Objectives

1. To introduce the equipment and techniques necessary for collecting semen from stallions.
2. To gain knowledge of safe and effective stallion-handling procedures.
3. To present a live demonstration of semen collection from the stallion.

Equipment

Semen is collected from stallions primarily by allowing them to ejaculate into an artificial device which provides an appropriate stimulus to the penis. The stallion is allowed to mount an estrous mare, or an inanimate object called a "dummy" or "phantom," and his penis is deflected into a device that simulates the temperature and pressure of the vagina, typically called an artificial vagina or AV. It is also important to remember that the stallions’ ejaculate contains a gel plug that needs to be separated from the fluid portion of the semen during collection. This is typically done by placing a filter on the collection container that is attached to AV. By preventing the mixing of the gel plug and fluid rich portion of the collection, it allows the producer to further process the semen without it coagulating. If an estrous mare is used, timing can be important, for that stallion may mount and enter the mare before the operator can deflect the penis into the collecting device. Alternatively, a "breeder's bag" or condom can be placed on the stallion's penis and he is allowed to breed a mare naturally. A third method of collecting semen is by manual masturbation. Electro-ejaculation, commonly used to collect semen from bulls and rams, cannot be used with stallions as they do not tolerate the voltage.

1) Breeder's bag - This device is just what it sounds like, a condom. In use, it is fitted on the stallion during sexual excitement, after he has been washed. The stallion is then allowed to breed the mare, and as he dismounts, the condom is removed and taken into the laboratory for filtering and evaluating the semen.

**Condom**

**Pro's** - Breeder's bags are relatively inexpensive, and require relatively little skill or experience to use. For this reason, breeders who have only a few mares to breed may consider the breeder's bag as an option. Breeder's bags are ruggedly built, and can be used more than once, if washed carefully. They do not require much training of the stallion. You simply put it on the freshly washed penis and allow him to breed a mare naturally. The breeder's bag is inexpensive.
Con's - The very fact that you use a breeder's bag on an untrained stallion makes it less safe to use. Remember that it has to be put on when the stallion is sexually excited and interested only in breeding the mare. He may not tolerate having his penis manipulated at that time. In addition to this caveat, an estrous mare is required for him to mount and breed. In a small operation, with only a few mares, this may mean having the stallion breed the mare you want to inseminate. Alternatively, it may require maintaining a quiet mare that can be induced to estrous behavior by estrogen administration, but that negates the occasional use of a breeder's bag. Finally, the semen is dirtier when collected in a condom, no matter how well you wash the penis, some dirt is likely to be scraped off the penis while putting the condom on, or taking it off. This dirt is in the same place as the freshly ejaculated semen. The semen can be filtered in the laboratory, but that is usually several minutes after ejaculation. Although inexpensive, proper hygiene dictates that a given condom should not be used on more than one stallion. Therefore, if you have more than one stallion, you need more than one breeder's bag.

2) Artificial vagina - The artificial vagina (AV) is a device which attempts to mimic conditions of temperature and pressure in the vagina. The stallion is allowed to mount a mare, or phantom and his penis is deflected into the artificial vagina held parallel to the natural position of his penis, and slightly to one side. There are several designs of AV shown below, but the basic design consists of an inner chamber that can be filled with water and/or air to provide appropriate temperature and pressure. The chamber, usually constructed of rubber, is supported in some rigid material, such as leather (Missouri Model), aluminum (Colorado Model, Japanese Model), or plastic (Colorado Model, Roanoke Model). The AV can be fitted with a filter so the semen passes through it at ejaculation. A poly-bag or other collection device catches the filtered semen.
Pro's - The AV provides a relatively clean, filtered semen sample. It is somewhat safer than applying a condom, because it is not put on the stallion until he has already mounted a mare or dummy, and his attention is focused. Most stallions adapt to ejaculating in an AV relatively quickly, requiring little training time. The temperature and/or pressure in the inner chamber can be changed to provide conditions to which a given stallion will respond. If used for multiple stallions, the entire apparatus need not be changed, just the inner liner. Thus, the Colorado Model, for instance, can be prepared and used to collect several stallions, changing only the inner liner, filter and collecting bag for each stallion. Disposable inner liners are available, and can be used with many stallions, although some apparently do not respond well.

