REPUBLIC OF ALGERIA DEMOCRATIC AND POPULAR

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY OF TIARET

INSTITUTE OF VETERINARY SCIENCES

ARTIFICIAL INSEMINATION OF FARM ANIMALS

Course Handbook for 5th-Year Veterinary Students

By AYAD Mohamed Amine
Assistant Professor

Academic Year 2024-2025

ARTIFICIAL INSEMINATION

OF

FARM ANIMALS

Contents

Contenu

Preface

This text was motivated by our desire to create a valuable contribution to the existing literature on one aspect of farm animal reproduction and biotechnology. This handbook has been carefully prepared to serve as a foundational guide for veterinary students who wish to explore the field of artificial insemination in farm animals, such as bovines, mares, and small ruminants. The material aims to give readers a comprehensive understanding of the principles, techniques, and practical applications of artificial insemination, which is an integral component of modern animal reproduction and management.

This handbook will guide you through both the basic principles and advanced methodologies associated with artificial insemination. The sections are structured to build upon your foundational knowledge and equip you with the skills necessary to perform these techniques with precision and confidence. Whether you are encountering this topic for the first time or seeking to refine your expertise, this resource will be extremely helpful in both your academic studies and future veterinary practice.

As you progress through the material, I encourage you to take your time and approach it with curiosity and a commitment to excellence. The knowledge and practical skills you gain will not only enhance your understanding of reproductive technologies but will also have a significant impact on your professional development as a veterinarian. Veterinary medicine is a constantly evolving field, and your mastery of these techniques will play a critical role in promoting animal health and productivity.

ARTIFICIAL INSEMINATION

Artificial insemination (AI) is a key technique to increase reproductive efficiency and the genetic improvement in animals. The first report of artificial insemination dates back over 220 years; since then, numerous studies have been published, and it is widely used in domestic animals. Despite the advances of embryo transfer programs in vivo and in vitro, artificial insemination still represents the main form of assisted reproduction in many herds, being one of the lowest cost strategies to improve genetic merit in the farms. Artificial insemination has many advantages over natural breeding, such as health benefits, control of infectious diseases; genetic improvement, e.g. use of genetically improved males; reproductive management improvements; and more stringent control of the zootechnical and economic features of the herd, e.g. standardized management and animals.

Comprehensive AI training for veterinary students is important, not only in the acquisition of a valuable skill but also in preparation for potentially assuming the role of an advisor to farm and companion animal producers. Veterinarians who are able to perform AI may also meet client demands for AI. Understanding the AI process, from male acquisition to collection, evaluation, cryopreservation, and distribution of semen, appropriate storage and handling of frozen semen, and correct AI technique is critical for building a strong foundation as a veterinarian (Dalton, J.C. et al., 2021).

Therefore, this chapter aims to present the main advances of artificial insemination and timed artificial insemination, highlighting the technical parameters, advantages, and influencing factors, and to discuss practical and current strategies for the improvement of the herd via artificial insemination programs (Morotti, F et al., 20121).

Artificial Insemination

Artificial insemination involves the collection of semen from a male, usually of superior genetic merit, followed by the transfer of that semen in to a female at a time of ovulation in order to result in fertilization (Davies, 1999). It has become one of the most important techniques ever devised for the genetic improvement of farm animals

History of Artificial insemination

Use of AI in animals is a human invention and more recent. Undocumented tales exist of Arabs obtaining sperm from mated mares belonging to rival groups and using the sperm to inseminate their own mares. However, our story starts with recorded history, where facts are available to document noteworthy achievements. Consequently, the story is related chronologically. Much of the development of AI occurred before the 1980s when electronic networks became available, so earlier references are included. The developments that made AI the most important animal biotechnology applied to date include improved methods of male management and semen collection, evaluation, preservation, and insemination. Detection of estrus and control of the estrous cycle in the female also were important. The development of AI is a remarkable story of tireless workers dedicated to the pursuit of knowledge, to the replacement of fiction with facts, and the application thereof.

Leeuwenhoek (1678) and his assistant, Hamm, were the first persons to see sperm, which they called “animalcules.” Leeuwenhoek did not have an advanced formal education, so he did not study Latin, the scientific language of the day. language of the day. However, he was a clever, capable individual who ground lenses so precisely (one still exists today with 270 magnifications) that sperm were visible. His published paper (Leeuwenhoek, 1678) amazed, and perhaps amused, the reigning king of England, who regularly read papers submitted to the Royal Society, where Leeuwenhoek’s paper was published. Another century passed before the first successful insemination was performed by Spallanzani (1784) in a dog, which whelped three pups 62 d later. Another 100 yr passed before Heape (1897) and others in several countries reported that AI had been used in isolated studies with rabbits, dogs, and horses (Foote R. H., 2010).

In 1899, Ivanoff embarked on groundbreaking efforts Russia to establish artificial insemination (AI) as a viable procedure. By 1907, his pioneering work extended to encompass research on AI across various species, including dogs, livestock, rabbits, foxes, and poultry. Notably, Ivanoff not only conducted extensive studies but also contributed significantly to the development of AI by devising semen extenders, training personnel in the selection of superior stallions, and effectively utilizing AI to enhance their offspring (Ivanoff, EI., 1922). In 1938, Milovanov introduced pioneering strategies for the selective breeding of ovine and bovine species. Among his notable advancements was the development of functional artificial vaginas, which continue to be employed in contemporary animal

husbandry practices. This innovation represented a significant enhancement over preceding methodologies, such as the extraction of semen via female specimens.

Dr. Ishikawa, a scientist from Japan, received mentorship from Ivanoff. After working with Ivanoff in 1912, Dr. Ishikawa brought artificial insemination (AI) methods for domestic animal husbandry to Japan. The Japanese government started AI programs aimed at cattle and poultry in the late 1930s (Nishikawa Y., 1959). However, the adoption of AI progressed slower than expected, initially focusing on horses. The Ivanoff study, which was published in 1922, helped other nations learn about AI methods from Russia. Walton's seminal book on AI, released in the West in 1933, further popularized the technique. Walton achieved a milestone by successfully using ram semen to inseminate ewes, a technique later transported to Poland. Eduard Sörensen founded Denmark's first cooperative dairy AI association in 1933, motivated by the groundbreaking work in Russia. The association's efforts were fruitful, as 59% of the 1,070 cows produced calves. EJ Perry of New Jersey established the country's inaugural AI cooperation in 1938 (Ombelet WJ., 2015). A major turning point was reached on November 1, 1939, when the first artificially inseminated rabbit was displayed at the 12th Annual Graduate Fortnight of the New York Academy of Medicine. Gregory Pincus, an American scientist, achieved this milestone by employing in vitro fertilization techniques. He fertilized an ovum extracted from the ovarian follicles of a female rabbit using a saline solution. Subsequently, he transferred the fertilized ovum into the uterine cavity of another female rabbit, where it served as an ectopic incubation environment (Foote RH., 2010).

Through rectovaginal immobilization of the cervix, Danish veterinarians invented a method for injecting semen deeply into the uterine body or cervix. This method offered the remarkable advantage of significantly achieving fewer fertilization sperm, with notable breakthrough. Another significant advancement in Danish artificial insemination was the introduction of semen packaging using straws (Sørensen E., 1940). Subsequently, Cassou began commercial production of straws in 1964, and today they are widely utilized worldwide (Cassou R., 1964). Some important landmarks regarding Artificial insemination are given in Table 1.

Table 01. Important dates in the history of Artificial Insemination (Foote RH., 2010).

Year	Description of Event
1677	Leeuwenhoek discovered spermatozoa.
1780	Spallanzani successfully inseminated a bitch
1799	Hunter used AI for a woman.
1803	Spallanzani observed that chilling sperm did not kill them.
1899	Ivanoff initiated organized AI research in Russia
1902	Sand recommended AI in Denmark, but no program was started.
1912	Ishikawa organized AI research in Japan
1914	Amantea devised the first artificial vagina for use in dogs.
1930s	Organized AI began in Denmark and the USA and quickly spread.
1937	Danish had established rectovaginal insemination, reducing sperm required
1940	Phillips developed phosphate-buffered egg yolk for preserving bull sperm
1941	Salisbury and others developed citrate-buffered egg yolk for preserving bull sperm.
1948	Almquist and Foote reported independently on the value of antibiotics in semen extenders to control microorganisms and increase fertility
1949	Polge et al. discovered that glycerol-protected sperm during freezing
1950s	Powerful tools for progeny testing were developed by Henderson and Robertson.
1954	Waterloo (Canada) was the first organization to use frozen semen 100%.
1957	American Breeders Service developed liquid nitrogen tanks and services for frozen semen.
1963	Davis et al. (Cornell) developed Tris-buffered egg yolk-glycerol for fresh and frozen sperm, used later for many species
1970	AI was used commercially for superovulated cows and embryo transfer and provided the initial framework for many breeding strategies.
1990s	Sexing bull sperm was improved with limited potential application.

