

# A Cool Way to Enhance Your Breeding Program

By Cynthia Rodzen, on behalf of Hamilton Research, Inc.

## History of Transported Cooled Semen

In 1981, Meg Douglas-Hamilton and Mary Lou Cabot, co-owners of the renowned Hamilton Farm, were certain there was a more efficient and cost effective way to breed horses. Because mares needed to be at the stud farm for insemination, breeding season was always tough on mares, especially those with foals at their sides. Even after the mares were in foal, being vanned back to their home could be risky and result in loss of the foal. In addition, the mare traffic, quarantine procedures and limited stall space often resulted in a logistical nightmare for the stud farm. There had to be a better way.

As the story goes, the idea of transporting stallion semen to the mare, instead of the mare to the stallion, came to Meg in a dream one night. After waking up her physicist husband, Diarmaid, and describing her idea, it was agreed that the concept was possible - but it needed to be a simple process which the small breeding farm could easily use. That ruled out using frozen semen but would it be possible to extend the viable life of stallion semen without freezing? There was only one way to find out - to give it a try.

After rigorous in-house research and testing at top U.S. veterinary schools, the patented Equitainer® became a reality and Hamilton Equine Systems was formed. However, the adventure was just beginning. The \$64,000 question was "now that the system was developed, how would breeding farms be convinced to try this new process?" It wasn't easy. Breeders were apprehensive about the new technology and reluctant to change their ways. Thus, it was decided that the only way to prove the reliability and success of the Equitainer was to use it to breed mares to the Hamilton Farm stallions.

The pivotal field trial occurred in 1983 and involved three different stallions and the breeding of 50 mares. The results were irrefutable - with a total conception rate of 90% over three cycles, the Equitainer was deemed a success! Based on the amazing results of this field trial, gradually equine breeders began using the Equitainer as part of their A.I. programs. Soon, breed registry after breed registry approved the use of transported semen and the Equitainer is now a trusted partner for breeding farms around the world.

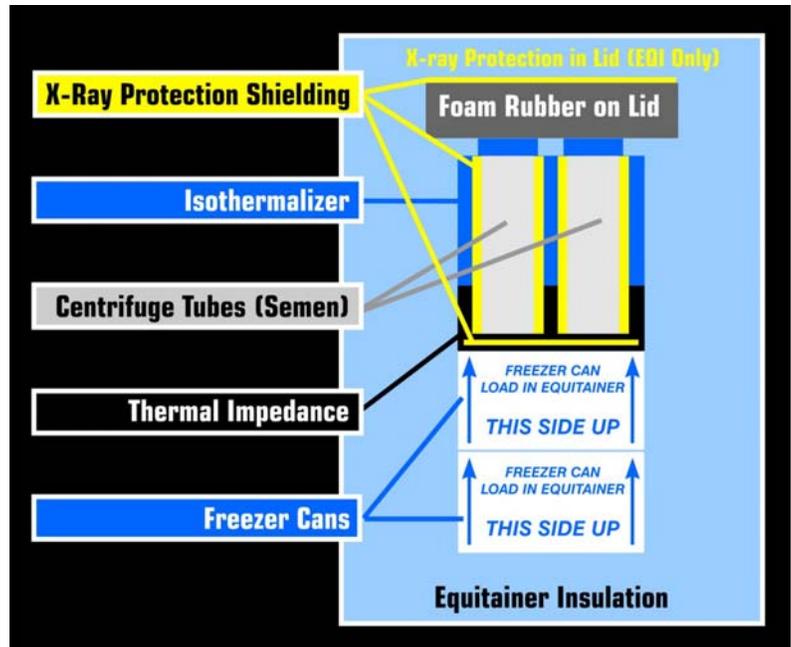
With the irrefutable success in breeding horses with transported semen, the canine world took notice and by the 1990's more and more breeding kennels began offering transported semen as an option for their customers. To address the specific needs of dog breeders, Hamilton Research introduced the low-cost disposable semen shipper, The Rover®, in 2002.



## How the Equitainer Technology Works

So just how does the Equitainer cool the semen sample at the optimum rate and maintain it at a final temperature for the best fertility outcomes? Well, this is where it starts to get a bit technical, so please bear with us!

