Setting Up For Semen Collection and Transport
By Jos Mottershead
http://www.equine-reproduction.com/articles/setup.htm

One of the most commonly asked questions that I hear is "what do I need to get set up to collect for transported semen?"

Collection of semen and the preparation for transport or on-farm A.I. is not difficult, although care, knowledge and attention to
detail is required in all stages. There is also a financial requirement in that there is some basic equipment that is essential, and if
one chooses to get "fancy" (or lazy!) there is a whole host of other equipment that can be bought as well. Semen is fragile and if
not handled in a responsible and educated manner, will very quickly lose its viability. So much can cause harm - exposure to
sudden changes of temperature, sunlight, many toxic substances - even air and water under the wrong circumstances are toxic to
sperm. In fact, it's one of life's ironies that seminal plasma itself is toxic to sperm over an extended period of time! So assuming
that you have either completed a course, or "hung out" with your friendly neighbourhood equine veterinarian, let us look at what is
going to be essential equipment:

Artificial Vagina
Generally semen is collected using an artificial vagina ("AV") although there are other methods that are discussed elsewhere
on this site. Once you've chosen the A.V. that you feel is suitable for you and your stallion, you will have to get the associated
equipment required to fill it and use it. This would include such items as a thermometer capable of registering up to about 60°C
Celsius; disposable liners if you've chosen to use them (very convenient, and save on cleaning the AV, and reduce the danger of
cross-contamination if more than one stallion is being collected); a suitable lubricant - you should note that not all lubricants are
suitable, the "bacteriocidal" lubricants may be spermicidal. We actually use mineral oil, which is cheap, and about as harmless as
you can find although it cannot be used if a disposable liner is not being used as it will cause the breakdown of the latex liner of
the A.V.; disposable rectal sleeves (the sort used for palpations) to apply the lubricant to the interior of the A.V.; collection bottles
and filters, depending upon what model of A.V. you are using; a funnel to assist in filling the A.V. with water; and a plastic gallon
jug, which is useful for putting the water in that you are going to fill the A.V. with, and which allows the temperature of the water
to be taken prior to filling.

Microscope
Next on the list is required laboratory equipment. Undoubtedly at the top of the list is a microscope. If you read veterinary
journals, you will find that a "phase contrast" microscope is always recommended for semen analysis. Then when you go and
check the price of such a creature, the great plans for "collecting at home" often come to a grinding halt, as their price starts at
about $3,000! Fear not! For the average "on farm" user, doing basic collections and semen analysis, an ordinary "bright light"
microscope will suffice. You may come across advertisements for microscopes with "heated stages", and also see recommendations that your microscope for semen analysis have one. Again, this is in the ideal world, and as long as you turn your
microscope on ahead of time, there will be some warming of the stage by the light source. You must be aware at all times
however, that your microscope stage is not maintaining a perfect 37°C's, and make adjustments accordingly. If you are looking at a
semen sample that has just come from a cooled transporting container, then it will of course have to be warmed! Likewise, if your
stage tends to be hot, don't be surprised to find the sperm on the microscope slide "clumped" and immotile after 10 minutes
exposure!

Incubator or Water Bath
As sperm is very heat (or cold) sensitive, you will need an incubator or water bath. This piece of
equipment is used to keep anything that is going to come into contact with the semen at body
temperature (38°C Celsius). As this will include the microscope slides and cover slips, an incubator is
easier to use than a water bath. Other items that will need to be stored in the incubator (or water bath)
will be the semen extender; any glassware that is to be used, such as a measuring beaker; and at times
the semen itself.

Glassware
I mentioned glassware, so maybe I should elaborate a little on that. You will need something to measure the volume of the
ejaculate. A glass beaker is good, or a disposable plastic one. The advantage of the plastic one is that you will not have to sterilize
after each use, merely throw it away. In the long term of course, the disposable route is more expensive (although unless you are
very sure of your sterilization techniques, it is far preferable). The collection bottles that come with the "Colorado" A.V. are
graduated, and will give a close enough estimate for the average collection situation, as will the baby bottle attachment that is used
with the "Missouri" A.V. If however you are using all-in-one disposable liners with either of these systems, or are using another
collection method, then you will still require the graduated beaker. A 250 ML beaker will suffice in most instances - remember
you may want to mix the ejaculate and extender in it, so go larger rather than smaller. We use disposable baby bottle liners in lieu
of much of the container glassware, they do not however offer accurate measurement of the ejaculate volume although they are cheap and sterile. Microscope slides and cover slips would also be included in the "glassware" category.

