Mutations that affect bovine fertility

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By studying the genetic origin of a recessive disease affecting the Holstein breed, a team from GIGA has discovered how this affects the fertility of these dairy cows. Up to the present time, early embryonic death of homozygous individuals could not be attributed to the impact of the mutation responsible for the brachyspina syndrome on fertility levels in bred cattle. This discovery has opened a new channel of research in the fight against the decrease in the fertility of cattle populations.

Breeding involves reproduction, and the very essence of reproduction depends on the fertility of bred animals. High fertility, therefore, represents a major challenge for all types of breeding. The consequences of a drop in fertility levels are self-evident: a reduction in herd numbers and in stock farming products followed by inevitable negative economic and social impacts.

In the case of dairy cattle and more particularly the Holstein breed, the situation is particularly worrying. For decades, fertility in this breed which is the most widespread in the world has been steadily declining. Fertility, which can be estimated from a set of parameters such as the number of calves born, the calving interval (or the time necessary for a cow to have two calves), the age at which the cow is culled, that is to say the moment when the farmer decides to stop using it for breeding purposes, and many other factors, is expressed in the form of an index. "This index combines all the criteria which are involved in measuring fertility and all of these criteria have diminished during recent decades", explains Carole Charlier, senior research associate with the FNRS and project leader at the GIGA Unit of Animal Genomics.

In collaboration with the Merete Fredholm team from Denmark, Carole Charlier recently identified a mutation which partly explains the decrease in the fertility of Holstein dairy cows. The results of this study have been published in the journal PLoS ONE (1).
The brachyspina syndrome under the spotlight

Carole Charlier and her colleagues are working on a set of recessive diseases in cattle. The object of their research is to identify the genes and mutations that cause these diseases in order to develop genetic tests which make it possible to identify individuals that carry these mutations and therefore avoid risky crossings (see: Domestic animals, the new Eldorado for geneticists). She continues, "We were engaged in a standard study of a recessive disease and that work led us to the discovery of an effect on the fertility of Holstein-
Friesian cattle. We were trying to identify the gene and mutation responsible for the brachyspina syndrome, an extremely rare syndrome which is observed, almost anecdotally, in the calves of this breed. The few recorded cases of this congenital disease, described in 2006 by our Danish collaborators, involve stillborn calves which present a series of defects. The principal defect and the one that gives its name to this syndrome, is a deformed and shortened spine. Other defects involve the internal organs, limbs, ribs etc. The symptoms observed in the Holstein-Friesian calves are very similar to those observed in the Fanconi Anemia condition in humans which is the human equivalent of this disease.

By taking advantage of next-generation high-throughput DNA sequencing technologies which are accessible through the GIGA-Genotranscriptomics platform (directed by Wouter Coppieters), the research by the team from Liege led them to the FANCI gene which is a well-studied gene that is well-known for its key role in the repair mechanisms of DNA. "It is part of an entire complex which is essential for correcting errors that occur in the genome and develops in the same way as an entire series of genes that are involved in human cancers", continues the researcher. More precisely, her team has succeeded in showing that the mutation causing the brachyspina syndrome in the Holstein-Friesian breed is a 3.3 kb deletion in this gene.

A rare recessive disease that is not so rare in fact

Once the causal mutation was identified, the researchers, principally Wanbo Li, conducted a population study based on their discovery. There was great surprise when the results of this study showed that more than 7% of the animals were carriers of this mutation in the Holstein-Friesian breed! "As it is one of the most popular dairy cows in the world, we should have observed many more cases of sick animals at birth", the researcher points out. To reconcile these two seemingly contradictory observations, the scientists postulated that individuals that carried two copies of the mutation would die during gestation. Carole Charlier continues, "thanks to quantitative
data on the fertility of deletion carrier and non-carrier bulls in the FANCI gene, we have been able to confirm this hypothesis". Thus a large majority of homozygous mutant calves (both of whose parents were carriers) die at the embryonic stage which results in a significant reduction in crossings involving two carrier parents.
3.3 Kb deletion in the FANCI gene. (A) Sequence and position of the deletion in the

(B) Gel electrophoresis showing the deletion across DEL.

(C) Gel electrophoresis showing the deletion within DEL1 and DEL2.
Following these observations, Carole Charlier’s team is currently developing an entirely new research project aimed at detecting other mutations which behave in the same way, that is to say mutations which are responsible for early embryonic death. "This type of mutation affects the fertility of animals without the farmer being any the wiser as he does not see any calves dying at birth. It is more likely that each livestock population shows other deleterious mutations of this kind which have an insidious effect and a largely underestimated overall impact on fertility" she explains.

**Tracking diseases that cause early embryonic death**

Rolling up their sleeves to take on this new research project which is the main subject of Wanbo Li’s doctoral thesis, scientists have selected bulls that are mainly used for artificial insemination worldwide. "It is also thanks to the GIGA-Genotranscriptomics platform that we are in the process of sequencing their exome, that is to say the coding part of their genome, in order to identify mutations that are potentially deleterious. These mutations can be deleterious for different reasons, notably because they introduce a stop codon, lead to a delay in the reading phase or because they affect the splicing of genes for example", Carole Charlier continues.

Because the bulls used for artificial insemination are handpicked and perfectly healthy, potential deleterious mutations that the team from Liege is searching for hide in their genome in the heterozygous state that is to say in single-copy only. "We are going to try to identify them and make a list of these mutations. The idea is to then develop genetic tests making it possible to examine each population in detail, statistically, we will then be able to estimate the frequency of these mutations in the different populations", adds the researcher. If, in the case of the brachyspina syndrome, scientists discover that the frequency of carrier animals of a mutation is relatively high but no living animal presents two copies of this, they will know that this mutation causes early embryonic death. The impact of the mutations that are identified as having an effect on bovine fertility could then be traditionally estimated by comparing the fertility data of carrier and non-carrier bulls.

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