

Artificial Insemination methods

The inseminating cow considers the last step of AI. technique and its involved putting the collected semen from the bull into the reproductive tract of cattle.

There are two methods of artificial inseminate cow which include:

- 1- **Surgical methods:** by labrascope or through the laparotomy
- 2- **Non-surgical methods:** include three methods
 - 1- **Vaginal insemination:** the earliest insemination was accomplished by simply inserting a tube into the vagina and depositing seen at the mouth of cervix.
This method is simple but need large number of sperm with low conception rate.
 - 2- **Cervical insemination:** accomplished by inserting sterile speculum into vagina with the use of light source and the inseminating instrument can be inserted in to the opening of cervix (1-2 cm) this method is far superior than vaginal method but usually gives 10-12 percent unit are lower conception rate than the recto-vaginal method with large number of sperm and its need sterilize the equipment between the inseminations.
 - 3- **Intra-uterine insemination (The recto-vaginal inseminations):** this technique is the most commonly used method to artificially inseminate cattle. it have high conception rate with small number of sperm, its need some experience for used.

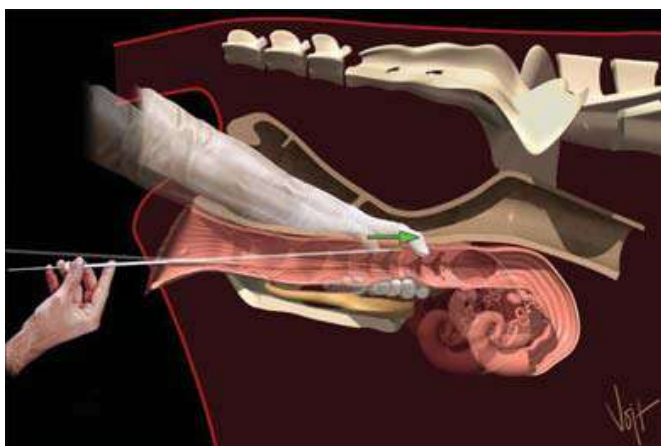
Steps of recto-vaginal Insemination in cattle

- 1- **Estrous detection:** The cow must be in estrus or heat before she is artificial inseminated.
Estrus is the time period during the estrous cycle when the female will allow breeding to occur.
The best signs that a cow is in estrus are stand to amount by other cow or bull, the other signs are riding other cows, restlessness, bellowing, vaginal discharge.
- 2- **Timing of Insemination:** For conception to occur, insemination must take place at the correct stage of the cow's estrous cycle.
Bovine spermatozoa require a few hours in the female reproductive tract to capacitate and become capable of fertilization and they remain viable for about 24 hours.

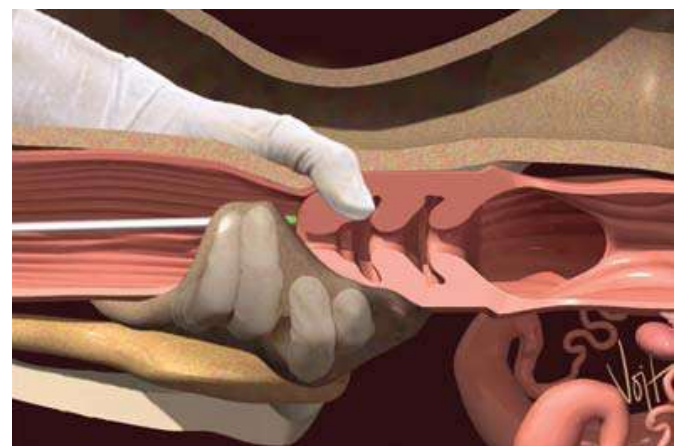
The egg is most likely to become fertilized if it contacts viable spermatozoa within about 6 hours of ovulation, and has maximum life of about 12 hours. Thus the timing of AI must be fairly precise.

The optimum time to inseminate is 12 hours after the beginning of standing estrous.

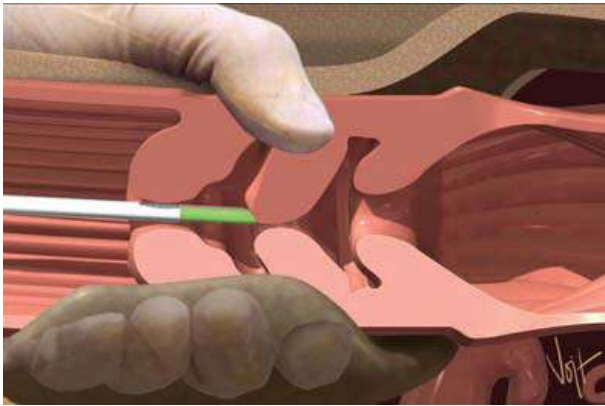
- 3- **Restraint animal:** for Safety of both the animal and the inseminator and for easy used of inseminate.
- 4- **Thawing the Semen:** The straw should then be placed in warm water bath for 30 seconds. The straw is then removed from the bath and dried off with a paper towel to prevent water from coming into contact with the semen.
- 5- **Loading the AI Rod:** The sealed end of the straw is then removed with sharp scissors and the straw loaded into a specially made 'gun' being secured in place by a clean, new, disposable plastic sheath. The straw in the insemination gun should be insulated, especially in cold weather.
- 6- **Cleaning:** The skin around the vulva should be cleaned with paper towels to avoid contaminating the cow when inserting the AI rod into the vagina.
- 7- **Inserting the Rod:** the AI technician grasps the cervix per rectum, pulls it towards him, and holds it level so that the insemination gun can be introduced through the vagina into the cervical os. The tip of the pipette is passed just through the cervix so that semen is injected into the uterine body.
- 8- After the semen has been deposited, the technician may also massage the tract to stimulate release of Oxytocin which aids in semen transport. And then the rod should carefully be removed from the reproductive tract.