**Sperm Counting Device**

After the microscope and the incubator, probably the next most important piece of equipment is some form of counting device to establish the concentration in millions per ML (abbreviated as: number x 10⁶/ML). There are a variety of commercially available units, some of which will also calculate the required volume to establish the correct insemination dose. Two of these that we have found reliable are the "IMV Micro Reader" (IMV International, Minneapolis, MN) and the "Counterpoint Sperm Counter" (Hamilton Research Inc., S. Hamilton, MA). These devices all operate on the principal of a spectrophotometer, which is a machine that measures the light transference through some sort of medium. In fact, you can use a spectrophotometer to do the job. The "Spec 20" model is the standard of the industry. If you do elect to go with a spectrophotometer, you will have to get it calibrated for equine semen, and Colorado State University will do that at a minimal cost, and with little delay.

If all of the above units are outside your pocketbook range when starting (they are priced at about $1,500 and up), then you will have to resort to the somewhat more time-consuming, albeit actually more accurate, hemacytometer (various manufacturers, cost is around $100). In fact, once you have become familiar with the hemacytometer, and have had some practice, you should be able to establish concentration levels within 10 minutes. A calculator will also save a lot of time and mathematical effort when calculating insemination doses and extender rates if you are not using a counting device that does the math for you.

Syringes or pipettes will be required for handling the semen, and for getting the correct amount for calculating concentration. If you are using syringes, they should be of the all-plastic, non-spermicidal type (Air-Tite, Vineland, NJ), and not the normal rubber-plungered type.

**Semen Extenders**

There are a variety of semen extenders available commercially, the majority of which are based on Dr. Bob Kenney's tried-and-true non-fat-dried-milk-solid (NFDMS) "Kenney" formula. The only variable in the majority of these is what the antibiotic is that is added to it (if in fact there is one). It is advisable when first collecting your stallion each season, to split his ejaculate and store it in several different extenders (i.e. different antibiotics) to establish which he seems to be most compatible with. There is usually a little variation, although sometimes it is negligible. The two antibiotics that I favour the most are Amikacin Sulfate (which is what the "EZ Mixin' CST" extender from ARS is, as well as other commercially produced extenders), and Ticarcillin (although in Canada this has now been replaced by "Timentin" which is a mixture of Ticarcillin and Clavulanic Acid). I tend to stay well away from using Polymixin B. Sulfate in any but on-farm inseminations, as I have found it seems to be more detrimental to longevity that others. Another commonly used antibiotic is Gentamycin, although I have run across some that have the same concerns about the use of this as I do the Polymixin B. Sulfate. Another extender arrived on the US market in 2003 - INRA '96 (IMV International, Minneapolis, MN). This extender has some unique properties in that it can be successfully used at up to 15°C with some stallions; at low extension ratios (as low as 1:1 semen:extender); and aerobically (i.e. there is no need to remove all the air from the shipment). We now always include this extender in any pre-shipping evaluation we perform on a stallion. Essentially, for the beginner, it is best to go with one of the many commercially available semen extenders, rather than attempting to mix your own.
Semen Transport Containers

Without a doubt the best of these is the "Equitainer" (Hamilton Research Inc.). It is also the most expensive initially, but will last for many, many years, and so become by far the cheapest in the long run. (I know of several farms that are still using the old "blue bucket" type that was the original prototype developed almost 20 years ago!). The Equitainer is the most reliable as far as insulation properties against heat or cold is concerned, and it's tough plastic shell is immensely durable and will handle the rough handling of courier companies and airline baggage handlers. The Equitainer is the system we use at Equine-Reproduction.com Services.

