

by **HEATHER SMITH THOMAS**

An important aspect of evaluating a stallion's fertility is the scrutiny of the semen sample, so as to determine whether the horse has adequate sperm count and normal sperm. A laboratory test can show whether a stallion's sperm is normal enough to get mares in foal, as well as pinpointing problems that can interfere with fertility.

The evaluation of a young, unproven stallion can be of value before he goes to stud, and the evaluation of older stallions may detect problems so that there is time to do something about them before a number of mares are fruitlessly bred and much of the breeding season is lost.

Semen Check

A semen evaluation looks at the volume of ejaculate (which should be at least 50 to 150 cc. in a mature stallion), sperm count per cc, percentage of normal and abnormal sperm, and the pH of the semen. On average, a normal stallion produces 35,000 spermatazoa per second and then up to 70,000 per second during the peak of the breeding season. Thus the average stallion produces three to six billion sperm per day. By collecting the horse daily for eight days to deplete semen stores, the daily sperm production can be estimated afterward. He can also be collected two or three times daily (after depletion of stored sperm) to see if his fertility is adequate for that kind of breeding schedule.

The color of semen can give an indication as to concentration of sperm. Milky color usually means good concentration (sperm count of 250-500 million per cc). A count in the range of 250 million or more is desirable; a count of 50 million or less may mean questionable fertility. At least 65 percent of the sperm should be normal. There are always some of abnormal size and shape (immature cells, abnormal cells with curled tails or deformed heads, etc.) but if defective sperm numbers exceed 30 to 40 percent, the stallion may have fertility problems.

A high number of immature or primitive cells may mean some testicular degeneration caused by infection, nutritional deficiency, hormone imbalance, or damage caused by drugs. Drugs such as steroids, often used for treating lameness, leg problems or other conditions, can contribute to infertility by causing a shedding of too many immature cells from the tubule linings.

The mobility of sperm (whether they are active and moving) is also

Infertility in Stallions: Evaluation of Semen and Sperm

a factor to be noted. The number of living sperm should be at least 60 percent or higher immediately after collection, and 30 to 50 percent of the living sperm should be actively moving. All semen samples will have some dead sperm present, but if the percentage of dead and inactive sperm is too high, the horse won't be very fertile. If after eight hours at room temperature (70 degrees), 10 percent of the sperm are still alive with two to five percent still active and moving, the test is considered satisfactory.

All samples contain some white blood cells, but an abnormally high white count could mean infection in the reproductive tract. The pH of semen should be slightly alkaline, ranging from 6.9 to 7.4. If semen is highly alkaline and there are also a lot of white blood cells present, the stallion probably has an infection. The presence of too many white cells, and a change in pH, can be more conclusive than a culture for determining whether an infection is present. An infection in the tract, or injury to the testicles causing bruising and inflammation, can make him infertile.

Whether or not a complete fertility evaluation is conducted before (or periodically during) the breeding season, microscopic evaluation of a "dismount sample" (two to three ml. of semen that drips from the urethra following ejaculation) can give important information after each breeding. It can confirm if

sperm were actually ejaculated. This is crucial, since it is possible for a stallion to periodically show all the normal indications of ejaculation while putting forth only fluid. And evaluation of the dismount samples can show the percent of active sperm, or any changes in motility. This simple and inexpensive practice can help ensure that a basic requirement for conception has been met (delivery of adequate number of viable sperm into the mare's reproductive tract). The mare should receive a minimum of 500 million active sperm to have a good opportunity to

conceive, but this is no problem for most stallions, since the average stallion ejaculates enough sperm during one cover to breed 15 to 16 mares.

Testicular Degeneration

The most common cause of reduced fertility in stallions is testicular degeneration, in which sperm production is decreased. There are fewer seminiferous tubules producing sperm, and sometimes there is degeneration of spermatocytes (the parent cells of sperm). Degeneration of the tubules will cause an increase in the percentage of defective sperm and decreased motility of live sperm, and there may also be other cells in the semen that are not normally present (such as spermatids and spermatocytes—immature cells and parent cells).

The degeneration of the testicular tissue is usually followed by atrophy. The testicle becomes smaller and softer, and eventually has very little sperm production and becomes much reduced in size—in the final stages becoming small and firm. Testicular degeneration can be caused by a number of things (heat in the testicle, overdose of an antibiotic or some chemical, or even genetics) and some of the detrimental effects may be very temporary or cumulative, or long-lasting and permanent. The serious and advanced stages can be detected by



palpation of the testicle, but by that point in time the damage is far advanced.

Most stallions suffer some degree of testicular degeneration as they get older; it is a normal process of aging, and may or may not reduce fertility until the stallion is quite old—depending on the individual. But degeneration can be hastened by other factors. By the time the problem can be detected by palpation, it is too late. In order to diagnose degeneration early, you have to pick up the clues from the semen sample (abnormal sperm and numbers) and other techniques such as measuring the testicles and predicting what the daily sperm output should be.