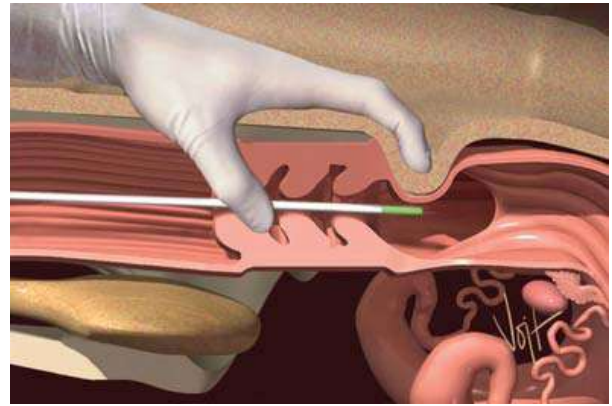
Grasp the cervix and push it forward to straighten vaginal folds.



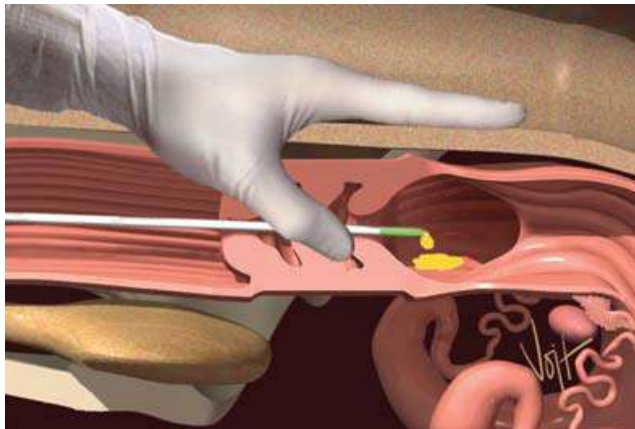
Grasp the external opening to the cervix with the thumb on top and the forefingers underneath to close the fornix and guide the gun tip into the cervix.



Using the flexibility of your wrist, twist and bend the cervix until you feel the second ring slide over the gun tip.



Use your index finger to check gun placement (1/4 inch past the end of the cervix) before depositing semen.



Push the plunger slowly so that drops of semen fall directly into the uterine body.

SPERM TRANSPORT IN THE FEMALE TRACT

Species differences exist in the sites at which the ejaculate is deposited in the female reproductive tract during copulation.

In cattle and sheep, the small volume of semen is ejaculated into the cranial end of the vagina and onto the cervix.

In horses and swine, the voluminous ejaculate is deposited through the relaxed cervical canal into the uterus.

SPERM IN THE FEMALE TRACT

Within the female tract spermatozoa are lost by:

A. Phagocytosis by neutrophils (WBC): the uterus and vagina

- High estradiol increases the number of WBC
- Neutrophils in the mucosa of the vagina and uterus attack foreign microorganisms
- Spermatozoa are foreign to the female and thus some sperms are killed by neutrophils

B. Physical barrier including the cervix.

Cervix is a Physical barrier to sperm transport it serves several function including:

- Removal of non-motile sperm
- Removal of some abnormal sperms
- may also serve as a reservoir for spermatozoa

In the cow cervix two type of mucus:

- 1) **Sialomucin:** low viscosity and helps forward movement
- 2) **Sulfomucin:** high viscosity and washes sperm out!

Low viscosity environment in the valley of the cervix creates "**privileged pathway**" for healthy spermatozoa

Distribution in the Female Sperm Reproductive Tract

Three stages are recognized in sperm transport in the female reproductive tract:

- 1- short, rapid sperm transport;
- 2- colonization of reservoirs; and
- 3- slow, prolonged release.

RAPID TRANSPORT:

Immediately after insemination sperm penetrate the micelles of the cervical mucus where some are quickly transported through the cervical canal.

This phase takes 2 to 10 minutes and may be facilitated by sperm motility as well as increased contractile activity of the myometrium and mesosalpinx during courtship and coitus.

COLONIZATION OF SPERM RESERVOIRS:

Massive numbers of sperm are trapped in the complex mucosal folds of the cervical crypts. This process is facilitated by the fact that the micelles of the cervical mucus direct sperm to the cervical crypts where the reservoir is formed.

Fewer leukocytes are found in the cervical secretions compared with those of the vagina or uterus; this suggests that less phagocytosis of sperm takes place in the cervix.

The more sperm that enter the cervical reservoir, the more that will reach the oviduct, thus increasing the chance of fertilization. In addition, the larger the reservoir, the longer an adequate population of sperm will be maintained.

SLOW RELEASE AND TRANSPORT:

After adequate sperm reservoirs have been established within the reproductive tract, the sperm are released sequentially for a prolonged period. This slow release, which involves the innate motility of sperm and the contractile activity of the myometrium and mesosalpinx, ensures the continued availability of sperm for entry to the oviduct to effect fertilization of the egg. However, various anatomic and physiologic barriers prevent massive numbers of sperm in the ejaculate from reaching the site of fertilization presumably to avoid polyspermy, which is lethal to the fertilized egg.