There are several "disposable" containers available for use with transported semen as well, such as the "Clipper" and "Equine Express II". These are constructed using a cardboard box with a Styrofoam type insulation. The manufacturers recommend they be used no more than 5 times, and fewer if damage is evident. The trouble with these systems is that they tend to have poorer insulation qualities to start with, and then if they are subject to rough handling in transit, they can develop "microcrack" which may not even be visible. These microcracks then act as a heat/cold vector to the inside, and hence allow the semen temperature to drastically alter. The price certainly is a bonus initially (usually around $30, as opposed to Equitainer's $240), and some farms simply add the cost into the stud fee, and don't bother about trying to get the mare owner to return them (getting shipment containers returned can be a nuisance occasionally). Both the Clipper and the Equine Express II allow for the packaging of the semen in the insemination syringe, which may be attractive to some, but means you will have the added expense of providing the all plastic non-spermicidal syringes. If using one of the other systems, you will require baby bottle liners and elastic bands to package the semen in. A "permanent" marker is also required to write identification on the outside of the bag or the syringe.

"Outside" Equipment for the Collection Process

The first consideration for "outside" equipment is whether you are going to use a breeding phantom (phantom mare or dummy), or a live "jump mare". If you are going to use a phantom mare, then obviously that will have to be bought or constructed. I have built many very serviceable breeding phantoms using sections of telephone pole. Regardless of the type of "mare" you are using, the stallion should have his penis washed off prior to a collection. For this you will require a source of warm water, a bucket, a sterile liner for the bucket (disposable plastic bags will usually suffice), and a sterile plastic cup.

Whether you are using a breeding phantom or a "jump mare", it is quite possible you will need somewhere to "tease" the stallion, to encourage him to "drop" for the washing process, and then to gain an erection prior to collection. Depending on the stallion's temperament, this teasing can be achieved over a stall door or through stall bars, or it may be that a proper teasing rail is preferred - especially with rowdier stallions that may become too used to teasing over a stall door, and attempt it each time they pass one. If using a breeding phantom, some stallions may require the tease mare to be in a close proximity to the phantom, as they may become distracted if the distance is too great, and loose their erection.

The above is a list of basic equipment that the person collecting semen for on-farm or cooled-transported use will need. It is however, just that, and there are many other pieces of equipment that can make the process more efficient, easier, and a whole lot more expensive!

Other pieces of equipment that you could find of use in the future may include such items as centrifuges, a slide warmer, a refrigerator, a water bath (if you have an incubator for regular use), various sizes of pipettors, and if you have money to spare, the "holy of holies": CASA, which is "Computer Assisted Semen Analysis" - priced at $25,000 the last time I checked!

THE ARTIFICIAL VAGINA

http://www.equine-reproduction.com/articles/avs.htm

The "A.V." is the tool most commonly used for semen collection from stallions for use in artificial insemination programs, for the semen to be frozen, or for analysis. There are several types of artificial vagina available commercially. Although there are differences, they all follow the same general design format of having a tubular inner liner, usually of latex, which is surrounded by a fillable water jacket encased in a harder outer shell. Attachable at the distal end is some form of collection apparatus to capture the ejaculated semen, this apparatus will often include a filter of some sort to remove the gel fraction of the ejaculate and any detritus such as smegma. Probably the two most common models seen in use in North America are the Colorado and Missouri models. The Colorado model has been modified by other companies but retains a similar basic design. Each AV model has different advantages over another, and many technicians, and indeed stallions, develop a preference for one or another model. These are some of the commonest advantages to each model that we and others have noted:

**COLORADO STYLE**

- Manufactured by: NASCO
- Retains water temperature longer in cold weather
- Is more durable under extreme use
- Liner is cheaper to replace when worn or damaged
- If not too long, allows the stallion to ejaculate well clear of the heated liner, thereby avoiding danger of sperm damage by heat shock

**MISSOURI**

- Manufactured by: NASCO
- Is cheaper to purchase initially
- Is lighter and therefore easier to handle when filled with water
- Will allow for the addition of air to make the liner tighter
- Allows a less direct manual stimulation of the penis, which can be preferable with some stallions

**FRENCH (INRA MODEL)**

- Manufactured by: IMV International Corporation
- Extremely light weight - can be held with one hand
- Has 2 handles (second handle not visible) making it extremely easy to handle
- Holds marginally more water than the Missouri or Roanoke models, but not as much as the Colorado, making it lighter to handle, but maintaining good heat retention properties
- The removable/adjustable latex hood (seen at the right end of image) enables the use of a single AV for many different size stallions
- The latex hood also allows for the less direct manual stimulation of the glans of the penis during collection if required
- Liner is cheaper to replace when worn or damaged

