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Texas A&M research produces tools to study stallions' subfertility

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Subfertility of breeding stallions — meaning the horses are less able to sire foals — is a well-recognized problem that has caused multi-million-dollar losses in the equine industry, experts say. Texas A&M researchers believe they are making progress in solving the problem by using an approach that might provide tools and resources necessary to study subfertility without causing stallions the angst of providing testicular samples for testing.

They have developed protocols to isolate RNA—which carries the information of genes in DNA — from stallion sperm and testis biopsies so that genetic factors associated with this condition can be identified.

Researchers from the Departments of Veterinary Integrative Biosciences (VIBS) and Large Animal Clinical Sciences had their results published online in the journal *Theriogenology*. The leading author of the published research is Dr. Terje Raudsepp, an assistant professor from VIBS, and the first author is Dr. Pranab J. Das, a postdoctoral research assistant from the same department.

The RNA isolation technique from stallion sperm is believed to lead to the discovery of fertility biomarkers that could improve breeding procedures and raise thoroughbred race horses.

"Because of the structure of horse breeding, where one stallion covers many mares, the economy of the breeding industry is more sensitive to the fertility of the stallion than the mare," Das says.

"During past decades, several organized studies have been conducted to understand the role of various environmental, behavioral and physiological factors affecting fertility in horses," adds Raudsepp, the project leader. "However, very little is known about the genetic factors associated with stallion fertility, and genetic factors of male fertility involve the interplay of hundreds of genes."

Obtaining testis tissue by surgery could harm the horse, so the research team studied an alternative — RNA isolation from sperm — which is non-invasive.

But RNA isolation from sperm has several challenges, the researchers say. "RNA quantity in a sperm cell is low, and sperm are highly condensed cells," they explain.

The Texas A&M team overcame these challenges and developed protocols that are specific to the species and to the various sources of sperm, including fresh ejaculates, flash frozen, cooled ejaculates and others.

"The team has started to identify the genes whose messengers are present in stallion sperm and to associate these genes with known fertility issues and sperm functions," the researchers say. "This knowledge is necessary for the next stage of research, which aims to identify differences in gene profiles between normal and subfertile or infertile stallions.

"Now, breeding stallions are selected mainly on the basis of their pedigree, looks and performance," the researchers add. "But in the future, we may be able to select them based on their reproductive potential. The published paper on RNA isolation from stallion sperm sets a necessary foundation to initiate these studies."

Provided by Texas A&M University