



## Handle With Care

Artificial insemination has been a boon to Standardbred breeders. It has eliminated potential injuries and the spread of disease associated with live cover, and has reduced demand on a mare owner's time and checkbook. The use of cryopreservation, or freezing, of semen also enables stallions to breed overseas mares and stand at stud while continuing an active racing career. It also may act as an insurance policy should the stallion become unable to breed. Although breeding with transported semen requires twice as many total sperm to inseminate a mare as does breeding with freshly collected semen, conception rates can be as high as with fresh semen if everyone involved works as a team. "Breeding with cooled transported semen is a team effort requiring the expertise of qualified professionals," advises the American Association of Equine Practitioners. "The goal is to produce a healthy foal in the most efficient, effective way. To prevent disappointment, undue expense, and loss of valuable time, you must do your part to ensure success. "The ball starts in the stallion owner's court, and then is passed to the agent who prepares the semen for transport. The agent then hands it off to the air freight carrier, which delivers it to the mare owner, who hands it to a farm representative or veterinarian for insemination of the mare. Stallion owner a stallion owner is not simply offering the services of his stallion in today's high-tech breeding industry. Instead, he is marketing a product that must be properly handled to ensure that it is potent, free from disease, and delivered to the customer on a tight time schedule. This requires that the stallion's semen be evaluated to determine how much sperm he produces, how well the sperm can swim, and if they are shaped properly to penetrate the egg. On top of these basic considerations, the sperm must be rugged enough to withstand cooling and transport, or even freezing. Additionally, the farm office must be well organized to quickly and efficiently process each mare owner's request to ship semen so that it arrives at the optimal time for insemination. Since the reputations of their stallions depend on the conception rate achieved through transported semen, most farms have semen collected and processed by a veterinarian with expertise in this area, or enlist the services of a commercial agent that specializes in handling semen. Semen processor Select Breeders Services, headquartered in Coloma, Md., specializes in state-of-the-art processing of collected semen. SBS collects semen, tests it to determine its quality, then either cools or freezes it for shipment, or stores it in frozen form for later use. SBS President Paul Loomis said that the biggest mistake semen processors make is not using enough extender to protect the sperm. "They need to measure the sperm concentration in the seminal fluid—not guess—and add the appropriate amount of extender," he said. "For this, the single most important piece of equipment is a sperm counter, followed by a good microscope." Loomis explained that sperm counts fluctuate from day to day because of a wide number of variables. Cooled semen that is not diluted properly may suffer the harmful effects of seminal plasma and temperature shock that the proper amount of extender would otherwise buffer. Antibiotics in the extender also protect against growth of bacteria. SBS establishes the optimum dosage of sperm needed for conception for each stallion in the program by subjecting his semen to a 24-hour cooling trial to determine how many sperm are motile after it is rewarmed for five to 10 minutes. If, for example, half do not survive cooling, the shipment dosage is doubled to provide adequate, viable sperm for insemination. The procedure for freezing semen is even more delicate. "We split a stallion's semen and do what we call a 'test freeze,'" said Jonathan Clark, an SBS technician. "We have several different protocols and different extenders in which we can freeze the semen. We try to find the best procedure that gives us the best post-thaw motility. What we at our company consider a threshold post-thaw progressive motility is greater than 30 percent. This is measured by an automated semen analyzer. If it is below that, we don't recommend that the stallion owners market the semen. "Clark said the big question that has yet to be

answered is why some stallion's semen freezes well and some does not. "It is very stallion-dependent," he said. "A lot of people in the research community, including Colorado State University, Texas A&M, and University of Florida, are still trying to find out what stallion A possesses that stallion B might not possess that his cells are not able to withstand the rigorous procedure of cryo-preservation." SBS also cultures semen for disease and routinely adds an antibiotic—Gentamicin, Amikacin or a form of penicillin—to the shipped semen to inhibit the growth of bacteria. "The only virus that we look for is Equine Viral Arteritis," Clark said. "If that is in the semen, and it is not known to be there when people breed their mares, EVA is known to cause abortion. The other thing we do is a bacteriological check post-thaw when we do the motility check. We culture the semen to see that it is pathogen-free. We do add antibiotics with all of our freezing media, because even in a natural breeding instance, there is a bacterium on the stallion's penis, and we try to clean that up and make sure it doesn't get into the mare." Air freight carrier Keith Feister, broodmare manager of Winbak Farm in Chesapeake City, Md., said he believes that the air freight carrier can be the weak link in the relay race to get semen to its destination. "A lot of people take the container to the airport, drop it off, and say 'Good luck,'" he noted. "Someone who is very diligent will track those flights." Feister, an avid reining horse exhibitor in his spare time, stands a Quarter Horse stallion and has first-hand experience with lost semen shipments. "My wife has saved three or four shipments from missing connecting flights just by keeping after the carrier and following up on the tracking numbers," he said. "A lot of people don't even do that, because it takes time, but in a year you can get a couple extra mares bred that may have ovulated before the dose got there simply by calling and seeing that the containers make the connecting flights. "We've had the best success with US Air and United. Delta has been nothing but bad news for us. We do occasionally use Federal Express, too, but the same thing applies. You need to use the tracking numbers and check on the shipment at 8 a.m. to see if it has arrived. If not, you've got a couple hours for them to locate the shipment and get it there in time." Diligence paid off for Feister, who reported his stallion's statistics at 115 conceptions in 130 mares—all by shipped semen. "I think that's about as good as you're going to do," he said. Feister does not use United Parcel Service, because it will not insure a shipment of semen. "We insure all of our shipped semen," he said. "At least if it is lost, the mare owner gets something in compensation. For the \$5 it costs, it's worth it. We have at least one mare owner every year that gets an insurance check. They go through all the time and expense, so they should get something back if the carrier loses it." According to Loomis, some stallions don't cool well beyond 12 to 20 hours. Those stallions should be shipped counter to counter on airlines, so you can collect the stallion in the morning, get the semen on a plane, and breed the mare the same day. "If a stallion owner is going to ship semen, he has to be willing to make those adjustments," he said. "It's a nuisance, because it is much more convenient to stick it outside the door and have Fed-Ex pick it up. But if you have a stallion that doesn't cool well, this is a necessity." One stallion in the SBS program produces a lot of sperm, but of very poor quality that does not ship well. "What we've done in his case is require that the mares either be shipped to us or to a facility close by, and in some cases we've done airline shipments where it can be there in six to eight hours," Loomis said. "We've ended up getting pregnancies with this stallion by tripling the dose of sperm we would typically use to deliver an adequate count of normal sperm. We've had great success with this stallion. We limit the number of mares he's going to breed, because we know that we're only going to get a couple of doses out of each collection."