Advantages of artificial insemination

1. **Genetic Improvement**: AI allows for the use of superior sires, leading to genetic progress in livestock populations (Nogalski et al., 2021); and superior stallions, facilitating genetic progress without the need for physical transportation (Duchamp et al., 2022).
2. **Disease Control**: Reduces the risk of transmitting diseases and injuries associated with natural mating (Kumar et al., 2023; Panzani et al., 2021).
3. **Increased Reproductive Efficiency**: AI enables efficient utilization of superior males, optimizing breeding programs (Bonilla et al., 2022; Samper et al., 2023).
4. **Cost Savings**: Reduces the need for maintaining expensive breeding males on farms (Lassaad et al., 2023)
5. **Flexibility and Convenience**: Provides flexibility in timing and logistics of breeding (Franco-Jimenez et al., 2021; Alcántara-Neto et al., 2022).
6. **Management of Subfertility**: Helps manage fertility issues in mares and stallions through specialized semen handling and timing of insemination (Hinrichs, 2021).

Disadvantages of artificial insemination:

1. **Technological Expertise Required**: AI requires skilled personnel and equipment, which may not be available in all locations (Aerts et al., 2022; Squires et al., 2023).
2. **Higher Initial Costs**: Setting up AI facilities and purchasing semen can be expensive (Zorrilla et al., 2023; Stout, 2022).
3. **Reduced Genetic Diversity**: Overuse of a few highly selected sires can reduce genetic diversity within populations (Oom et al., 2022); Semen handling and storage can affect sperm viability and fertility rates if not managed meticulously (Sieme et al., 2021).
4. **Handling and Storage Issues**: Semen handling and storage can affect fertility rates if not managed properly (Cuesta et al., 2021).
5. **Labor Intensive**: Despite technological advancements, AI procedures can still be labor-intensive compared to natural breeding (Roca et al., 2023).
6. **Legal and Ethical Considerations**: In some jurisdictions, regulations regarding AI in horses may restrict its use or impose additional requirements (Varner et al., 2023).

7. **Behavioral Considerations**: Artificial insemination may not address natural mating behaviors and social interactions important for horse welfare (Aurich., 2021).

Artificial Insemination Technique: Cattle

Though artificial insemination (AI) is common in the cattle industry and often performed by farm managers or employees, it should not be assumed that AI is an easy technique or that all of those performing AI are proficient in the technique. Success rates differ, as can sometimes be evidenced by a difference in conception rates between technicians.

In developing the manual skills needed for insemination, trainees should work with numerous reproductive tracts and receive considerable practice inseminating a variety of live cows. Developing the skill to thread the insemination rod through the cervix should not be the only objective. AI training programs should also emphasize the importance of sanitation and the perfection of skills to consistently identify the proper site of semen deposition and to accurately deposit the semen. In addition, trainees should obtain a good understanding of reproductive anatomy and appreciate the essentials of a sound reproductive management program.

Reproductive anatomy

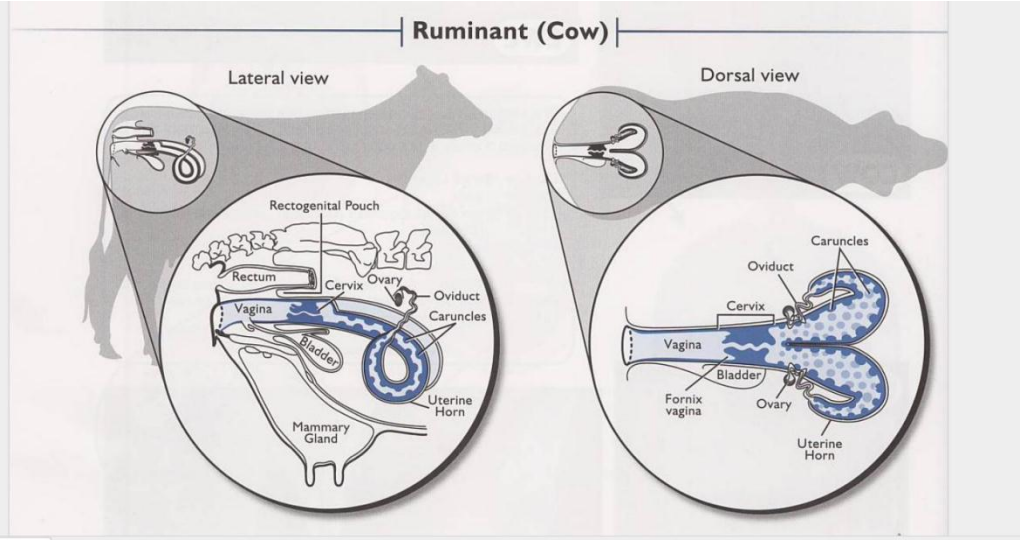


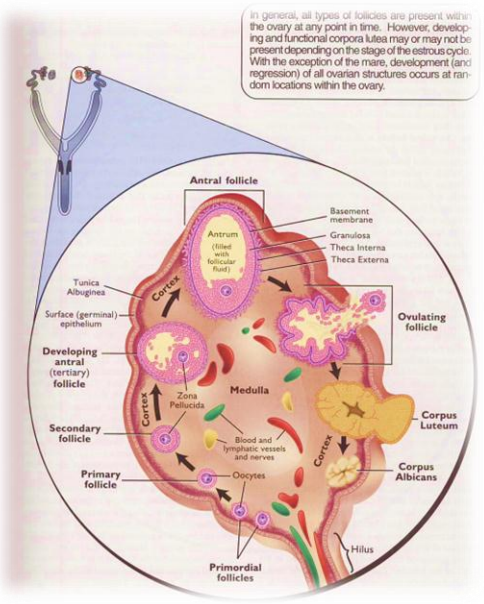
Figure 1. Lateral/ Dorsal view of cow
(P.L. SENGER 2005)

Introduction

The anatomy of the reproductive system in the cow is functionally grouped into the components associated with oocyte production and transport and those involved with gestation and copulation.

Ovaries

The cellular machinery for oogenesis and steroid production is found in the ovary (Figure 2,3). The ovary consists of a cortex and medulla. The medulla is composed of connective tissue, lymphatic vessels, blood vessels, and nerves. Surrounding the medulla is the cortex. The cortex contains the ova surrounded by follicular cells within the connective tissue stroma (Ross, M et al., 2003). Exterior to the cortex, the ovary is covered by the dense fibrous tunica albuginea and a superficial epithelium (Schaller, O. and Constantinescu, G., 1992). Because the ovary in the cow descends further from its embryologic origin near the kidney than other species, it is positioned closer to the pelvis. The consequence of this ovarian location and the attachment of the short mesovarium is that the uterine horns bend ventrally and caudally (Budras, K.D., 2003). (Figure 1,4).



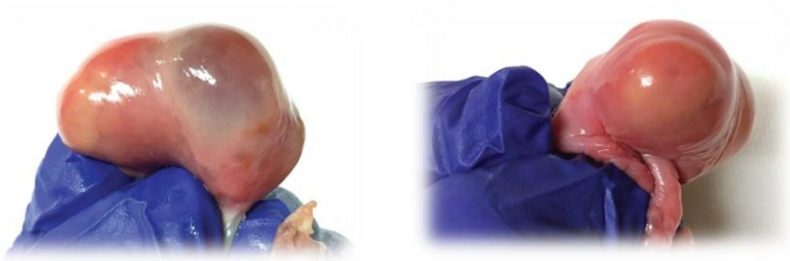


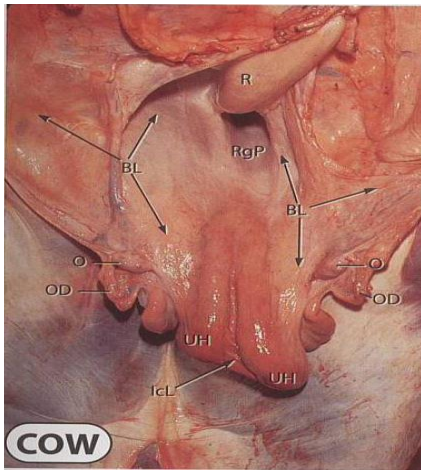
Figure 2 : the major structures of the ovary present

Figure 3 : *An ovary with a dominant follicle (Left), a corpus luteum (CL) present (Right) .*

Transport and Gestation

The reproductive system of the cow is designed to transport spermatozoa toward the ovary and to transport an ovum toward the spermatozoa (Figure 1). The parts of this tubular

system include the vestibule, vagina, cervix, uterine horns, and uterine tubes (oviduct) (Ben Nabors., 2021).



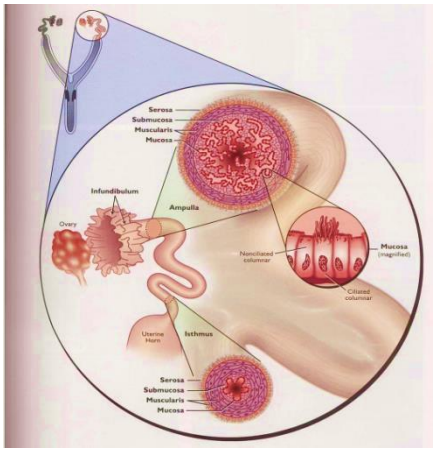
The intestines have been removed so that the reproductive tract is in full view. The tract is suspended by the broad ligament that is attached dorsally and is continuous with the peritoneum.

