

## An Update on Arthrogyposis Multiplex in Cattle

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During the past eight weeks (since September 8, 2008), researchers at the University of Illinois and the University of Nebraska, in collaboration with the American Angus Association, have been investigating the genetic condition called Arthrogyposis Multiplex (AM), commonly referred to as "Curly Calf Syndrome (CCS)" in Angus cattle. At this time, we feel it is important to provide some details concerning the results of this research and provide answers to some frequently asked questions that we have encountered.

We are pleased to announce that the research has been very successful thus far and we are nearing completion of our work. Using breeder reports on AM affected calves as well as DNA samples obtained from these calves and many of their parents, we have been able to identify the gene and mutation that we believe is responsible for this genetic abnormality. From this information, we have developed an accurate DNA-based diagnostic test that can be used to assess an individual's status for AM. As part of test development, we have been able to provide the genotypes and AM status of 736 AI sires and feel that it would be beneficial to release these results to the industry.

These sires were used as an essential part of our research for three purposes. Firstly, a broad cross section of genetics representing the Angus breed is necessary to assess the validity of any diagnostic test that is developed. This assessment is based on the principle that because AM is a lethal abnormality, and because we assume a recessive mode of inheritance, there should not be any living animals that are homozygous for the mutation. Secondly, if our assessment is correct and we have identified the mutation that causes AM, then the results of testing would provide genotype information for all of the sires submitted. This would allow breeders to assess the genetic risk of their breeding programs and prepare for implementation of future testing programs. Lastly, this sampling provides an overall assessment of the frequency of the mutation within the entire Angus population and provides insight for the development of policies aimed at management within the population.

The naming scheme that we have chosen to use for this test identifies animals as having one of three possible genotypes. If an animal is homozygous for the normal variant (called an allele) we refer to them as **AM-Free (AMF)** indicating that they have been tested for the causative mutation and been found to be "free" of the mutation. Therefore these animals are unable to transmit it to any of their offspring. If the individuals are tested and found to be heterozygous or "carriers" for the mutation, meaning that they possess one normal allele and one mutant allele, they are referred to as **AM-Carrier (AMC)**. These animals pass the mutation on to approximately half of their offspring. Although affected calves are rarely tested, they would be homozygous for the mutation and referred to as **AM-Affected (AMA)**. This nomenclature is based on several concepts. Firstly, although the term "curly calf" certainly describes the outward appearance of some of these calves, the term is also problematic for a couple of reasons. Because of the tremendous global success of the Angus breed we need to consider the international implications of the terminology that we use. For example, in Australia the term "curly calf" is commonly used to describe a form of arthrogyposis that is caused by viral infection and not genetic mutation. Thus, in Australia the "curly calf" term might cause some confusion between the two forms of the disease, e.g. infectious or genetic. Secondly, the term used should have relevance to the more technical aspects of the disease, particularly for veterinary professionals who might encounter the condition as part of their practice. In these

instances, if they are unfamiliar with the condition it is more likely that they will investigate it using the medical descriptors and not common terminology. Lastly, there is a precedent for describing these recessives that has scientific merit and is already in place within other organizations. In this case we have decided to use the term Arthrogyriposis Multiplex (AM) as the technical name for this condition. This reflects not only the most obvious characteristic of the condition, but also indicates there are multiple abnormalities associated with the condition. Additionally, AM also fits well with a gene-based naming system that will become more obvious as the results of the research are presented in the scientific literature.

In regard to the accuracy of the test, there are two distinct components that contribute to how the DNA test performs. The first component involves the scientific data that underlie the test. The second component is the design and execution of the diagnostic assay performed as part of the testing procedure. From a scientific standpoint, the AM test is based on the presence of a specific change or mutation in the DNA sequence of specific genes of an animal. For AM, this change is the complete deletion of a segment of DNA that encompasses two different genes (a gene is a sequence in the DNA that encodes a protein). One of these genes is expressed at a crucial time in the development of nerve and muscle tissue. The mutation results in no protein being produced from this gene and therefore it is unable to carry out its normal function, referred to as a loss-of-function mutation. After identifying the specific mutation, experiments were conducted to validate the relationship between the mutation and AM. Based on these experiments, we believe that the scientific basis of the test is accurate; in other words, testing for this specific mutation will lead to correct classification of any animal. In regard to the diagnostic assay that is used for determining an animal's AM status, the test has undergone several optimizations that also lead us to believe that the test is highly reproducible and accurate. However, as with any task requiring human intervention, errors can occur. Of course, we do our best to limit any errors that occur by automating portions of the test and appropriately incorporation of testing controls. Although we cannot guarantee perfection, we estimate the assay may have an error rate of around 1 in 10,000. Therefore, for the listed AI sires we believe the following results to be accurate. These results are being released prior to the availability of commercial testing services. We intend to facilitate access to commercial services within the next 30 to 45 days.