

# Artificial Insemination in Horses

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**T**he number of horses produced in the United States has increased greatly in recent decades. Many have been produced through the use of artificial insemination (AI), particularly among the breed registries that permit the use of such technology. Successful use of AI in a horse breeding program requires that horse owners, breeding technicians and breeding farm managers understand the procedures associated with AI.

Artificial insemination requires instruction and skills developed under the supervision of trained personnel before attempting it on a farm. In some states, AI must be performed by or under the supervision of a licensed veterinarian. Before incorporating this practice into a breeding management program, breeding managers should learn the regulations in their states. Therefore, the purpose of this publication is to increase awareness of the factors involved and expertise required in an AI program.

Factors to consider before using artificial insemination include:

- ◆ The physical facilities
- ◆ Equipment required

- ◆ The number of stallions and mares in the program
- ◆ Access to veterinary personnel
- ◆ The amount of time that can be committed to the breeding program.

Breeding horses by AI requires considerable management. The timing of mare insemination is dictated by follicular dynamics, so mares must be evaluated at specific intervals. Breeding managers must develop a relatively strict routine to ensure that insemination takes place at the most appropriate time to achieve conception.

## The Mare

The mare is seasonally polyestrous, meaning that she will have a number of regular estrous cycles but only during the long-day season of the year. In the Northern Hemisphere, a mare normally has cycles of approximately 21 to 23 days from April through September. If not pregnant, she will have six to eight estrous cycles during the breeding season.

Most mares go through a short transitional period in the fall before entering anestrus, the time when they do not have estrous cycles or



Estrus in mares can be detected by placing a stallion in a holding pen while the mares are allowed to exhibit behavioral signs without the influence of a handler.



Characteristic signs of intense estrus: lifting tail, squatting (posturing) and urination.

show behavioral estrus. Anestrous mares typically go through a lengthy transitional period in late winter and early spring as they re-enter the breeding season (long days). During this season, the mare's estrous cycle consists of two phases — estrus, when she is receptive to the stallion, and diestrus, when she is disinterested or even negatively aggressive toward the stallion. These phases are governed by hormonal activity and the influence of hormones on the reproductive structures.

Mares should be evaluated before the start of the season to determine that they are sound for breeding. Then specific criteria must be met in deciding when to inseminate a mare during an estrous cycle.

The first of these is detection of behavioral estrus. Mares demonstrate receptivity to the stallion that coincides with and is governed by the follicular activity on the ovaries. Signs of behavioral estrus are often defined by a numerical system. Although scoring systems may differ, they generally begin with a low number that indicates no interest or aggression toward the stallion and proceed to a higher score to indicate an intense display of receptivity. The Texas A&M University Horse Center assigns scores to mares according to the following table of characteristics associated with teasing behavior. There are other similar scoring systems, and a horse breeder should use the system that works best in his situation.

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| <ul style="list-style-type: none"> <li>0 – resists stallion, pins ears, vocalizes, kicks at stallion</li> <li>1 – indifferent toward stallion, tolerates presence of stallion</li> <li>2 – interested in stallion as evidenced by advancing toward stallion, lifting tail</li> <li>3 – stands close to stallion, sometimes in a squatting position, some urination and eversion of clitoris (winking)</li> <li>4 – squatting, frequent urination, eversion of clitoris, leaning toward or onto stallion</li> </ul> |
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In most mares, intensity of the physical signs increases until ovulation of the follicle and then ceases relatively abruptly within 1 to 2 days. Generally, once a mare demonstrates receptivity to the stallion, she has passed the first of three criteria required for breeding.

The behavioral signs should be correlated with the presence of a growing or mature follicle, an appropriate uterine environment and a relaxed cervix, all of which are determined either by palpation or ultrasonography. Before the development of ultrasound technology, a mare's reproductive tract was evaluated by rectal palpation of the ovaries, uterus and cervix and by visual examination of the cervix using a vaginal speculum. Palpation and visual examination are valid tools that continue to be useful in reproductive tract evaluation.

**NOTE:** Palpation and ultrasound should not be attempted by any untrained person. These skills are developed through intensive practice under the supervision of trained technicians. Mares can be seriously injured during this examination, and great care must be taken to ensure safety of both animals and people.