The Equitainer technology works by a method called "thermal conduction," in which heat is transferred between two solid objects in contact with each other. (While the term "heat" may seem contradictory to what you might expect in a cooling system, it simply means that heat is removed from the sample, instead of coldness being sent into the sample.) Thus, when the isothermalizer containing the sample is placed in contact with the Freezer Cans, heat is drawn out of both the isothermalizer and the sample it contains. The x-ray shielding, which both lines the isothermalizer wells and isothermalizer bottom, assists in this heat transfer process. This is why it is crucial that there is direct contact between the sample and the isothermalizer, and that both wells of the isothermalizer are filled. However, unless this heat removal process is controlled, the sample will be cooled too quickly. This is where the isothermalizer truly comes into play. The vinyl base of isothermalizer acts as a "thermal impedance" that precisely controls the rate at which heat is removed from the sample. Without the use of a thermal impedance barrier, the sample may cool too quickly, resulting in "cold shock" to the sperm. Cold shock has been shown to dramatically reduce the viability and fertilizing capacity of equine sperm.



The Rover technology works exactly the same way. However due to its differences in structure and components, the Rover does not require use of a thermal impedance to achieve the proper cooling rate. With its unique internal design, the cooling rate inside the Rover is regulated and is not affected by its orientation during shipping. Other disposable shippers rely on random air movement within the container to cool the sample, which means that the position of the container during transport affects the cooling process. Unlike these other shippers, the cooling inside the Rover is governed by the proven Equitainer

technology and is not dependent on air movement. Thus, the cooling rate of the Rover is always strictly controlled – regardless of whether it is upside down or right side up during shipping!

## Advantages of Transported Cooled Semen and AI

### For the dame owner

- No need to ship the dame to the stud. This is especially crucial when the weather is too hot or too cold, or if you have a high-stress dame who does not fare well when shipping by air. It is also less costly to ship the semen than it is the dame.
- Unlimited choice of studs from which to choose. You no longer need to be restricted by geographical location, which opens up a wider field for your pedigree. Now it is easier to breed to a stud that will improve upon your dame.
- The stud's show schedule doesn't interfere with breeding. Even if the stud is not home, he can be collected and the ejaculate shipped to you.
- Less worry for you about the health and well-being of your dame.
- Behavioral problems of the dame do not interfere with breeding.
- Less risk of sexually transmitted disease.

### For the stud owner

- More dames available to improve upon your stud's pedigree - allowing you to choose dames based on breed standards rather than geography.
- Your stud's show schedule does not have to be interrupted, as may happen for natural breeding.
- When breeding by AI, semen quality and quantity can be checked whenever a collection is performed. Thus, you can monitor the reproductive status of your stud and notice potential problems immediately.
- Behavioral problems of the stud do not interfere with breeding.
- Less risk of sexually transmitted disease.

## Setting Up Your Transported Cooled Semen Program

There is a bit of legwork that must be performed before you can begin shipping your studs' semen to paying customers. Of course, you will need all the required equipment, but in addition you will need to test each stud's semen for its ability to withstand cooling and shipping.

### Semen Collection Equipment

Collection equipment for canine sperm is relatively simple and can be purchased in prepackaged kits. Typically kits will include a collection cone, one 15cc centrifuge tube, one docking ring (for attaching the end-cone to the centrifuge tube) and one pair of gloves.

### Extender

Canine semen will not survive cooling and transport if it is not first extended with a nutrient rich media. The most common extender is a skim milk and



glucose recipe, which has been used very successfully in both horses and dogs for over 25 years. The Kenney Extender may be mixed, separated into individual 10ml doses, and frozen until ready for use.



### Semen Evaluation

It is critical that the semen is evaluated for both motility and concentration each time before it is shipped to your client. Sending poor quality sperm not only wastes your customer's time and money, but also will reflect negatively on your transported semen program. If you are planning on shipping a lot of semen, the purchase of a microscope may be a wise investment in the long run. For smaller operations, a small portable microscope called the Handycop® is an affordable alternative for making a quick assessment of semen quality.



### Performing a Test "Shipment"

For each stud you plan on breeding by transported semen, it is highly recommended that a simulated test shipment be performed. The semen must be collected, extended and packaged just as you would for an actual shipment. The shipping container should then be kept at room temperature for 2 days. At both 24 hours and 48 hours post collection, an aliquot should be removed and the motility checked and compared against the initial motility. (*Important Note: It is critical that the semen aliquot being evaluated is warmed to body temperature before analysis for proper motility estimation.*)

You may also want to consider taking advantage of the semen evaluation service offered by Hamilton Research. Using a high end computer-assisted semen analyzer, Hamilton Research will provide a detailed motility analysis report, including a live video image, on semen shipped to them in a Rover or Equitainer. The analysis is performed at both 24 hours and 48 hours post collection.