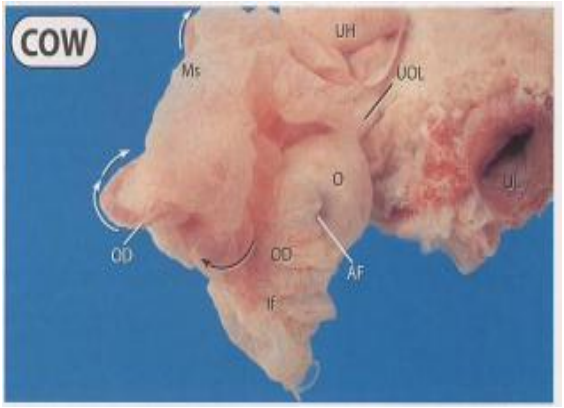
BL= Broad ligament
CX= Cervix
IcL= Intercoronual ligament (Dorsal IcL seen here, Ventral IcL out of view.)
O= Ovary
OD= Oviduct
R= Rectum
RgP= Rectogenital Pouch
UH= Uterine Horn

Figure 4. Caudal view of the reproductive tract (Reproductive tract in situ)
(Pathways to pregnancy and parturition, second revised edition. P.L. SENGER 2005)

Uterine Tube

The uterine tube is arranged like a funnel near the ovary. The funnel-shaped end, or infundibulum, contains processes, the fimbriae, which collect the ovum on ovulation (Figure 5). The ovum is then transported through the abdominal opening of the uterine tube located at the base of the infundibulum (Nickel, R et al., 1971). The ampulla of the uterine tube is the region adjacent to the infundibulum where fertilization takes place. The isthmus, the continuation of the uterine tube from the ampulla toward the uterus, is relatively long due to the meandering course it takes before ending at the uterine opening where it releases the ovum into the uterine horn (Nickel, R et al., 1971).





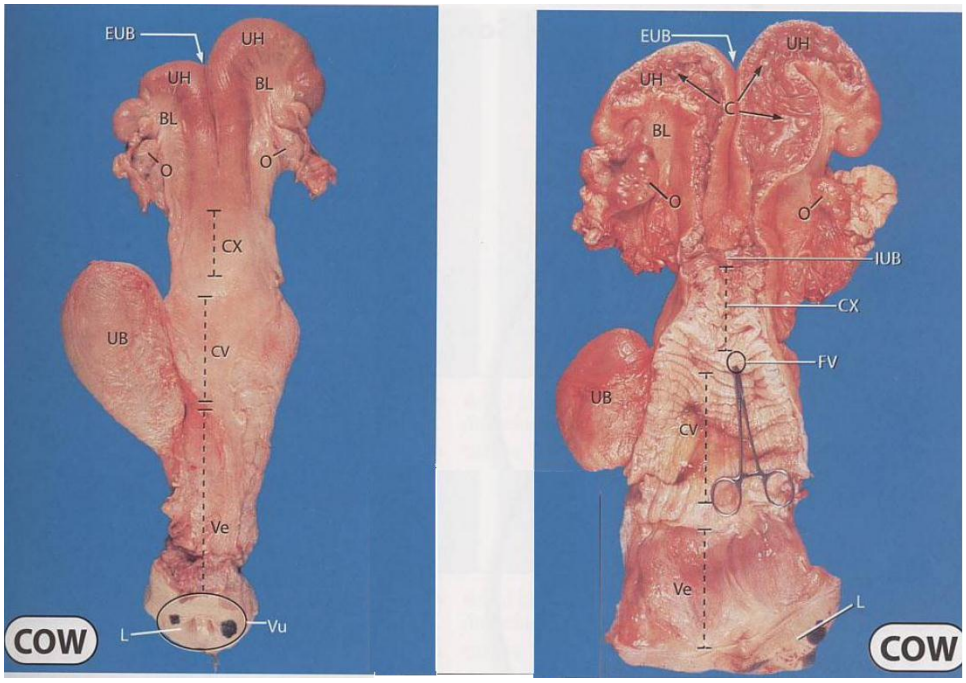
Relationship of the mesosalpinx to the oviduct in the cow, ewe, sow and mare. The infundibulum is a delicate membrane-like component of the oviduct that is in close apposition to the ovary. Arrows indicate the direction of oocyte/embryo transport within the oviduct toward the uterus.

AF	= Antral Follicle
CA	= Corpus Albicans
CL	= Corpus Luteum
H	= Hilus
If	= Infundibulum
M(OB)	= Mesosalpinx forming an ovarian bursa
Ms	= Mesosalpinx
O	= Ovary
OD	= Oviduct
OF	= Ovulation Fossa
UH	= Uterine Horn
UL	= Uterine Lumen
UOL	= Utero-Ovarian Ligament

Figure 5: the oviduct and its components
(P.L. SENGER 2005)

Uterus

The uterus consists of a body and two horns (Figure 6). The body is short, beginning immediately after the cervix ends. The horns branch from the body but are joined together by the peritoneum, giving the appearance that the body is longer than it truly is. As the horns progress cranial they divide at the intercornual ligaments, each turning abruptly ventrally, then proceeding caudally, and finally ending dorsal to the ovary (Budras, K.D., 2003) (Figure 4). From external to internal, the uterus can be divided into three layers: the perimetrium, the myometrium, and the endometrium (Budras, K.-D. 2003). The perimetrium is the continuation of the abdominal peritoneum onto the uterus. The myometrium constitutes the muscular layers, which can undergo substantial hypertrophy (Pineda, M. and Dooley, M., 2003). The endometrium is the internal epithelial lining of the uterus and is arranged into two distinct regions, caruncular and intercaruncular (Mullins, K. and Saacke, R., 2003). The caruncles are raised mucosal regions of the endometrium that are highly vascularized. The caruncles of the uterus join with the cotyledons of the fetal placental membranes to form the placentomes of a cotyledonary placenta (Nickel, R et al., 1971).



BL= Broad ligament (mesometrium)
C= Caruncle
CX= Cervix
EUB= External Uterine Bifurcation
FV= Fornix Vagina
IUB= Internal Uterine Bifucation
L= Labia
O= Ovary
OD= Oviduct
UB= Urinary Bladder
UH= Uterine Horn
Ve= Vestibule
Vu= Vulva

Figure 6. Dorsal view of excesed reproductive tract (P.L. SENGER 2005)

Cervix

The cervix is located between the body of the uterus cranially and the vagina caudally (Figure 6). It is a firm, muscular, sphincter-like structure that acts as a barrier separating the external genitalia from the internal genitalia (Pineda, M. and Dooley, M., 2003). Characteristic of the cervix are the three to four circular folds projecting into its lumen. The arrangement of the cervical musculature and mucosa is responsible for this characteristic architecture (Figure 7,8). The superficial mucosa is arranged in longitudinal folds punctuated by circular folds that interdigitate to form a series of ridges and interlocking notches when the cervix is closed (Nickel, R et al., 1971). This arrangement effectively seals the external environment from the internal uterine environment.

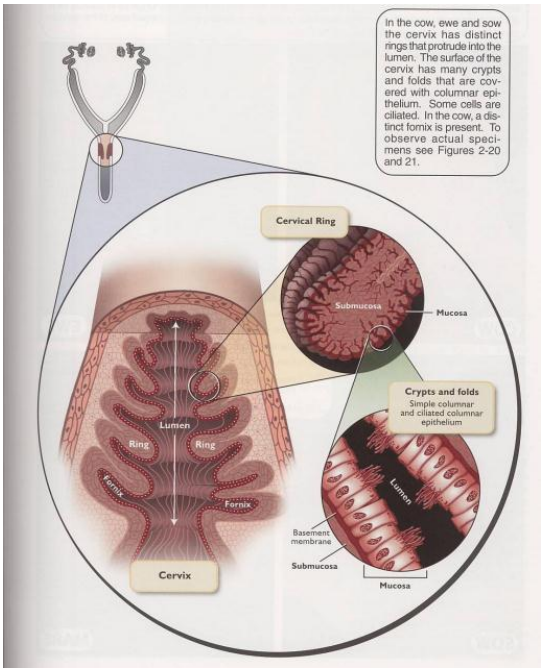


Figure 7: A Schematic of the Cervix

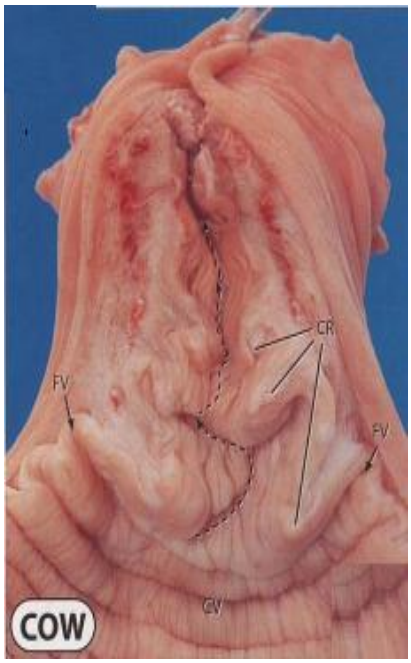


Figure 8: Excessed Cervical Tissue (P.L. SENGER 2005)

CR= Cervical Rings

CV= Cranial vagina

FV= Fornix vagina

Vagina

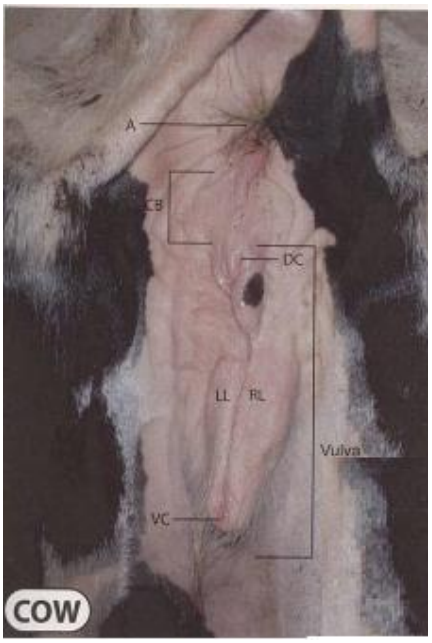
The vagina is positioned between the caudal extent of the cervix and border of the vestibule at the external urethral orifice. The cervix projects into the lumen of the vagina caudoventrally, causing the dorsal vaginal fornix to form a deeper recess than the ventral fornix (Nickel, R et al., 1971).