Con's - The AV requires more "set-up" time than does a breeder's bag, and requires more skill in preparing the AV. Furthermore, stallions react differently to the conditions of the AV, and the operator needs to assess a given stallion's response, so that the AV can be changed appropriately. The AV and associated equipment is expensive, approximately $500.00 for Colorado Models, $150.00 for Missouri Models, and $350 for Roanoke models. As with Breeder's bags, AV's should not be used interchangeably for different stallions, necessitating purchase of several separate inners. The AV, especially the Colorado Model, can be heavy and cumbersome. If collecting by having the stallion mount an estrus mare, a heavy AV can slow the operator down, giving the stallion time to enter the mare.

3) Manual manipulation - Semen can be collected from stallions by manual manipulation of the penis. This requires relatively little equipment, gloves and a collecting device.

Pro's - This method does not require any equipment set-up or cleaning. An estrous mare or dummy is not necessarily required. The stallion can be collected while he is standing with all four legs on the ground, although some stallions may respond better with an estrous mare visually accessible.

Con's - This method requires more skill and experience by the operator, and may require more training of the stallion. In an industry still occupied primarily by human males, taboos about masturbation may make this technique unacceptable.
Collection of Semen

In this lab, we will collect semen from stallions using an artificial vagina (AV) and having the stallion mount a "phantom dummy." After the AV is prepared, the stallion will be allowed to tease the mare. When he obtains an erection, he will be led away and his penis washed with warm water to remove dirt and debris. After he is washed, he will be led toward phantom. The operator with the AV will be behind and to the right of stallion-handler. The operator moves with the stallion handler, remaining behind until the stallion mounts the phantom. Many stallions "wing-out" with their front feet when mounting, and the AV operator who moves in too quickly can get hurt. The stallion handler should keep this in mind. It is important to remain out of stallion's way until he mounts phantom, but to move in immediately when he mounts. This is even more important when a live "jump" mare is used. The operator should hold the AV in left hand, and guide the stallion's penis into AV with right hand. The operator should be careful not to grab the penis, but rather guide it, with the hand held open, palm-up. Squeezing or grabbing the penis may cause him to lose the erection and dismount, particularly with inexperienced stallion.

Once the penis is in the AV, the natural angle of the penis should be maintained, that is, hold the AV at about a $45^\circ$ angle, with the collection-end up. Hold the AV firmly, forcing it toward his pelvis as he thrusts. This may require both hands. Many people turn to face the stallion's rear at this point, to help hold the AV against his thrusts. This is OK when using a phantom, but never turn your back when collecting from a live jump mare! It is usually not the stallion that hurts people when collecting semen, it is the mare. Be careful of the placement of your feet. The stallion may dance on his hind feet, and it can be very uncomfortable if he steps on your foot! If it is possible, it is a good idea to keep the right hand, open, and palm-up, on the base of the penis. There are two reasons for this.

1) You can feel the urethral pulses as he ejaculates, and will know, unambiguously, that he has ejaculated. Some stallions do not "flag" (pumping up-and-down motion while ejaculating) their tail when ejaculating, and some stallions have learned to fake ejaculation and will flag without ejaculating.

2) You can provide some pressure to the penis if the stallion is not responding to the AV, enhancing the sensation and helping him ejaculate. This employs some of the principles of manual manipulation in combination with use of the AV.

When using a phantom, operators may find it difficult or impossible to do this, because stallions tend to crowd the phantom. In that case, one must rely on the AV to provide the necessary sensation. The Missouri Model is soft enough that the operator can sometimes provide more pressure by squeezing through the leather case, or by placing one hand (the left) between the leather case and the rubber liner. As the operator may not be in a position to feel the urethral pulses, or see the tail flagging, it can be helpful for the stallion handler, or an observer to provide this information, by announcing, "flagging." The AV operator should then gently lower the collecting end of the AV so that semen will run down into the collection container, instead of running out the back end. Keep the AV on the stallion, collection end down, until the penis loses it's erection and/or the stallion dismounts. Hold the AV upright, collection end down, and assess the collection. Unless it is very cold, we will not use an insulator around the collection bag, and the results of the collection can be inspected immediately. The bag can then be removed from the AV, closed by whirling in a circle and clamping the wire tabs, and transported to the laboratory for microscopic inspection.
Processing of Semen for Insemination

The semen sample should be kept warm as it is carried into the laboratory for evaluation. The sample can be poured into a warm & clean graduated cylinder to ascertain the volume of the collection. There are also collection containers that are fitted with a both a filter and graduated marks on the side of the container for instant volume determination. There are also disposable AV liners that have a filter on the end of the liner that can be used to collect the semen. Therefore, transferring the sample to a graduated cylinder is not required for measurement. The volume of semen collected from the stallion ranges between 3 to 70 mL. The semen sample will be processed and evaluated for the following items.