## The Stud Contract

To avoid legal woes, it is vital that a Transported Semen Stud Contract is signed by your customers. This will likely be different than a basic stud contract as more factors come into play with transported semen than with on-site A.I. The contract contains conditions as to what the stud owner promises to deliver, what the responsibilities are of the dame owner, and any limitations of the agreement. Points to consider include requirements for notification of when a semen shipment is needed, how many semen doses will be offered, whether a veterinarian is required to inseminate the dame, how semen quality issues will be addressed, and refund policy.

## **The Dame Owner's Responsibilities**

Even if your stud has great semen quality, much of the success of breeding with transported semen rests on the shoulders of the dame owner and the dame's veterinarian. If the dame is inseminated at the wrong time, inseminated improperly or has a physical issue such as an infection, a successful pregnancy will be next to impossible to achieve.

The timing of insemination is the most crucial factor to successful breeding with transported semen. In general, insemination with cooled semen should occur 2 to 5 days post-ovulation. While an approximation of approaching ovulation can be determined by observation of behavioral changes and vaginal cytology, the recommended method of accurately determining ovulation is by measurement of blood progesterone level<sup>1</sup>. A baseline progesterone level is first determined during the first 5 days of proestrus (indicated by vulvular swelling and blood tinged discharge). 11 to 13 days after the first sign of vulvular swelling and bleeding, the progesterone level of the dame should be tested every two days until an initial rise is detected. Testing then should be performed daily until results indicate that ovulation has occurred.

While there are sophisticated assays that can be performed for determining progesterone levels, the Target® Canine Ovulation Kit is a reliable and cost-effective alternative. The Target® test can be performed right in the veterinarian's office (no need to send out to a reference laboratory) and each kit contains 12 tests.

The dame owner should contact you as soon as the progesterone level indicates that ovulation has occurred. This will give you enough time to collect the semen and ship it to arrive within the optimum insemination window.



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<sup>1</sup> Hoffmann, B and Schuler G. Semen Collection, Preservation and Artificial Insemination in the Dog. Proceedings of the 29<sup>th</sup> World Congress of the World Small Animal Veterinary Association. (2004)

## Preparing for Collection

Before you collect the semen, all collection, processing and packaging materials should be ready for use. Any equipment that will come in contact with the semen should be at least at room temperature.

- Make sure that the freezer pack for the shipping container is solidly frozen (at least 24 hours in the freezer).
- The extender should be mixed freshly, or thawed if previously frozen, and warmed to 37°C (99°F). If this is not possible, it may be warmed to room temperature (70°F). *It is vital that the warm semen is not placed into cold extender as this will have a detrimental effect on sperm motility.*
- Prepare the collection device.

## Collecting the Stud

*Note: AKC registration requires that only a licensed veterinarian may extract and extend semen. Check with your individual registry for details on their requirements.*

Canine semen is collected using hand pressure and massage. If the male has been manually collected before, the use of a teaser dame is usually not required. However, for young or nervous dogs, the presence of a teaser dame may be helpful.

If you have never performed a semen collection, your veterinarian will be able to help you learn the proper collection technique. In addition, there is extensive information on the Internet regarding semen collection from dogs. A very brief outline of the collection process follows:

- Using brisk massage, manually stimulate the penis through the prepuce (the outer fur-covered sheath).
- When erection occurs and the prepuce retracts, place the latex collecting cone over the penis.
- Tightly encircle the penis covered with the collection cone with the fingers to simulate the vulvular constriction of the dame that occurs during natural mating.
- Ejaculation occurs in three fractions: the pre-sperm fraction, the milky sperm rich fraction and the clear, prostatic fluid fraction. Only the first two fractions should be collected.

## Processing and Packaging the Semen

Visually examine the semen for color. The semen should be a pearly white. If it shows a yellow color, the semen has been contaminated with urine, which is detrimental to the sperm. A red color indicates blood in the semen, due to trauma, prostate problems or infection. The semen should be discarded in both latter cases.

