

NEW BLOOD TEST TO DETECT PREGNANCY IN MARES

By Heather Smith Thomas

Detecting pregnancy in horses is not as simple as the home test kit used in humans, but it's getting easier—with a new test that merely requires a blood sample.

A few years ago a blood test to detect pregnancy in cows became commercially available, with technology developed by Dr. Garth Sassar at University of Idaho. He founded a company called BioTracking and created a blood test called BioPRYN. There are now 25 affiliate laboratories in the U.S. and Canada that handle the blood samples.

BioTracking recently introduced a commercially available blood test for mares that can detect pregnancy with high accuracy any time between 70 days post breeding and 2 weeks before foaling. According to Jeremy Howard, sales manager at BioTracking, "We made it available in September 2008. At this point about 80 percent of the samples have been sent in by veterinarians." Some breeders draw the blood themselves and send the samples, but the average horse owner has the veterinarian draw the blood and send it to the lab.

This is an ELISA test (enzyme-linked immunosorbent assay test) that looks for estrogen molecules in a blood sample. There are three major estrogen molecules (steroid hormones) that play a role in reproduction in mammals—estradiol, estrone and estriol. One form of estrone is estrone sulfate, and one source of this hormone is the fetus and placenta—which produce high concentrations of estrone sulfate that appear in the blood and urine of a pregnant mare.

Estrone sulfate concentration is very low in the blood serum of geldings, stallions and non-pregnant mares. Research in pregnant mares showed that during the first month of pregnancy the blood level of this estrogen conjugate is very low (below 5 ng/mL), then rises briefly between days 38 and 42. This rise is due to estrogen produced by the CL (corpus luteum) in the mare's ovary.

The estrone sulfate concentration in the blood plasma gradually increases to a relatively high level (12 n/mL of plasma) by day 70 of gestation. From that time on, the hormone concentration continues to increase and remains significantly higher than that in non-pregnant mares. When evaluating a blood sample, mares with a concentration above 8 to 10 ng/mL are considered pregnant and those with lower levels are reported as not pregnant.



Blood samples are usually taken from the jugular vein, since this is an easy place to collect the 2 ml necessary for the test. Samples can be definitive for determining pregnancy any time after 70 days, but a sample taken 2 weeks or closer to the actual foaling may not be reliable, since estrone sulfate levels may drop just before foaling.

A blood sample should be collected into a "red top" vial, and refrigerated after collection until it can be sent to the lab. Ice packs or cool packs are required during shipment. "At this point in time we are only running the horse tests every other week, generally on Fridays, and then report the results back by phone, e-mail, fax or mail, depending on the sender's preference," says Howard. The cost is \$25 per test.

Some horse owners choose the blood test rather than an ultrasound check, because the blood test is more cost effective and easier to perform. "Veterinarians usually do an early ultrasound (which can

detect pregnancy in mares earlier than the blood test)," says Howard. Most owners want to do that to make sure the mare is actually pregnant or can still be rebred that season if she's not, or to make sure she's not carrying twin embryos—since one can be terminated at an early stage and help ensure a successful pregnancy for the remaining embryo.

"This is especially true in some of the more valuable mares that are being closely monitored. Then many owners/veterinarians choose to use the blood test after that—instead of the routine ultrasound monitoring every 2 weeks or once a month—to check on the health status of the fetus and pregnancy," says Howard. The blood test is simpler and less expensive, and can give a clue as to the viability of the fetus.

The fetus is the main source of estrone sulfate in the mare's body. A normal concentration of estrone sulfate indicates that the fetus is healthy and viable. If the plasma level of this hormone drops lower than normal, this means the fetus is stressed or dead. Thus the blood test can help monitor fetal health and viability.

"The blood test is great for fetal monitoring, rather than having to load the mare to take her to the clinic for ultrasound," says Howard. There are also some small breeders who are not doing intensive monitoring of pregnant mares, who may choose to use this test just to find out if a mare is pregnant or not.



Dr. Garth Sassar, University of Idaho, founder of BioTracking and the BioPRYN blood test for detecting pregnancy, hard at work in the laboratory.