**ROANOKE**

- Manufactured by: Roanoke AI Labs.
- Extremely light weight - can be held with one hand
- Allows for great bell pressure on penis glans, which is preferred by some stallions
- Shortness permits manual stimulation of the upper shaft of the penis at the sensitive pressure point located close to the preputial ring
- "Spit-off" pressure relief valve automatically adjusts water pressure to required level
At Equine-Reproduction.com we have used all of the above models, but generally prefer the French Model, as we find it is convenient in size and weight to handle, while providing rigidity and good heat retention properties. We also particularly appreciate the double handles, and the ability to move the latex hood to adjust the length of the AV to enable its use on a variety of different sized stallions.

Many farms and semen collection centers use a sterile liner in the artificial vagina. This liner is usually made of a thin plastic of some sort, and is especially useful if one is collecting semen from different stallions, as it pretty well eliminates any chance of cross-contamination of any kind, either bacterial or seminal. Even if not collecting multiple stallions, it is still extremely convenient, as it eliminates the need for removing and cleaning the latex AV liner after each use, and aside from the time savings, this will also assist in extending the length of time before the latex liner becomes worn out. Often stallion owners will advise us that some stallions do not tolerate a disposable liner, but we have found that changing the internal temperature or pressure of the AV, or switching to another brand or style of liner that may be of a different thickness or "softness" will solve this situation. Some of the sterile liners available commercially, notably those designed for use in the Missouri model AV, come with a built-in filter for gel and detritus, which can be very convenient. If one is going to be using the AV a lot however, it may be preferable to buy your liner in a straight continuous roll, and cut off the desired length at the time of use, as this will prove a lot cheaper.

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The actual process of artificial insemination in a mare is not complicated, and can be learned very rapidly. Unlike artificial insemination in cattle, which involves guiding the insemination pipette through the cervix via manipulation per rectum, equine AI is carried out completely vaginally. The equine rectum is not as durable as a bovine's, and it is for this reason that rectal palpation by the lay person is not encouraged by most veterinarians and teaching facilities. Insemination per vagina does mean that probably the most important part of the process is the preparation, during which the perineal area is cleansed as for surgery, most commonly using an iodine preparation such as "Betadine".

The mare is preferably secured in breeding stocks, and her tail is wrapped and deflected to one side where it should remain throughout the whole insemination process to avoid contamination of the scrubbed area. The perineal area is scrubbed using a surgical preparation scrub, starting centrally, and working outwards. Once the outer (unscrubbed) section is reached, the disposable paper towel or cotton is discarded, and a new piece used to return to the center to scrub outwards again. The final scrub should involve a little greater pressure to remove any surplus surface scrub liquid remaining, so that it may not carried into the vagina during AI.

Once the mare is prepared, the semen is removed from the shipping container and the insemination sample is slowly drawn through the insemination pipette into the syringe. As elsewhere with the process, sterility is important here too. Note that nobody is touching the pipette, which once the syringe is filled will be placed back into it's sterile packaging until it is later introduced into the mare's uterus to inseminate.

Using a sterile lubricated gloved arm (the glove having been reversed to ensure maximum cleanliness), the inseminator will introduce his arm into the mare's vagina, palpate the cervix, which should be found on the bottom (ventral) surface of the head-ward (cranial) portion of the vagina. The closer the mare is to ovulation, the more relaxed the cervix becomes. In the center of the cervix will be found a small depression, which is the opening to the uterus. One of the most apt descriptions of a cervix is that it is like a "small volcano" in shape. The inseminator will gently introduce the index finger through this depression, and then using the finger as a guide, slide the insemination pipette along it and into the uterus. It is important not to force the pipette at any point, as internal damage may occur if that is done.

The gloved thumb is placed over the end of the pipette prior to it's introduction into the vagina. This protects it from picking up any contaminants which may subsequently be inseminated into the uterus along with the semen.

With the pipette introduced into the uterus as far as possible without any resistance, the plunger of the syringe is slowly depressed, introducing the semen. Before removal of the pipette, the syringe may be unhooked and rehooked so that 2 cc of air may be introduced behind the semen in order to clear the pipette of the remaining semen. It is important that excess air is not introduced into the uterus, and a very small portion of the semen should remain at the very end of the pipette when it is removed from the uterus. The arm should then be slowly withdrawn from the vagina.
The Equine Uterus
http://www.equine-reproduction.com/articles/uterus.htm

This is a view of the equine uterus from above, and behind.