Charles C. Love, DVM, PhD, and other researchers at the Hoffman Center for Reproductive Studies at University of Pennsylvania's New Bolton Center (Kennett Square, PA), have in recent years developed a set of formulas that can predict reproductive performance and diagnose testicular degeneration in its early stages.

Before this method came into use, fertility was usually evaluated by measuring scrotal width or figuring the daily sperm output. For instance, a stallion's ejaculate may contain 100 million sperm per cc of gel-free semen and be considered quite fertile (since he needs to provide only 500 million live and mobile sperm to give a mare optimum chance for conception, and thus less than seven cc of his semen would be adequate for an artificial insemination). But if this stallion were previously capable of producing more than 400 million sperm per cc, then the present 100 million count could be indicative of a serious and advanced degenerative condition. Just because a stallion's sperm count is high enough to impregnate mares doesn't mean it's normal output for him as an individual; you need more facts for proper evaluation.

Now with the formulas developed at New Bolton Center, which combine the information concerning size of testicles and daily sperm output to come up with the stallion's daily production per gram of testicular tissue, breeders and veterinarians can get a true picture of the stallion's reproductive abil-

ities. This leaves less room for misinterpreting a sperm count. A horse might have large but diseased testicles or small healthy ones (both could be producing the same numbers of sperm but you wouldn't know which condition you were dealing with).

Monitoring Daily Sperm Output

There are many advantages to checking a stallion often, especially during the breeding season—the best time to check daily sperm output. A yearly evaluation doesn't tell you much, especially if it is prior to the breeding season. The quality of a stallion's semen in the off season is much different from what he produces when in regular use. To assess him fairly, he should be monitored closely during breeding.

This also gives you background information on the efficiency of his testicles, and you'll be able to tell if his production decreases in the future, or see some early warning signs of problems that might indicate a degenerative process is occurring. You may see changes in sperm numbers, motility, and quality (percent of normal to abnormal cells).

New Tests for Sperm Quality

A sperm cell must be able to successfully travel through the mare's reproductive tract to find the egg, penetrate the egg's surrounding cumulus, bind to the zona pellucida (the thick, transparent layer or envelope surrounding the ovum), and lose its acrosome (the cap-like structure at the front of the sperm's nucleus, which contains enzymes which help it enter the ovum).

Only a small percentage of sperm are capable of doing all these things, according to Stuart Meyers, veterinary researcher at the University of California, Davis. Some sperm may be good at one or two of these jobs, but few can accomplish them all. Traditional semen analysis doesn't give much clue as to which sperm can do the job. But new tests adapted from human fertility work may be able to more accurately evaluate equine sperm.

At the 1993 Symposium on Equine Reproduction in Brazil, Dr. Meyers presented information on new sperm tests that can help detect subfertility in stallions. The Zona Pellucida Binding and Penetration Test measures the ability of

the sperm cell to bind and penetrate the envelope surrounding the ovum. The Sperm Chromatin Structure Assay (a test which is already being done routinely at the New Bolton Center, University of Pennsylvania) looks for structural integrity of the sperm's DNA and can check for percentage of damaged or fragile sperm.

The Acrosome Reaction Evaluation checks for sperm that mature too quickly and lose their acrosome cap (and have their acrosome reactions before they get to the oviduct; the acrosome reaction is a process in which enzymes and proteins are released to help them penetrate the ovum). This test also checks for sperm that are unable to acrosome-react.

The Hemi-Zona Assay checks for sperm that show a normal ability to bind to the zona pellucida, acrosome-react and penetrate the ovum. The Cumulus Penetration Test checks the sperm's ability to penetrate a field of hyaluronic acid in the laboratory—since fertilization of an egg depends on sperm being able to penetrate the halo-like cumulus of hyaluronic acid that surrounds the ovum.

Many of these new tests will become useful in evaluating semen and pinpointing problems with subfertile stallions. Fertility is a complex thing, and it may take a battery of tests to know what is going on with reproductive abilities of a given individual.

Proof in the Pudding

Semen tests are very useful, but the ultimate test is whether or not the stallion gets his mares in foal. Occasionally a horse will test very well in the laboratory but still have low fertility in the breeding shed. And libido is just as important as semen count and viability. A stallion's libido will be the major factor in how many times a stallion will mount and ejaculate within a given period. Good management can help preserve or enhance his libido. Overuse, a bad experience, incompetent handling, etc., can be very detrimental.

Most breeding farms, after fully evaluating a stallion's semen in the laboratory, will test breed him to a few mares

Continued on page 122

HORSE CARE Continued

before declaring him a sure breeder. The actual performance at stud (whether he is able to get is mares in foal) is the real proof of a stallion's fertility.