Vestibule

The vestibule is a small area in the cow that originates at the urethral opening and ends caudally to blend with the labia of the vulva.

Vulva

The labia of the vulva are located on either side of the labial fissure (Budras, K.D., 2003) (Figure 9). The labia meet dorsally, forming the dorsal commissure, and again ventrally to form the ventral commissure. The clitoris is found just cranial to the ventral commissure (Budras, K.-D. 2003).



A= Anus

CB= Cutaneous Bridge

DC= Dorsal Commissure

VC= Ventral Commissure

RL= Right Labia

LL= Left Labia

Figure 9: External Genitalia
(P.L. SENGER 2005)

Preparations for insemination and sanitation

Preparation

Before the day of AI, it is important to make sure that all AI equipment and supplies are stocked and ready for use. In addition, make sure that all equipment is cleaned and sanitary. Contaminants such as dirt and manure can be damaging to sperm as well as potentially infectious bacteria into the reproductive tract.

Before putting on a shoulder-length sleeve, wrists and fingers should be free of any item (jewelry, hair ties, watches) that may be abrasive to the rectal lining of the cow or heifer. Place a shoulder-length sleeve on your palpating arm. Most technicians choose to palpate with their non-dominant hand while manipulating the AI catheter with their dominant hand. In addition, some technicians prefer to use an additional latex or nitrile glove on the outside

of the sleeve to improve fingertip dexterity. Apply non-spermicidal lubricant to the palpating hand (Jordan Thomas.,2021).

Preparation and Handling of Catheters for Artificial Insemination of Cattle

Proper semen handling is essential in order to achieve maximum conception rates with artificial insemination (AI). Reduced conception rates can and will occur if semen is handled improperly before, during or after the thawing process (Jordan Thomas and Carson Andersen., 2021**).**

Temperature gradient in tank neck

To retrieve a straw, raise the canister until the cane tops are a few inches below the opening of the tank (Figure 10). The temperature of liquid nitrogen is -320 degrees F; however, there is a temperature gradient in the neck of the tank (Table 2). Damage to sperm occurs at temperatures as low as -110 degrees F. Damage to sperm will not be reversed even when returning frozen straws back into the liquid nitrogen immediately. Therefore, it is critical to not raise the straws above the frost line. The temperature drastically increases above the frost line, and raising straws above this point can cause damage to straws that are not intended for immediate use (Jordan Thomas and Carson Andersen., 2021**).**

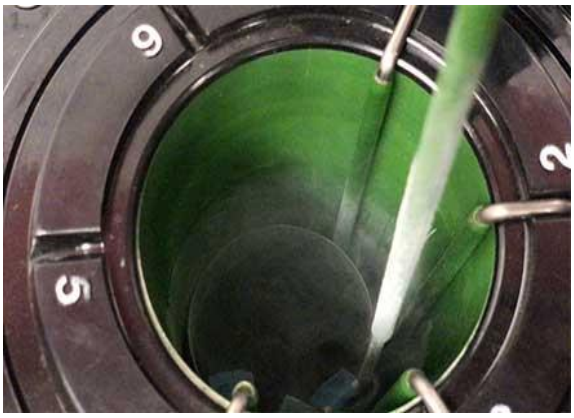


Figure 10. Raising a canister to just below the frost line in the neck of a liquid nitrogen tank. (Jordan Thomas and Carson Andersen., 2021**).**

Table 2. Temperature gradient in the neck of a liquid nitrogen tank.

Distance in neck of the liquid nitrogen tank	Range in temperature (degrees F)
Top	+36 to +54
1 inch from top	+5 to -8
2 inches from top	-40 to -51
3 inches from top	-103 to -116
4 inches from top	-148 to -184
5 inches from top	-220 to -256
6 inches from top	-292 to -313
Danger zone above the frost line. Damage to sperm occurs at temperatures as low as -110 degrees F. Adapted from Saacke 1978, Proceedings: Conference on AI of Beef Cattle.	

Retrieving the unit

After identifying the cane needed, bend the identification tab to a 45-degree angle to allow for easier removal of units. If the canister is raised into the neck of the tank for more than eight seconds, lower the canister back into the tank to allow for it to cool again for 20–30 seconds before raising it again. This helps to prevent partial thawing of straws that are not intended for immediate use.

Remember, fingertips are a source of heat, so it is critical to only touch the straw or straws you will remove from the cane for thawing. It may be helpful to use forceps if removing only one straw for thawing or to avoid touching other straws. In addition, forceps can allow for retrieval of straws while keeping the cane further into the neck of the tank where the temperature is lower.

After retrieving the desired straw or straws, lower the cane immediately back into the canister. In order to avoid losing canes within the liquid nitrogen tank, make sure the canister is raised partially into the neck of the tank before releasing the cane (Jordan Thomas and Carson Andersen., 2021).

Thawing

Exact thawing procedures may be more or less critical based on the type of extender used in production of the units. However, as a general rule, straws should be thawed for a minimum

of 45 seconds in 96 degrees F water. Clean water should be used in the thaw bath. Before retrieving a straw from the tank, use a thermometer or a thaw card thermometer to confirm the water in the thaw bath is at the correct temperature.

Time considerations

Straws can remain in the thaw bath for some time. However, it is recommended that semen be deposited into the cow or heifer within 10 minutes of thawing. Therefore, it is suggested to only thaw the number of straws that can be used within that time.

Thawing multiple straws

Multiple straws may be thawed at once, but attention to detail is critical if thawing multiple straws in the same thaw bath. First, it is critical to keep straws identified within the thaw bath in order to avoid confusion, especially if using straws from multiple bulls. Second, straws should not be allowed to stick together in the thaw bath during thawing, as this results in uneven or incomplete thawing of straws. Use caution as to the number of frozen straws placed into the unit at any one time. Each straw is essentially a small ice cube that results in a slight temporary cooling of the water in the thaw bath. In general, it is recommended to thaw no more than three straws in a single thaw bath at any one time. Consider using multiple thaw baths to keep up with a rapid AI process, or to avoid confusion if using multiple bulls (Jordan Thomas and Carson Andersen., 2021**).**

Electric thaw baths

Electric thaw baths (Figure 11) provide an efficient way to thaw straws. Electric thaw baths warm the added water and keep the water at a constant temperature. If using an electrically heated thaw bath, make sure the unit is turned on and the unit indicates it has reached the correct temperature. If hot rather than cold water was placed into the unit, be aware that the water could actually be too hot. Use a thermometer or a thaw card thermometer to confirm the temperature of the water. Occasionally, electric thaw bath units may require calibration to maintain water at the recommended temperature (Jordan Thomas and Carson Andersen., 2021**).**

.

Figure 11. Thawing a straw of semen in an electronic thaw bath. Note that the thaw card thermometer indicates a temperature of 96 degrees for the water in the thaw bath

Preparing an AI catheter

When handling semen, it is important to keep the workspace and equipment free of any potential contaminants that can be damaging to sperm. Keep the sheaths and AI catheters in a place that avoids contamination from dirt, dust, or manure until it is time to use them.

Thermal protection

The AI catheter should be warmed prior to straw insertion to avoid cold-shocking the sperm. An electric AI catheter warmer can be used to warm catheters before loading. Electric AI catheter warmers (Figure 12) can also be used to maintain thermal protection of loaded catheters after they are prepared. If there is not an AI catheter warmer available, catheters can be tucked inside a shirt and maintained at body temperature until the straw is ready to be inserted. In addition, catheter sheaths can also be tucked into a shirt to keep warm if it is cold outside.

Figure 12. Catheters in a warmer prior to being prepared.

Loading the catheter

When inserting a straw into an AI catheter, work in a sheltered area out of direct sunlight. Work quickly to avoid exposing the straw to temperature swings.

- First, pull the plunger (Figure 13) of the warmed AI catheter back approximately 4 inches to allow for insertion of the straw into the warmed AI catheter.

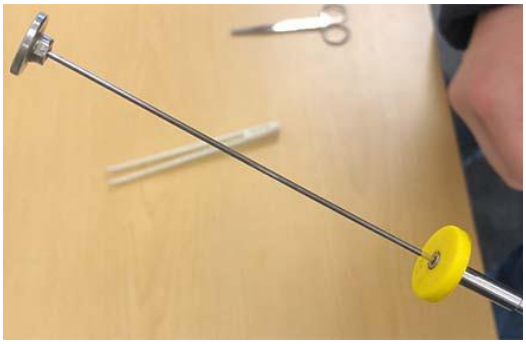


Figure 13. Pulling back the plunger of an AI catheter prior to loading.