1. **Volume & appearance** Determined by pouring the semen into a clean graduated cylinder and a visual analysis of sample to make sure it is not contaminated with dirt, blood, or pus.

2. **Motility** The percent of sperm that are moving forward is an estimate based on visual observation of several microscope fields. As this is an estimate, there can be operator error and even highly trained operators will differ somewhat in estimating motility. Nevertheless, sperm motility remains one of the most useful estimates of sperm viability after a variety of handling procedures. It may be necessary to dilute the semen sample somewhat to estimate motility if the spermatozoa are heavily concentrated. Semen is typically diluted in and a commercial extender (E-Z mixin) or similar skim milk diluent. A 1:20 dilution is made.

3. **Concentration** Estimated using either a hemacytometer, spectrophotometer, or commercial unit. Commercially available instruments need to be calibrated, but calibration solutions are usually provided and computer-assisted interpretation is done. These instruments would be handy if you collect stallions frequently and need to evaluate the semen often, but are expensive and probably not justifiable for smaller operations.

Calculating an Insemination Dose.

Once the volume of semen collected, concentration of sperm per mL, and percent progressively motile are known, the total number of motile sperm per ejaculate can be calculated.

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\text{Sperm # per ejaculate} = \left( \frac{\text{concentration}}{\text{mL}} \times \text{volume collected} \times \frac{\text{percent motile}}{100} \right)
\]

This value represents the total numbers of sperm that are probably capable of fertilizing an oocyte. It is recommended that each mare be inseminated with 500 million motile sperm. Therefore, the number of insemination doses per ejaculate can be calculated:

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\text{Insemination doses per ejaculate} = \frac{\text{total number of motile sperm}}{500 \times 10^6}
\]

If fresh semen is used for on-farm inseminations, it can be extended and approximately 2 to 10 inseminations can be achieved from a single stallion ejaculate. If fresh semen is extended, cooled and shipped, a single collection will typically yield between 2 to 5 inseminations from a single stallion ejaculate.
Shipping Equine Semen.

Development of passive cooling containers, like the “Equitainer” and the “Equine Express” shipping container allow liquids to be stored and/or shipped at a predetermined temperature (5°C) for up to 48 hours (See picture below). The Equitainer contains small cans that contain a coolant, which sits on top of the insulated container where the semen is stored. The Equine Express uses an ice pack that has a styrofoam barrier between it and the semen, which is usually stored in syringe. In both instances the semen is cooled passively.

Passive cooling prevents a rapid decrease in temperature that can cause cold shock and kill the sperm cells. To maintain sperm motility during and after cooling the cooling rate should be between 0.1 to 0.05°C/minute. Storage temperatures of 4 - 6°C are better than 0°C. The containers have been used to ship semen great distances without the significant losses to the integrity of the sperm cells that would occur from freezing. Furthermore, semen from certain stallions apparently freezes better than others, whereas cooled semen appears to cause fewer losses. Fertilizing capacity of cooled semen is usually maintained for 24 hours, and has been reported after as long as 96 hours. In general, stallion owner, mare owner, associated veterinarians, and farm managers should plan for insemination to occur within 24 hours of semen collection.

This new technology requires that the semen deposited in the shipping container be evaluated (for motility and concentration) by a competent person at the time of shipping and again when received. Although quality of shipped semen deteriorates somewhat, it is imperative that the recipient breeder evaluate it again to make sure it is usable. Often stallion owners send a double dose to make sure there is sufficient material, raising the question of whether or not to put it all in the mare at the time of insemination. Recent discussions at veterinary and scientific meetings have generated the consensus that 1) the mare is the best incubator for semen and 2) once the passive cooling container is opened, the conditions which favor sperm survival have been lost. Hence, the extended semen should be transferred directly into the mare’s uterus upon removal and evaluation of the sample form the shipping container.