In the past decade, ultrasonography has become a routine procedure for making breeding decisions as well as for pregnancy determination. It provides knowledge of the size and consistency of follicles, the condition of the uterus and the relaxation of the cervix. This information is necessary in timing insemination.

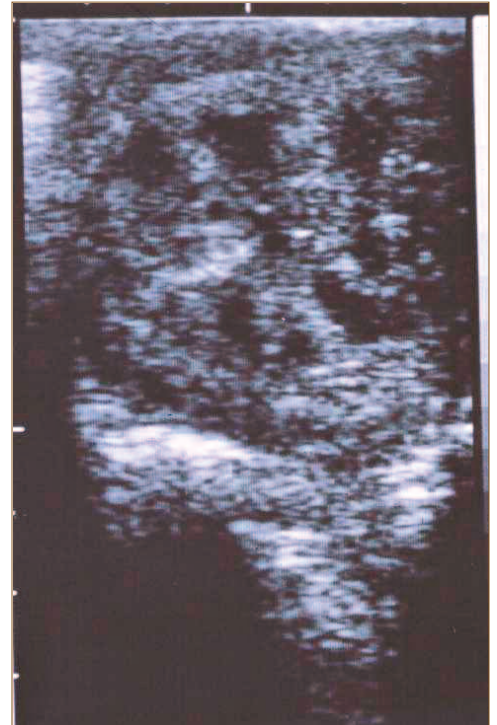
In the week before ovulation, the follicle grows approximately 3 mm per day (Pierson and Ginther, 1985). Mares are generally inseminated when the follicle is larger than 35 mm in diameter, assuming that other characteristics relating to estrus and cervical relaxation are present. Therefore, even when mares are palpated or examined by ultrasonography only once or twice per week, the breeder can still time insemination based on expected follicular growth, behavioral signs and cervical evaluation. If the follicle does not ovulate within 2 days or if the mare continues to demonstrate signs of intense estrus, she should be inseminated again because sperm are viable within the mare's tract for only about 48 hours.

Although presence of a mature follicle is a necessary criterion for insemination, it is also important to evaluate the uterine environment. Ultrasonography of the uterus will show whether it is under the influence of estrogen, which produces a good environment for breeding, or progesterone, which produces an unacceptable state. An estrogen-dominated uterus has a characteristic ultrasonographic appearance shown in the illustration to the right. If the uterus does not have this appearance, further evaluation is needed to determine why there is inconsistency between follicular and uterine dynamics.

Finally, the cervix should be examined by digital manipulation or by using a vaginal speculum. During estrus the cervix should appear pink or reddish, should be open and flaccid (soft and loose) and may hang or lie loosely on the floor of the anterior vaginal vault. If the cervix is pale, positioned relatively high and is closed, the mare is most likely under the influence of progesterone and should not be bred. She is either pregnant or in diestrus or anestrus.

Mares should meet at least two of these three criteria before breeding:

- ◆ The mare should be receptive to the stallion. Although this generally coincides with increasing follicular growth, a small



Ultrasonogram of the uterine horn of a mare in estrus.

number of mares will not show estrus even when a large follicle is present. This is known as “silent heat.”

- ◆ A mature follicle should be present. Typically, the mature follicle is greater than 35 mm in diameter and has a “soft” consistency. However, ovulation of smaller follicles is not uncommon.
- ◆ The cervix should be open. Mares in estrus with a large follicle but a tightly closed cervix should be re-evaluated before being inseminated.

## The Stallion

Consistency in handling the stallion and collecting the semen influences the success of a breeding program. The stallion facility used for semen collection should provide consistency. If there is no shed or barn dedicated specifically for breeding, there should be one area where the collection always takes place. A familiar breeding routine will enhance a stallion’s performance and help prevent behavioral problems associated with breeding.

Use of a breeding phantom, or “dummy,” is also wise because it provides another measure of consistency for the stallion. However, a stallion can also be collected from a live or “jump” mare that is in estrus. This is relatively easy if done properly, although it introduces another variable and increases the need for good facilities and restraining equipment, such as breeding hobbles.

### Collecting the Semen



CSU model artificial vagina.