This is a view of the same uterus, from below. Here the urethral connection of the bladder can be seen clearly (the bladder is again displaced to the left), and the body and horns of the uterus are more clearly differentiated from the broad ligament.

In this image, the bladder, fat and broad ligament have been stripped away, clearly revealing the uterine body and horns.
On the left, the cervix is clearly exposed, and can be seen as the raised section between the forefinger and thumb of the hand.

In this image, the insemination pipette is seen being introduced through the cervix from the vagina, although the vagina itself has been removed. Note the index finger is introduced slightly (up to almost the first knuckle), and is being used to guide the pipette into and through the cervix, as it would when carrying out an insemination.

On the left can be seen the different textures of the uterine/cervical/vaginal tissue. The darker section at the top is the endometrium, the light section in the middle of the image is the cervix, and the lower section is the vagina.

The ovary (seen on the right) is roughly kidney-shaped, with the depression in the center of the lower side being the ovulation fossa, where the ova evacuated from the follicle (which "implodes" rather than "explodes" upon ovulation), exits on its way to the uterus after ovulation. The follicle shown here outlined in red is approximately 2 cm, the size being measured from the outer surface to the innermost extent (in this image therefore, from left to right). As a result, ultrasound is the only truly accurate method for establishing follicular size. Determination of size by rectal palpation relies on the assumption that the follicle is spherical in shape, and although this is often the case, it is not always so. Even if the follicle is spherical, it is difficult to determine the exact edge when using palpation. On the other hand, rectal palpation is once again becoming more widely used to determine how close to ovulation the mare is, as the consistency of the follicle softens dramatically close to ovulation, and this can only be established upon palpation. Ultrasound may detect either a "tear-shaping"; or a thickening of the outer membrane of the follicle, both signs of impending ovulation, but these signs are not always present.

With thanks to Canada's Equine Research Center for providing us with the reproductive tract for dissection.

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Power-pole Breeding Phantom

We have built quite a few of these very simple power-pole breeding phantoms for use in semen collection. They have worked every bit as well for us as the expensive pre-fabricated phantoms that we have also used, but at a fraction of the price.

We have called them “power-pole” breeding phantoms simply because the first ones we built were of power poles, but any suitable form of wood will suffice. Measurements are approximate and can be varied depending upon the size of the stallion that is being collected. A 56 inch “tail height” has been found to be a common height that works best for the widest variety of stallion sizes. Note that this height is including the padding.

We have found the slight incline to be of some use with younger stallions as a slight deterring factor in their traveling up the phantom as they are breeding. It can however be a problem for an older or sore stallion, with whom the resultant increase in the angle can put a strain on the back and make them uncomfortable.

The phantom’s legs can be of variable thickness depending upon the severity of work they face. A heavy, rambunctious stallion will demand heavier legs than a lighter weight pony. Whatever the diameter, they should be sunk into the ground for at least 24 inches. A collapsing breeding phantom can deter a stallion from ever mounting again!

The tops of the legs are “V’d” from front to back and the upper pole is secured by means of extremely long spikes being driven down through into the legs from the top. Other methods used with equal success have included strapping. Whatever method is used, it is essential that it is secure and leaves no sharp edges.

The top rear portion of the “rump” is angled for 6 inches or so from the back and upper surface. Once the superstructure is built, the entire upper pole surface must be padded. We have used old foam mattresses with great success. They are secured by means of rope and nylon straps. It is essential that all straps be as tight as possible otherwise slippage will occur while the phantom is being used. Great attention must be paid to the padding at the “tail” end over the flat section, where any pockets that may catch the stallion’s penis before it is deflected must be avoided. A non-slip, non-abrasive, non-burning outer cover is then placed over the padding. Leather is the best, but canvas covered with a piece of deep-pile carpeting has worked well. Beware of using materials that will cause friction burns on the inside of the stallion’s legs, as these will seriously deter him from remounting.

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Power-pole Breeding Phantom

Top secured by long spikes

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Definitely not to scale!

Entire upper portion should be padded with at least 4" of soft foam padding