- Next, remove the straw from the thaw unit and dry it in a doubled-over paper towel, as water is detrimental to sperm. The paper towel also serves to protect the straw from light, ambient temperature, and wind.
- Once dried, insert the cotton plug end of the straw into the warmed AI catheter, maintaining the paper towel around the catheter and straw (Figure 14).

Figure 14. Inserting the cotton plug end of a dried thawed straw into a warmed AI catheter.

- Using scissors or a straw cutter, cut approximately a quarter inch below the sealed end of the straw near the fluid line. Cut the straw at an approximately 90-degree angle (Figure 15).

Figure 15. Cutting the sealed end of the straw at a 90-degree angle.

- Slide the plastic sheath over the straw and catheter. Most sheathes are designed with a tip in which the straw will firmly "click" into place, preventing backflow (Figure 16). (Jordan Thomas and Carson Andersen., 2021**).**

Figure 16. A plastic sheath with its tip appropriately locked in place over the straw and catheter.

The AI process

It is essential to keep the tip of the AI catheter as sanitary as possible in order to reduce the risk of introducing bacteria or other contaminants into the uterus.

The success of AI depends on placing sufficient numbers of viable spermatozoa in the proper site at the optimum time relative to ovulation (Foote, R. H.,2010).

Insertion of palpating arm

Point your fingers and your thumb together (Figure 17) and gently insert your palpating hand into the rectum. With your free hand, use a paper towel to wipe the vulva free of any manure in order to avoid bringing in infectious bacteria. In addition, making a fist and applying downward pressure with your palpating hand will cause the vulva to slightly open, allowing for a clean entrance (Jordan Thomas and Carson Andersen., 2021**).**

Figure 17. Place the thumb and fingers of the palpating hand together in a small point, and gently insert the palpating hand into the rectum.

Insertion of the AI catheter

Insert the AI catheter into the vulva at a slight upward 30-degree angle to avoid inserting the catheter into the urethra and into the bladder (Figure 18). If you believe you have inserted into the urethra, pull the catheter back while not completely removing the tip of the catheter from the vagina. It is important to not remove the catheter once you have inserted it in order to prevent contamination of the tip upon removal. Begin to level out the angle of the catheter as you gently push the catheter forward into the reproductive tract.

Figure 18. Insert the AI catheter into the vulva at a slight upward angle (approximately 30 degrees) to avoid inserting the catheter into the urethra.

Moving the AI catheter in the tract

It does not take much force to move the AI catheter through the reproductive tract. Avoid applying too much forward pressure with the catheter, as this should be unnecessary and could cause irritation or injury. Vaginal folds will often cause resistance and prevent the

catheter from proceeding easily into the tract. If you believe the catheter is stuck in a vaginal fold, manipulate the reproductive tract with your palpating hand as described below while readjusting the direction of the catheter.

Bringing the catheter to the cervix

Once you have made progress into the reproductive tract with the AI catheter, palpate the reproductive tract for the cervix. Cervices will vary in size and shape, but all will have the dense feel of connective tissue, distinct from the feel of the vagina or uterus. The cervix is often described as feeling like a "**turkey neck**." Keep in mind that heifers will have smaller cervices than mature cows.

Bring the tip of the catheter to the opening of the cervix (Figure 19). The catheter may already be in contact with the cervix. If not, take a firm hold of the cervix and push it forward. This straightens out the reproductive tract and eliminates folds in which the catheter may be caught. Gently readjust the depth and direction of the catheter until you can easily guide it to the opening of the cervix (Jordan Thomas and Carson Andersen., 2021).

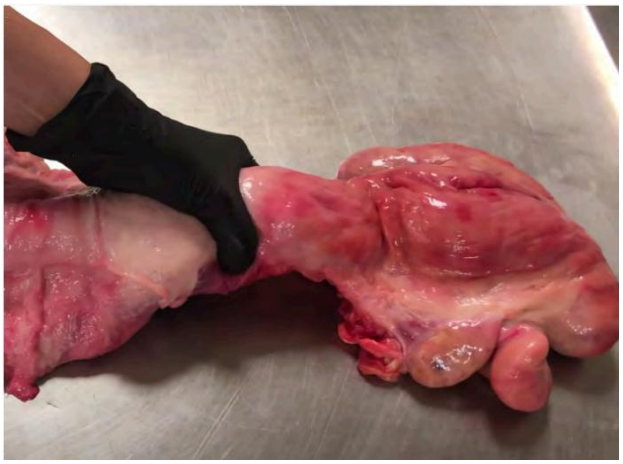


Figure 19. Palpating the cervix of the reproductive tract.

Inserting the catheter into the cervix

After you have brought the tip of your AI catheter close to the cervix, the next step is to insert the tip of the AI catheter into the opening of the cervix. This is often one of the more challenging steps in the AI process. Around the cervix, the vagina forms a blind-ended pocket called the fornix vagina (Figure 20). It is common for the tip of the catheter to be in the fornix rather than in the opening of the cervix. In addition, the fornix tends to stretch,

making the catheter appear to be advancing forward when it could really be above, below or to the side of the cervix. If you feel you are trapped in the fornix, pull the catheter back slightly and use your palpating hand to clamp down around the vaginal end of the cervix. This closes off the fornix and allows your palm and fingers to guide the catheter straight to the opening of the cervix. Due to the cervix being made of dense tissue and cartilage, you will feel a “dragging” sensation to the catheter once the tip is at the opening of the cervix.

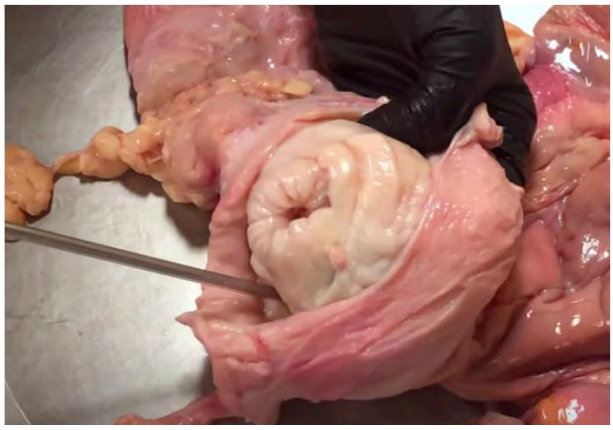


Figure 20. Around the cervix, the vagina forms a blind-ended pocket called the fornix vagina. As shown, it is common for the tip of an AI catheter to go into the fornix rather than the opening of thecervix. The palpating hand will need to close down the fornix so that the catheter can be guided into the cervix.

Passing the catheter through the annular rings of the cervix

If the cervix is far over the pelvic rim or hard to grip as you try to manipulate it, pull the cervix toward you after the catheter is near the opening of the cervix. You can also press the cervix against the rim of the pelvis to create more support. Once the AI catheter is in the cervix, you will need to pass the catheter through the annular rings. Most cows and heifers will have three to four annular rings. Passing the catheter through the rings can be very challenging in some animals, as the cervix is a tough structure and may require a lot of manipulation.

A key concept is to work the cervix back onto the catheter using your palpating hand, rather than pushing the catheter. After passing through one ring, maintain light forward pressure on the catheter with while you turn and angle the cervix to find the opening to the next ring. It is important to not apply too much pressure, especially if the tip of the catheter is almost through the cervix and close to entering the uterine body. Uterine tissue is very thin and can easily be punctured by the AI catheter if too much force is applied.

Depositing semen in the uterus

Depositing semen in the correct place is critical. Semen should only be deposited in the uterine body. If semen is deposited in the cervix instead of in the uterus, the vast majority of the sperm cells will actually flow back into the vagina rather than the uterus. To ensure that the AI catheter is in the correct place for depositing the semen, feel for the tip of the catheter with your fingers. If the catheter is still in the cervix, you will not be able to feel the tip of the catheter very well since the cervix is a dense structure. However, if the catheter is through the cervix and in the body of the uterus (Figure 21), you will be able to feel the tip of the catheter through the thin walls of the uterus. You want to clearly feel about a half inch to an inch of catheter in the body of the uterus. It is critical to avoid pushing the catheter farther into the uterus and down a uterine horn. Be gentle with movement of the catheter once through the cervix, as the uterine lining is very thin and prone to irritation. Once you are confident the tip of the catheter is in the correct location, deposit the semen. This should be done by depositing the semen relatively slowly (e.g., three to five seconds). In addition, keep the catheter in the same place while depositing semen, making sure not to pull back into the cervix until the semen has been deposited (Jordan Thomas and Carson Andersen., 2021).