For best results, extend the semen immediately after collection. The semen should be extended at least 1 part semen to 3 parts extender. For example, if 3 ml of ejaculate is collected, then 9 ml of extender should be used. Once the semen is extended, it can remain at room temperature while the motility and concentration is checked. The chart below shows normal semen values for dogs.

Parameter	Normal Value
Ejaculate Volume (without prostatic fraction)	1 – 6 ml
Motility	Greater than 70%
Concentration	100 – 500 million/ml

If the semen quality is acceptable, immediately package the semen according the shipping container instructions.

A good practice is to keep a small aliquot of the extended semen so that motility can be checked the next day. In this way, if the dame owner reports an issue with the semen quality, you will be able to determine if the problem is with the semen itself or if it is a shipping/handling problem. A small, tightly-capped tube (polypropylene) of the extended semen can be placed in a cup of warm water and placed in the refrigerator to simulate the cooling process that occurs in the shipping container.

### Reminder Sheet

It is wise to include a brief reminder sheet in the shipping container that highlights critical points such as:

- Do not leave the shipping container open for extended periods of time.
- Check the sperm motility before insemination.
- Motility must be assessed at 37°C for an accurate reading – if the semen is evaluated while cool, it will appear sluggish.
- You may inseminate the entire dose or inseminate half and save the second half for the next day. If the insemination dose is split between two days, store the sample overnight in the refrigerator (5°C).
- Gently invert the sample to mix before insemination.
- The semen does not need to be warmed prior to insemination.

You may also wish to include any stipulations regarding the procedure to follow if the semen quality is deemed unacceptable.

### Inseminating the Dame

*Note: AKC registration requires that only a licensed veterinarian may inseminate with fresh extended semen. Check with your individual registry for details on their requirements.*

The most common and least invasive method of artificial insemination is vaginal insemination. With vaginal insemination, semen is deposited just in front of the cervix through use of a thin tube called an insemination pipette.

### Vaginal Insemination Procedure

1. The dame is positioned with her rear raised or on a breeding ramp. Take care not to compress her abdomen.
2. A sterile, non-spermicidal syringe is attached to the insemination pipette and the extended semen sample drawn up through the tube into the syringe (it is best not to have any air in the insemination pipette).
3. Using a gloved, lubricated finger inserted into the vaginal canal, the insemination pipette is guided into the vagina and the semen gently deposited in front of the cervix.
4. The syringe is then removed to allow the semen remaining in the tube to drain. If the dame is positioned properly, backflow should not occur.
5. With the finger still in the vaginal canal, remove the insemination pipette.
6. The vaginal wall is then massaged (“feathered”) for several minutes to induce contractions.
7. Remove the finger and keep the rear of the dame elevated for 5 to 10 minutes to allow gravity to feed the semen down to the anterior vagina.
8. The dame should be restricted from excess activity and not allowed to urinate for one to two hours post insemination.

In some instances the veterinarian may chose to perform a surgical insemination directly into the uterus. This procedure may be beneficial if the semen sample has a low sperm concentration, if the dame is suspected of have a uterine or ovarian disease, or for certain Giant or Toy breeds.

### **Reasons for Failure to Conceive with Transported Cooled Semen**

Success rates with transported semen rely on more variables than natural breeding. Below are some of the most common reasons that dames may fail to conceive:

- Poor initial semen quality (low motility or concentration, abnormal morphology)
- Improper collection or handling of semen (collecting the third fraction prostatic fluid, improper preparation of extender, prolonged time between collection and extension)
- Exposure of semen to toxic materials (some syringes, plastics and lubricants are spermicidal)
- Exposure to temperature changes (placing warm semen in cold extender, compromised integrity of shipping container)
- Physical problems of the female that affect fertility (uterine or ovarian disease, infections)
- Improper timing of insemination
- Improper insemination technique

### **Parting Thoughts**

From the stud owner standpoint, offering transported cooled semen can be a valuable addition to your breeding program by allowing you to increase your market reach to distant geographic locations. Dame owners who were previously unwilling to ship their dames cross-country will now be part of your target market.

From the dame owner standpoint, transported cooled semen allows you to chose a stud anywhere in the country without the added stress and cost of shipping the female. You get to keep your dame at home, but at the same time match her to the best stud available, no matter his location.



Conception rates achieved with transported semen can be as good as, or even better than, those resulting from natural breeding, provided care is taken to follow protocols on both the stud and dame side of the process. Keep in mind that when using transported cooled semen, it is extremely important to maintain close communication between the stud and the dame owner.