There are two primary equine artificial vagina (AV) models that are popular and easy to use. Choose the model that is acceptable to the horse and maintains semen viability during the collection. The Missouri Model AV (NASCO, Ft. Atkinson, WI) is comparatively lightweight and flexible. However, it does not maintain temperature very long and if there is much delay between AV preparation and semen collection, the AV temperature may be too low for successful collection. The Colorado Model AV (Animal Reproduction Systems, Chino, CA) is more rigid and heavier but better maintains a consistent temperature, even when used outdoors.

The AV must be prepared in the same manner for each collection so that the temperature and pressure of the AV are the same each time. Many stallions become distracted or annoyed if there are significant differences in procedure, facilities, AV or handler from one collection to the next.

Each semen collection should begin by preparing the artificial vagina. It is much easier to apply lubricant to the inside of the AV before filling. Use a non-greasy, non-spermicidal, sterile lubri-

cant, applied to the hand of a disposable obstetrical sleeve and insert it one-half to two-thirds the length of the AV. There are a number of commercially available lubricants.

Leave the disposable sleeve in the AV to prevent the lubricant from drying and contaminants from entering. The bladder of the AV can be filled with hot water to the appropriate pressure necessary to stimulate the stallion's penis during semen collection. The AV temperature at collection should be 114 to 118 degrees F, so the water should be 125 to 135 degrees F when the bladder is filled. Then wait for the temperature in the AV to reach the desired range. While waiting, keep the AV in a warm, dry place.

The pressure of the AV should be such that a closed fist can be inserted into the AV all the way to the end where the glans penis of the stallion will enlarge during ejaculation. If the AV is too loose, the stallion will not receive proper stimulation. If it is too tight, it may cause pain that deters the stallion from completely ejaculating. If an unfamiliar stallion is being collected, both temperature and pressure will have to be evaluated and adjusted to determine the most suitable AV conditions for that stallion.

While waiting for the AV to reach proper temperature, the stallion can be brought to the collection area for teasing and washing of the penis. Many stallions become familiar with this routine and develop an erection as soon as they are brought to the collection area. This removes the need for a tease mare. Each stallion has its own preferences. Some may require more teasing for a complete ejaculation, while others may easily become over-stimulated to the point of becoming unmanageable or increasing semen volume too much.

**NOTE:** The increase in semen volume that occurs with increased teasing of a stallion does not yield a greater number of sperm cells in the ejaculate. Rather, it increases the volume due to increased emission of fluids from the accessory glands.

The stallion's penis should be washed only with warm water. Paper towels, cotton or gauze can be used, if necessary, to remove dirt and smegma. Stallions that are washed regularly tend to remain fairly clean, so the washing process may serve more to stimulate the stallion than to clean him. Do not use soap or cleanser as they disrupt the normal microfloral population on the surface of the penis and can allow potentially harmful pathogens to grow. After washing, the penis can be dried either by patting lightly with paper towels or by allowing it to dry on its own.

Collecting the semen is the next step in the AI process. If some time has elapsed between washing and collection, the stallion may have to be teased again to attain an erection. He should be led in



Missouri model artificial vagina (distributed by NASCO) being prepared for collection.



The washing process serves to clean the penis and prepare the stallion for the semen collection. The stallion should not be handled roughly during washing.

a controlled manner to the breeding phantom or jump mare to be used for the collection. If using a phantom, the stallion approaches from the rear. With a jump mare, his approach should be from the left side for the safety of both handler and stallion. The stallion should be well-mannered enough that he can be stopped at any time on his way to the phantom or jump mare. As the stallion mounts, his head should rest on the left side as this will make it easier for the handler to prevent the stallion from moving up toward the head or getting mounted sideways.

**Caution:** Stallion handling and semen collection should not be attempted by persons not experienced in handling horses. It should always be done under the direct supervision of an experienced and knowledgeable person who understands the procedure very well.



Semen collection into a CSU model AV using a phantom. Note the position of the AV during the collection.

The collector, who should be positioned somewhat to the side of the handler, can move in to deflect the stallion's penis to the left side and into the AV. Usually, as the stallion feels the pressure of the AV, he will begin to thrust into the AV and may try to move toward that side. The collector should be careful not to pull the stallion's penis toward the AV or otherwise be rough and cause the stallion to become distracted or uncomfortable with the procedure. As long as the AV is properly positioned and held steady, most stallions will "seat" themselves into the AV and continue thrusting until ejaculation. The collector can feel the pulses of the ejaculation by positioning the right hand at the opening of the AV beneath the stallion's penis. Also, the stallion will "flag" the tail several times as ejaculation occurs. At this time, the collection bottle end of the AV should be lowered so the semen is not lost.