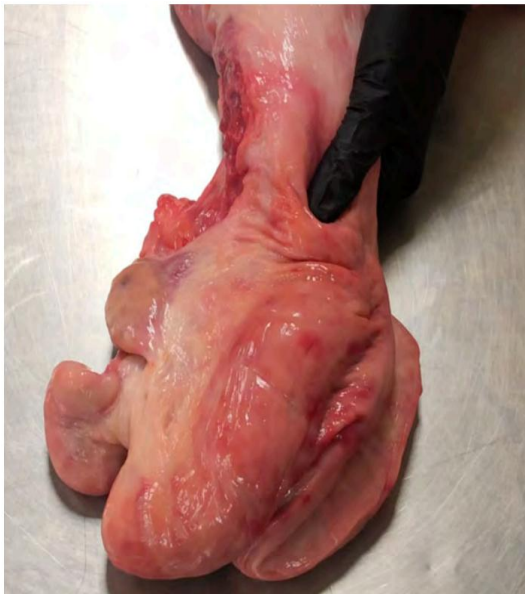
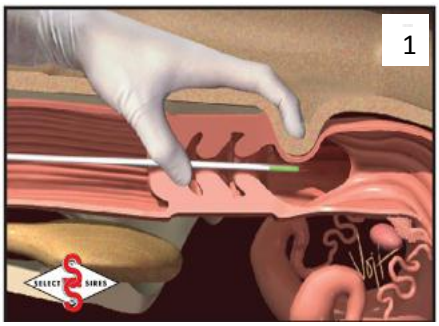
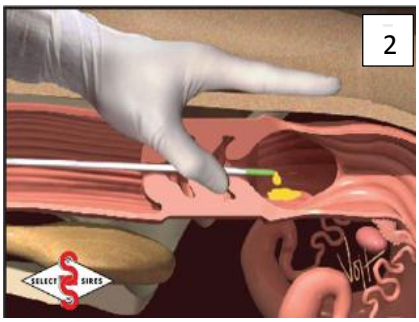
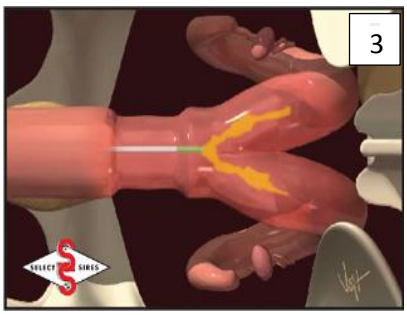


Figure 21. Semen should be deposited in the body of the uterus, with just a half inch to an inch of catheter in the uterus. Note that passing the catheter farther into a uterine horn is discouraged in order to avoid potential irritation of the uterine lining







1/ Use your index finger to check gun placement (1/4 inchpast the end of the servix) before depositing semen.
2/ Push the plunger slowly so the drops of semen fall directly into the uterine body.
3/ With proper IA technique and gun placement, semen will be deposited in the uterine body and contractions will transport spermatozoa forward to the horns and oviducts.

Some of the most important aspects to remember when inseminating a cow to attain maximum breeding efficiency are:

- Be gentle. Don't use too much force.
- Insemination is a two-step process. Get the gun to the cervix, and then place the cervix over the gun.
- Deposit the semen just through the cervix into the uterine body.
- Take your time.
- Relax.

Timing for AI

The reproductive records of cows are essential for AI. Additionally, the training of inseminators is a significant contribution to the successful commercial application of AI to dairy cattle breeding (Foote, R.H., 1981; López-Gatius, F., 2012).

In consequence, inseminator training and improvements in the act of inseminating remain topics of great interest (Dalton, J.C et al., 2021; . Lech, M et al., 2022; Koch, J et al., 2022, Mellado, M et al., 2022). However, the correct detection of estrus remains a challenge (Foote, R.H., 1975; Senger, P.L ., 1994; Diskin, M.G and Sreenan, J.M., 2000; . Roelofs, J et al 2010; Sumiyoshi, T et al., 2020) determining that some 20% of inseminations may be performed in pregnant cows(Roelofs, J et al 2010).

Behavioral signs of estrus are a predictor of the time of ovulation and a guide to the optimal time of insemination. It is generally accepted that ovulation occurs 10–12 h after the end of estrus. Cows may show a period of standing estrus of 8–20 h but become refractory to the

bull about 10–12 h before ovulation. Spermatozoa need to remain in the female reproductive tract for 6 to 8 h before they are capable of fertilization, and capacitated spermatozoa must be present in the fallopian tubes at the moment of ovulation (Hammond, J ., 1927; Laing, J ., 1945; Hunter, R.H.F and Wilmut, I., 1984; Hunter, R.H.F., 2008; Suarez, S.S., 2008). The risk of the ageing of gametes, particularly the postovulatory oocyte, becomes critical at the time of AI in cattle (Hunter, R.H.F., 1989; Hunter, R.H.F and Greve, T.,1997; Saacke, R.G., 2008). Owing to the challenges of correctly detecting estrus, hormonal protocols for fixed-time AI (FTAI) have been incorporated into routine intensive dairy farming reproductive management programs (Rabiee, A.R et al., 2005; Macmillan, K. L., 2010; Carvalho, P.D et al., 2018; López-Gatius, F., 2022). However, most AIs worldwide are performed at spontaneous estrus and hormone treatments have met with strong consumer opposition in some countries. In addition, the use of sex-sorted semen has increased enormously over the last decade (Vishwanath, R and Moreno, J.F., 2018; Marques, M.O et al ., 2018; Reese, S et al., 2021; Seidel, G.E., Jr and DeJarnette, J.M., 2021) and, in beef cattle, this practice has recently been recommended only in cows expressing estrus following FTAI protocols (Thomas, J.M et al., 2019; Perry, G.A et al., 2020).

The optimal time of AI was established using fresh semen in the classic studies by Trimberger and Davis (Trimberger, G.W and Davis, H.P., 1943) and Trimberger (1944). Highest conception rates (79%) were obtained when cows were inseminated in the middle to end of estrus (24 to 6 h before ovulation).

These findings led to the extensively used “a.m.-p.m.” guidelines. These state that cows detected to be in estrus in the morning (a.m.) should be submitted for AI that afternoon (p.m.), and cows in estrus in the afternoon should be inseminated the next morning.

However, recent findings suggest this interval should be reduced to 16 to 6 h before ovulation (Roelofs, J.B et al., 2006., Hockey, C.D et al., 2010; Furukawa, E et al., 2022). In other words, AI closer to end-estrus is a better insemination time. As ovulation occurs 10–12 h following the end of estrus behavior (Hammond, J ., 1927; Laing, J ., 1945; Hunter, R.H.F and Wilmut, I., 1984; Hunter, R.H.F), physiological indicators of late estrus such as ready for service may be simply and directly detected at AI (Sumiyoshi, T et al., 2014; Sumiyoshi, T et al., 2017; López-Gatius, F et al., 1996; López-Gatius, F., 2011). Thus, by better predicting the ovulation time, pregnancy rates to AI may be improved (Hockey, C.D et al., 2010).

Artificial Insemination Technique: Mare

One of the main goals of horse breeding is to get as many foals as possible from valuable parents within a short time.

Mares may be bred by natural cover or artificial insemination, depending on breed registry regulations, preference of the stallion owner and/or mare owner, location and availability of the stallion and mare, cost, safety concerns, experience of personnel, and other factors (Equine Reproduction Laboratory., 2022**)**

Artificial insemination (AI) is the technique used to transfer appropriately processed semen collected from a stallion into the uterus of the mare at the correct time in her oestrus cycle in order to obtain a single pregnancy. The semen can either be fresh, chilled or frozen. Many of the techniques used are common to all three types of semen. Fresh semen is usually collected, extended and stored in an airtight, light free container for up to 8 hours at room temperature. Semen that is to be used longer than 8 hours after but within 48 hours of collection should be chilled to 4ºC and stored for shipping in a special container. Semen that is required to last longer than 48 hours is frozen in liquid nitrogen at a temperature of -196ºC (Colin Tait., 2024)

Research data indicate that only a few spermatozoa get into the ampulla of the oviduct where fertilisation takes place. Moreover, only a few spermatozoa take part in the fertilisation process, but only one of them enters and fertilises the oocytes. Based on this observation, between 200 and 500 × 10^6 spermatozoa are used for artificial insemination (AI) in the practice. Morris (2004) reported that pregnancies were obtained after AI with a much lower number of spermatozoa (5 × 10^6). However, if the semen was deposited deep into the uterine horn, close to the opening of the oviduct, pregnancies were achieved with as few as 1 × 10^6 fresh spermatozoa. However, when frozen semen is used, the recommendation is to provide minimum 3 × 10^6 spermatozoa. Unfortunately, the spermatozoa of some stallions cannot be cryopreserved with a good survival rate. Minimum 35% of the spermatozoa must show progressive motility after thawing. Based on clinical observations, the semen of 30–50% of stallions has the appropriate number of motile spermatozoa after thawing (Scherzer et al., 2009; Aurich et al., 2016).

