150 animals. 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.

“Most of these animals are perfectly normal if they are simply a carrier 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,” explains Decker. 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.

Sometimes a stockman purchases 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 is a lethal allele inherited from both parents.

We’ve 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,” says Van Eenennaam. “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 mated with a heterozygous dam, we would expect ¼ of his offspring to be homozygous aa. “However, there are situations where we never find any of his offspring with aa. We only see 2/3 of his progeny with the AA genotype and 1/3 with the Aa genotype. If we never see progeny with the aa genotype, this suggests that this particular combination is some sort of lethal defect and the embryo does not survive. If we never find these aa animals in populations where we would expect to see some of them, based on the allele frequencies, then this combination is what we call a recessive lethal,” explains Van Eenennaam.
What you would see is a cow that looks like she didn’t get pregnant until the second or third round of 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 purposefully avoid the mating of two carriers for the same genetic condition. This would improve the chances that a cow could get pregnant on the first heat cycle,” she says.

“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. 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.

“So dairy researchers 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. In the absence of those gene products, the embryo died.” Any conceptus that inherits 2 copies of a mutant recessive allele for those genes would not survive.

“What we think is happening occurs very soon after conception,” says Decker. “If you examine cows with ultrasound 7 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 2 copies of a broken gene,” he says.

“There has been some research in Holsteins which 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.” The cow would return to heat, and you probably wouldn’t realize that she was briefly pregnant.

So instead of becoming pregnant early in the breeding season, she becomes pregnant at a 2nd or 3rd breeding, later in the season. That cow is more 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,” says Van Eenennaam. “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 started managing some of these problems in Holsteins a few years ago.

“We are now looking for genetic markers linked to fertility in beef cattle,” she says. This would be of great importance for cow-calf producers because fertility is such an important factor for profitability in this segment of the industry.

The current research project is sequencing many prominent beef sires (in different breeds) that have a lot of offspring. “We have tried to sequence the most commonly used
bulls (such as the most popular AI sires), so that we will be more likely to pick up the damaged genes that are floating around in those breeds. This effort is being led by Dr. Jerry Taylor in Missouri—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) in databases from humans and mice, and see if there is any overlap. Mutations in these essential genes might be the ones we’d predict to be problematic,” she explains.

“This grant project will develop a research chip in which we can check for all of the different predicted damaged/mutated genes. We are actually going to 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,” says Van Eenennaam.

Dr. Dave Patterson at Missouri is in charge of getting the 10,000 animals together as part of the Show Me Select heifer development program. The proposed outcome of this grant is a chip that includes all of the recessive alleles that do 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 Dr. Brian Kinghorn at the University of New England are simultaneously developing a computer software program to assign mate allocations (determine 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. It just means that breeders need to know the genotypes for those particular alleles. And it might be that the gene frequency for the mutant form is really low. If only 1 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 explains.

“We are now 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.

Purebred breeders need to know about this, because even though it is not like the inherited defects that show up as obvious problems—curly calf, fawn calf, dwarfism, hairlessness, calves with no legs, calves with spinal defects, hydrocephalic calves, etc.—it will 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. There is a reason why hybrid vigor exists,” she says. There are also many more genes to draw from, when crossbreeding, and less chance for doubling up recessive traits and lethals.

It is important to understand that all animals and humans have mutations. These occur all the time, and most of these mutations don’t have any adverse effects; the individual is healthy and normal. “The way it typically happens in nature, these recessive mutations are not doubled up very often. Cattle and humans, for instance, are
outbreeding species (not much inbreeding) 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,” says Van Enennaam.

Where that changes is in situations where populations are limited in their gene pool (a closed herd of livestock, or intentional linebreeding or inbreeding in a certain breed, for instance). “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. These situations are very common in purebred animals because there are certain very prominent founding sires or popular bloodlines that get doubled up,” she says.

“This is exactly the same thing that results with other genetic mutations that create abnormal phenotypes like curly calf. But you don’t see lethal recessives because the embryo’s genetic makeup is incompatible with life. It just looks like a skipped heat.”

We need to be sensible about how we manage our breeding decisions. “We know these lethals are out there, so we want to know which individuals are carrying which alleles. 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 lines of pedigrees these are coming from, you can make better selections about which animals to mate those animals to—at a very sophisticated level,” she says.

“At the very least, we can get an idea about the genetic load of a certain animal. Some will have more than others. There is some value in having fewer of the deleterious recessives. Sometimes carrier animals for known recessive conditions have been discounted at the marketplace. In this situation, however, we’re talking about one or two mutations out of 3 billion base pairs in the genome,” says Van Enennaam. It is important to not try 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.

“This has been a bit of a problem in how we’ve managed them up until this point, with people thinking they don’t want any carriers in their herd. If you made that decision about these 20-plus alleles, you might not have any animals left in your herd. So this is a matter of education, so people can realize they can manage this. Recessive lethals should always be expected, but they are usually at low frequency and can be managed—to avoid affected offspring,” she says.

“Identifying carrier animals, however, would be a way to enable breeders to avoid having embryonic losses. More cows/heifers would be more likely to settle on the first breeding—which has many benefits. This knowledge could be a help. Given the value of fertility in every cattle operation, even a 2% increase in conception rates on the first breeding is worth quite a bit.”

For people breeding purebred cattle, this is even more important, because there is more likelihood of these alleles doubling up. The stockmen who utilize crossbreeding are much less likely to have any problems, since there is less chance for the same defective genes to be inherited from both parents.
“Because it is recessive (and the animal can be normal with only one copy), it can creep up to a higher frequency in a certain herd or population of animals,” says Decker. This is especially true if the stockman uses a lot of inbreeding or linebreeding, which would double this up more often.

If a person is not really 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,” he says.

As we learn more, we can test cattle for these mutations. A genetic test gives us 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. We will have an EPD based on a genomic test,” says Decker.

“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 this project,” he says.