As the stallion dismounts, the AV is removed from the penis to be taken to the lab for semen evaluation. The stallion's penis can be rinsed with warm water, but it has to be done fairly quickly before it is retracted into the sheath. (The AV should be washed as soon as possible, but this can be done after mares are inseminated.)

Proper handling of the semen is essential to a successful breeding program. Care must be taken not to contaminate the semen or do anything that might decrease the viability of the sperm cells. In cold weather, spermatozoa can be easily shocked and become non-viable between the time of collection and arrival in the lab. If collection is done outdoors, use a collection bottle cover to protect the semen from cold temperature or sunlight. Once in the lab, the collection bottle is removed from the AV and placed in an incubator maintained at 98 degrees F (37 degrees C).

## Semen Evaluation

The semen should be evaluated as soon after collection as possible. Good managers maintain individual semen evaluation records for each collection performed. First, record the total volume, unless the semen was filtered as it was collected. If it was not filtered to remove the gel-fraction, this should be done next and the gel-free volume recorded. Within a few minutes, evaluate sperm motility with a microscope at 40X or 100X power since sperm cells quickly begin to lose forward motion and viability. Normal sperm have motility in the range of 60 to 100 percent (Evans, 2001).

To calculate an insemination dose, the concentration of spermatozoa in the ejaculate must be determined. Several instruments are available for this determination with a wide range of costs and capabilities. The least expensive is a hemacytometer, used with a microscope to count actual cells. The most widely used instrument is the Densimeter® (Animal Reproduction Systems, Chino, CA), which can also calculate the insemination dose. Several other less expensive sperm counters are reliable for determining spermatozoal concentration, but they have fewer features than the Densimeter®.



Evaluation of spermatozoal concentration in an ejaculate with the Densimeter®.

### Calculation of Insemination Dos

$$\frac{500 \times 10^6}{\text{Concentration} \times \text{Motility}} = \text{Insemination Dose}$$

#### Example

If the ejaculate had a concentration of  $200 \times 10^6$  (million) per ml and 75 percent motility, the following calculation would give the minimal dose for inseminating with 500 million motile spermatozoa.

$$\frac{500 \times 10^6}{(200 \times 10^6) \times .75} = \frac{500 \times 10^6}{150 \times 10^6} = 3.33 \text{ ml semen (unextended)}$$

Adapted from Pickett and Back, 1973

When the sperm motility and concentration are known, the insemination dose can be calculated. The optimum dose contains 500 million motile spermatozoa per insemination, although acceptable conception rates can be achieved with slightly fewer sperm from some stallions (McKinnon and Voss, 1993). If AI is performed with shipped, cooled semen, the recommended insemination dose is doubled to 1 billion motile sperm.

### Number of Inseminations per Ejaculate

If a number of mares are to be inseminated with an ejaculate, it is necessary to calculate the number of inseminations (with 500 million motile sperm) that can be made. Using the previously calculated insemination dose, an ejaculate of 50 ml would provide,

$$50 \text{ ml} \times .75 \times (200 \times 10^6) = 7500 \times 10^6 \text{ total motile sperm}$$

$$\frac{7500 \times 10^6}{500 \times 10^6} = 15 \text{ inseminations}$$

Adapted from Sorensen, 1976



A mild soap can be used to wash the perineal area of the mare prior to insemination.



Water is spermicidal so it is suggested that the mare be dried before insemination.

Once semen parameters have been established, the semen should be diluted with an extender containing antibiotics to maximize its longevity and decrease the growth of bacteria in the semen. Dilution ratios of 1:1 to 1:4 parts semen to extender can be used. Semen should be evaluated for motility again after it is diluted.

The total volume of extended semen placed in the uterus of a mare should be between 10 and 20 ml. Larger volumes of semen do not enhance conception and may reflux into the vagina (Samper, 2000). An extremely low volume may prevent the sperm from making their way into the oviduct. However, acceptable pregnancy rates have been achieved with low doses (.5 to 5 ml), as is usually the case when using frozen semen.

### The Insemination

Restrain the mare for insemination, preferably in a set of large animal stocks, so that forward, backward or side-to-side movement is limited. The tail should be held out of the way or tied to the side in some manner to prevent contamination of the external genitalia after the area has been washed.