Reproductive anatomy

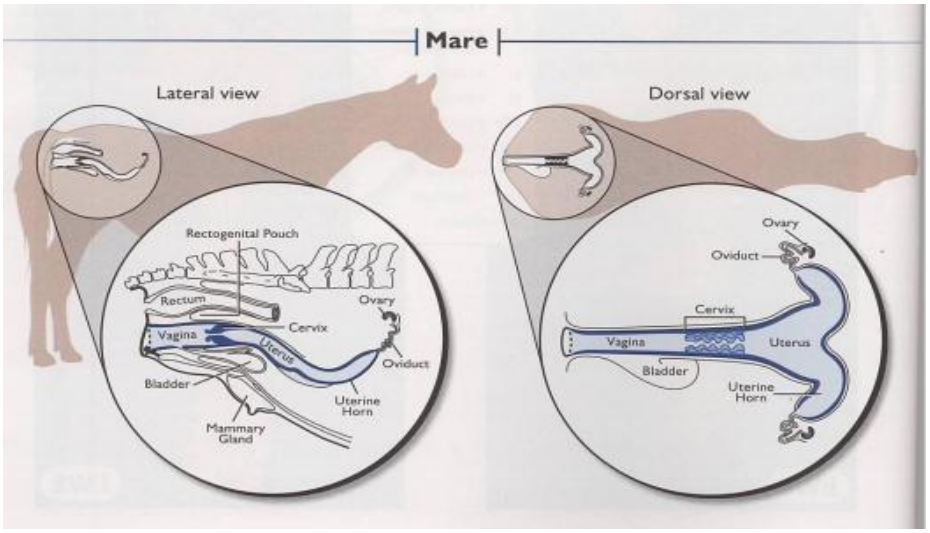


Figure 22. Lateral/ Dorsal view of cow
(P.L. SENGER 2005)

The female reproductive tract consists of paired ovaries, uterine tubes, and uterine horns contiguous with a uterine body and cervix, vagina, vestibule, and vulva. These organs, with the exception of the ovaries, are collectively termed the tubular genitalia. There is a continuous lumen from the cranial end of each uterine tube and uterine horn, via the uterine body and cervix, vagina, and vestibule to the labia of the vulva. The tubular genitalia provide a connection from the peritoneal cavity to the external environment.

Ovaries

The position of the ovaries is variable largely because of the extensive mesovarium that permits the ovary a wide range of passive movement. An ovary may lie on top of the intestinal mass in contact with the dorsal abdominal wall or it may be situated among the intestinal coils roughly on a plane through the fourth or fifth lumbar vertebra. The left ovary is caudal to the right, but it is closer to the ipsilateral kidney. The distance from the ovaries to tips of the uterine horns also varies. During pregnancy, the ovaries are pulled cranioventral as well as medial, an important position to recall when palpating per rectum.

The large ovaries (averaging 7–8 cm along the long axis) of the mare are reniform with a convex dorsal border attached to the mesovarium and a sharply concave ventral free border indented by an ovulation fossa (ovarian fossa 9). From the caudal pole of the ovary, the proper ligament of the ovary – a band of smooth muscle within the broad ligament –

extends to the tip of the uterine horn. The cranial pole of the ovary is attached, in part, to the uterine tube's infundibulum, which overlays the ovulation fossa.

The histological structure of the equine ovary is unique among domestic mammals in that it has a peripheral collagenous connective tissue vascular zone around a central parenchymatous zone, containing developing and atretic ovarian follicles, corpora lutea, and corpora albicantia. The parenchymatous zone surfaces at the ovulation fossa, and during folliculogenesis, maturing follicles move toward the fossa, enlarging to a diameter of 4.5–6 cm. Mature ovarian follicles become less turgid immediately before ovulation. A corpus hemorrhagicum commonly forms following ovulation. The developing reddish corpus luteum does not bulge from the ovary, but it may project slightly into the ovulation fossa. Later, the corpus luteum becomes yellow. The primary corpus luteum of pregnancy is supplemented by secondary corpora lutea that begin to form around day 40 of gestation, developing from follicles that continue to grow rapidly during the first couple of months of gestation. Luteinization takes place in follicles that have ovulated and in anovulatory follicles.11

Secondary corpora lutea are spherical, irregular, orgourd-like with a tract leading to the ovulation fossa.

Many corpora lutea have a central cavity.17 Corpora lutea continue to function until the sixth month of gestation, and then regression occurs.

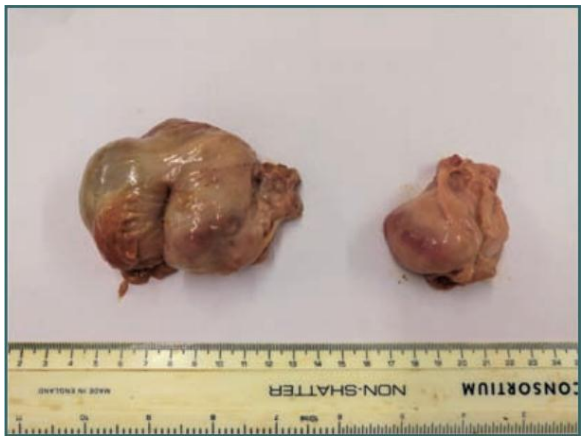


Figure 23. The ovaries of the mare. Note the difference in size between the ovary on the right (inactive) and the one on the left (active). The concave surface (position of the ovulation fossa) and the convex surface (the hilus, entry point for blood and nerve supply) of the ovary are clearly seen.

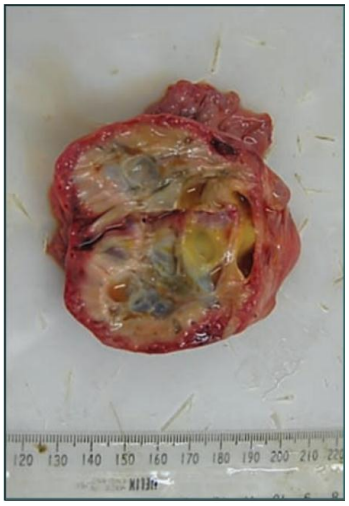


Figure 24. A cross section through the mare's ovary illustrating the outer (pink) medulla (structural) and the inner (cream and grey) cortex (gametogenic and steroidogenic).

Uterine tubes (oviducts or salpinges)

Each uterine tube consists of an expansive infundibulum covering the ovary's ovulation fossa, a highly tortuous ampulla about 6 mm in diameter, and a less tortuous isthmus half the diameter of the ampulla. The isthmus terminates at a small uterine ostium on a papilla within the end of the uterine horn. Based on measurements made with the loops effaced and free of the suspending mesosalpinx, equine uterine tubes are from 20 to 30 cm long with the ampulla constituting about half the length.(Dyce KM et al ., 1967; Sisson S., 1975).

Irregular fimbriae are present along the margin of the funnel-shaped infundibulum. Some are attached to the cranial pole of the ovary, allowing the rest of the infundibulum to spread over the ventral aspect and cover the ovulation fossa. At the edges of the fimbriae, their serosal covering is contiguous to the mucous membrane. The latter is highly folded, especially in the ampulla where secondary and tertiary ridges branch from longitudinal folds. The lining of simple columnar epithelium is intermittently ciliated. Ciliogenesis and ciliary motion (toward the uterus) reflect stages of the sexual cycle. A thin, wellvascularized lamina propria supports the epithelium. Inner, circularly disposed smooth muscle fibers are covered by outer, longitudinally arranged fibers that continue into the mesosalpinx.

The abdominal ostium in the center of the infundibulum is about 6 mm in diameter; the uterine ostium of the tube, 2–3 mm in diameter. (Sisson S., 1975) The inner circular muscle increases to form a sphincter at the tubo-uterine junction. Unfertilized ova are retained for a considerable duration in the uterine tubes (up to several months), parthogenetic cleavage occurring in some of them.(Van Niekerk CH and Gerneke WH., 1966).

The ovarian bursa of the mare is a peritoneal pouch extending from the ovulation fossa caudad to the cranial aspect of the uterine horn. Laterally it is bounded by the uterine tube and mesosalpinx. A fold of broad ligament containing the proper ligament of the ovary forms the medial wall of the ovarian bursa.

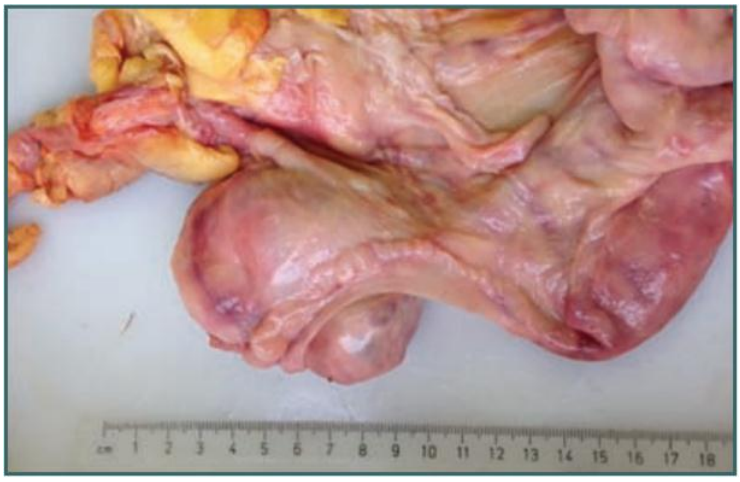


Figure 25. The convoluted Fallopian tube running through the mesovarian section of the broad ligaments, from the ovary on the left to the uterine horn on the right, illustrating the broader ampulla region of the Fallopian tube (on the left) and the more wiry and narrower isthmus region to the right.

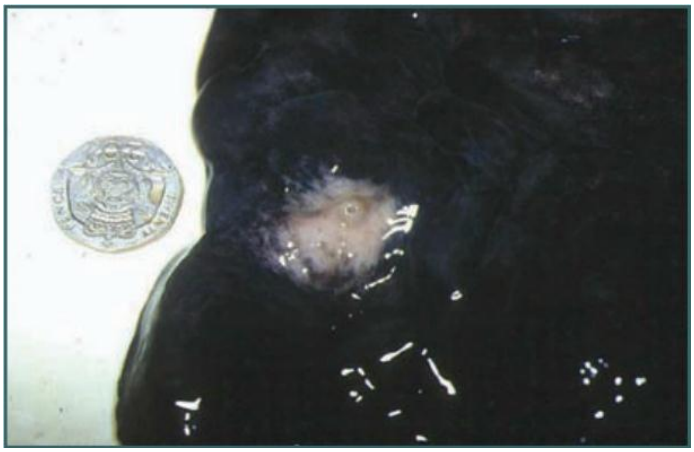


Figure 26. The utero-tubular junction in the mare, as seen from the uterine horn side (the dark colour of the uterine endometrium is not natural but serves to allow easier identification of the utero-tubular junction).