Terry Blanchard, D.V.M., who wrote the AAEP handbook on transported semen, urges the use of commercial semen storage systems for shipping. Commercial systems available in the U.S include Equitainer, Exquine Express II, Bio-Flite and EST Shipper. "Equitainers are extremely durable, and are the only reusable containers available commercially in the United States," he said. "Other containers are sold as disposable units. The disposable units are less expensive than the Equi-tainer, but have some potential disadvantages: cooling rate and storage temperature may fluctuate with environmental temperature, and the storage period for cooled semen storage of sperm may be relatively short. "There has been talk of X-ray machines at airports causing birth defects and, in fact, some shippers use Equitainers with added lead to protect sperm going through airport security, such as in Atlanta where they use twice the normal X-ray power. Loomis said this should not be a concern. "Theoretically, it is true that X-rays can cause mutation of sperm, but I don't think any of the X-rays that airlines are using are powerful enough to cause any of those kinds of changes," he said. "Certainly, airlines have been X-raying Equitainers for a long time. I don't know how many thousands of foals have been born

from cooled semen that has been X-rayed, and there is certainly no incidence of increased abnormalities as a result.” Mare owner Feister said the mare owner is the other critical player on the team. He advocated monitoring that the mare ovulates as close to insemination as possible to have the greatest likelihood of success. The mare should also be in the best possible reproductive health. “The convenience of cooled semen is that the owner can breed the mare at home; the flip side to that is that now you’ve taken the responsibility from the mare manager at the stud farm, who is experienced in doing that, and put it into the hands of the mare owner, who may not be experienced,” he said. “The biggest problem from the mare’s side is not having the ability to really manage that mare’s reproductive cycle properly. Oftentimes, the ability to breed your mare in the backyard leads to the problem of not having a teaser available to know when she’s in heat, and not having a reproductive veterinarian handy who does a lot of mare work. “The stallion owner ends up shipping a lot of semen to mares that aren’t ready to be bred, and there is not a lot the stallion owner can do, since the mare may be halfway across the country and out of his management control. This goes for cooled semen as well as frozen semen. You are going to get a better result whether you are breeding with fresh semen, cooled semen or frozen semen, if the people involved are skilled in those areas.” Communication between the mare management team and the stallion management team is the key to making transported semen work, Feister said, adding that if you give the stallion team enough notice, they can get you semen when you need it for the mare. By the same token, the stallion management team has to be honest in communicating with the mare owner about the quality of the semen that’s being shipped. “If you have a problem with either the stallion or the mare—a mare that is sub fertile or a stallion whose semen doesn’t cool well—and you are communicating about it, there are management techniques that you can use, such as shipping counter to counter or increasing the sperm number in each dose, that can improve your chances,” he said. “If people deny that there is a problem and just point fingers at one another, you are going to end up with a problem. The goal is to get the mare in foal. That’s what everybody wants to get done.” Inseminator Whoever inseminates the mare—be it a veterinarian or trained farm personnel—must be experienced in handling transported semen. He or she needs to know the mare’s reproductive history to ascertain if the follicle she produces is viable, and to be able to accurately predict the time of ovulation. In addition, before he inseminates the mare with the shipped semen, he needs to have the proper equipment to evaluate it and know what he is looking at. At the same time, whatever information the shipper has on the semen must accompany the shipment to enable the veterinarian to do his job properly. The bags should be labeled with the following: •the horse’s name •the date it was collected •what the semen dose contains •what kind of extender was used •what antibiotic is in the extender •what dilution rate was used •how many total sperm there are and what their motility was •instructions on how to handle the semen •instructions on how to manage the mare •instructions on how to inseminate the mare. “Breeding reproductively sound mares that don’t have an active infection and timing the insemination closely before the mare ovulates is the optimum situation,” Loomis added. Sometimes this apparently simple procedure is complicated when more mares than expected come into heat at once. “If a stallion manager has eight mares that are ready to breed on a particular day, he has two choices: Divide up the sperm and breed each mare with a suboptimum dose and none of them gets in foal, or select two or three of those mares that are close to ovulation and breed them only.” Loomis reminded mare owners that the adage “it only takes one sperm” does not apply when you are breeding horses. He urged them to work with the stallion manager and understand his predicament. “A stallion only produces so much sperm on a particular day,” he said. “Mare owners may be disappointed if they are not chosen to receive a shipment, but it is better to breed fewer mares and get them in foal than accommodate them all and get none of them in foal.” HB

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