The entire perineal area (vulva, anus and buttocks) should be cleaned with water and a mild soap. It is important to clean from the middle (vulvar opening) toward the outside (buttocks), taking debris away from the opening of the vulva. Wash the area with paper towels or by using the hand. After washing with soap and rinsing, repeat the washing until dirt and debris are not visible.

Use paper towels to dry the area, wiping first the buttocks and moving successively toward the center with each wipe. Be careful not to contaminate the clean area as it is dried.

The extended insemination dose should be placed in a sterile, non-toxic, disposable syringe (Air-Tite®) and kept warm and protected from sunlight until deposited in the uterus of the mare. The



inseminator places a hand into a sterile, disposable obstetrical sleeve and applies sterile lubricant to the back of the hand. The tip of the insemination pipette is held in the palm of the sleeved hand and the other end steadied with the free hand. The sleeved hand applies lubricant to the vulva and entry is made into the vagina by keeping the fingertips straight and together.

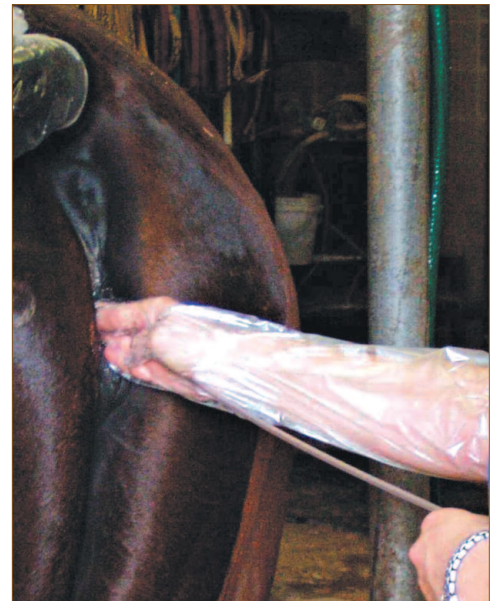
The hand is pushed in with an upward motion and may be rotated palm-down as the hand is inserted farther into the vagina. The tip of the pipette is held in the palm by the thumb, while the extended fingertips find the opening of the cervix and steady it for placement of the pipette. As the pipette is inserted through the open cervix into the body of the uterus, the fingers hold the cervix closed around the pipette to prevent backflow of semen into the vagina. The insemination dose is slowly expelled through the pipette (slow count of 10), followed by pushing 2 to 3 cc of air through the pipette to ensure that all the semen is expelled. The pipette is then pulled back through the cervix and held in the palm as it is removed from the vagina. As the hand is pulled out of the vulva, it should be in a downward slanted position to decrease aspiration of air into the vagina. The vulva may be wiped with a towel or rinsed with water to remove excess lubricant.

It may be necessary to inseminate a mare more than once during estrus, depending on the follicular characteristics and when ovulation occurs. It is not uncommon to have more than one insemination per cycle. Because sperm are viable about 48 hours in the mare's reproductive tract, the generally accepted practice is to inseminate every other day during estrus after the first insemination. Continuing to inseminate after ovulation does not increase the likelihood of conception and may contribute to early embryonic loss (Woods, Bergfelt and Ginther, 1990). Inseminating after ovulation is not recommended because conception rates are low (Samper, 2000).

### **The Decision to Use Artificial Insemination**

Horses have been produced by artificial insemination for decades with widespread use in the United States after 1950. Its use is permitted by all breed registries in the United States except the Jockey Club (Thoroughbreds).

With proper technical training, many horse owners can adapt their breeding facilities and programs to include AI. However, they must first decide whether the added costs and management commitment are offset by improved reproductive performance associated with AI. Both hands-on and technical training are required to perform AI successfully. Even minor errors in the use of AI technology can be detrimental to a breeding program.



Proper position of hand with insemination pipette prior to insemination of mare.



After insemination, the hand should be slowly withdrawn in a downward motion to prevent aspiration of air into the vagina.

There are numerous publications containing information on AI, including the following list. In addition, some colleges and universities teach seminars and short courses on equine AI techniques. For more information, contact Dr. Martha M. Vogelsang, Equine Science Section, Department of Animal Science, Texas A&M University.

## **Resources**

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