Uterus

The uterus of the mare is a hollow muscular Y-shaped organ joining the cervix and the Fallopian tubes (Figures 27 and 28). It lies in the abdominal cavity and is attached to the lumbar region of the mare by two broad ligaments, outfoldings of the peritoneum, on either side of the vertebral column. The broad ligaments provide the major support for the

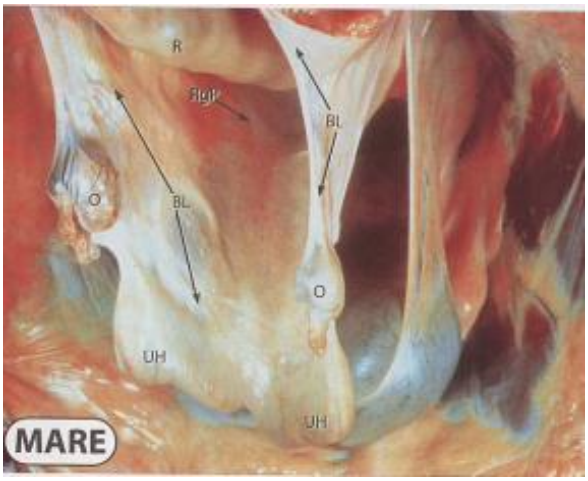
reproductive tract (Figure 27 and 28) and can be divided into three areas: mesometrium, attached to the uterus; mesosalpinx, attached to the Fallopian tubes; and mesovarium, attached to the ovaries (Ginther, 1992).

The Y-shaped uterus is divided into two areas: the body (caudal end) and the two horns (cranial end). The body of the uterus normally measures 18–20 cm long and 8–12 cm in diameter. The two horns that diverge from the uterine body are approximately 25 cm long and reduce in diameter from 4–6 cm to 1–2 cm as they approach the Fallopian tubes. The size of the uterus is affected by age and parity, older multiparous mares tending to have larger uteri which also tend to slope downwards into the abdominal cavity. The uterus of the mare is termed a simplex bipartitus, due to the relatively large size of the uterine body compared to the uterine horns (60:40 split). This differs from that in other farm livestock, where the uterine horns are more predominant. The lack of a septum dividing the uterine body is also notable (Hafez and Hafez, 2000; Frandson *et al*., 2009). In situ the uterine walls are flaccid and intermingle with the intestine, the only lumen present being very small and that formed between the endometrial folds.

The uterine wall (Figure 28), in common with the rest of the tract but most prominent here, consists of three layers: the perimetrium (an outer serosa layer) continuous with the broad ligaments; the myometrium (central muscular layer); and the inner endometrium.

The central myometrial layer is particularly evident in the uterus where clearly defined outer longitudinal muscle fibres, a central vascular layer and inner circular muscle fibres can be seen. It is this central myometrial layer that allows the elasticity for expansion of the uterus during pregnancy as well as providing the force for parturition. The inner endometrium is arranged in 12–15 longitudinal folds continuous with the folds of the cervix (Figure 31) and comprises the outer epithelial cells (epithelium) and inner submucosa of endometrial connective or stroma tissue with its associated endometrial glands and ducts (Figures 29 and 30). The submucosa can be further divided into the compact layer (stratum compactum), nearest the epithelium, and the spongy layer (stratum spongiosum), nearest the myometrium. Collagenous connective tissue cores support these endometrial folds. The activity and, therefore, appearance of the endometrial glands and the epithelial cells are dependent on the cyclical hormonal changes associated with the oestrous cycle. It is the endometrium that is responsible for supporting the early conceptus and for placental attachment and development (Ginther, 1992, 1995; Sertich, 1998; Kainer, 2011). Causey

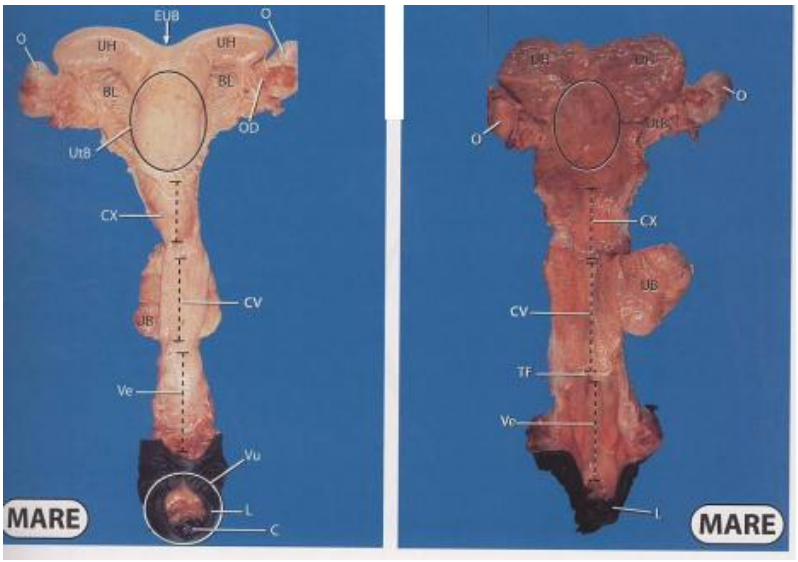
(2007) also suggested that within the uterine epithelium are mucus-secreting and ciliated cells that help eliminate bacteria, providing an additional defence against uterine bacterial invasion.



The intestines have been removed so that the reproductive tract is in full view. The tract is suspended by the broad ligament that is attached dorsally and is continuous with the peritoneum.

BL= Broad ligament
O= Ovary
R= Rectum
RgP= Rectogenital Pouch
UH= Uterine Horn

Figure 27. Caudal view of the reproductive tract (Reproductive tract in situ)
(P.L. SENGER 2005)



BL= Broad ligament (mesometrium)
C= Clitoris
CX= Cervix
EUB= External Uterine Bifurcation
CV= Cranial Vagina
IUB= Internal Uterine Bifucation
L= Labia
O= Ovary
OD= Oviduct
UB= Urinary Bladder
UH= Uterine Horn
Ve= Vestibule
Vu= Vulva

Figure 28. Dorsal view of excesed reproductive tract
(P.L. SENGER 2005)

Cervix

The cervix lies at the entrance to the uterus and is a remarkably versatile structure, normally providing a tight, thick-walled sphincter, hence acting as the final protector of the system, but is also able to dilate vastly to accommodate the passage of the fetus at parturition (Figures 29, 30 and 31). The walls of the cervix form a series of folds or crypts and are highly

muscular with collagenous connective tissue cores and lined by folded columnar epithelium containing mucus-secreting cells.

These crypts are continual with the uterine endometrium folds and enable the significant expansion required at parturition (Ginther, 1992; Kainer, 2011) (Figure 31). In the sexually inactive, dioestrous state, the cervix is tightly contracted, white in colour and measures on average 6–8 cm long and 4–5 cm in diameter; cervical secretion is minimal and thick in consistency (Figure 30). The muscle tone and, therefore, cervix size, along with its mucus secretion are again governed by cyclic hormonal changes. During oestrus muscle tone relaxes under the influence of increasing oestradiol, decreasing progesterone and increasing prostaglandin (PG) E (PGE) concentrations. These act on the collagen matrix, separating and dispersing the collagen fibres, which decreases tensile strength and so relaxes the cervix (Kershaw *et al*., 2005). In addition there is an increase in secretion, so easing the passage of the penis into the entrance of the cervix. The oestrous cervix appears pink in colour and may be seen protruding or 'flowering' into the vagina (Figure 30).

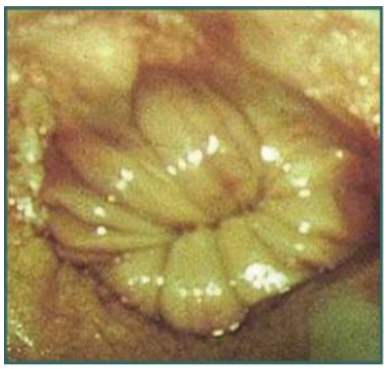


Figure 29. The dioestrous cervix is retracted, presenting a tight seal against entry into the Uterus

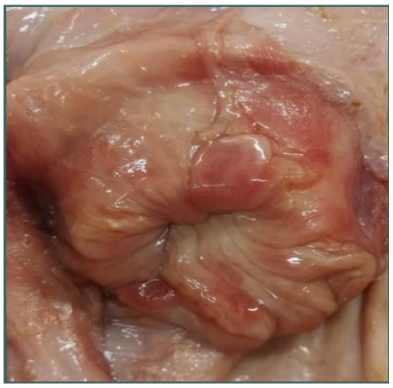


Figure 30. The oestrous cervix is relaxed 'flowering' into the vagina presenting a less effective seal but facilitating the entry of the penis into the cervix for sperm deposition.

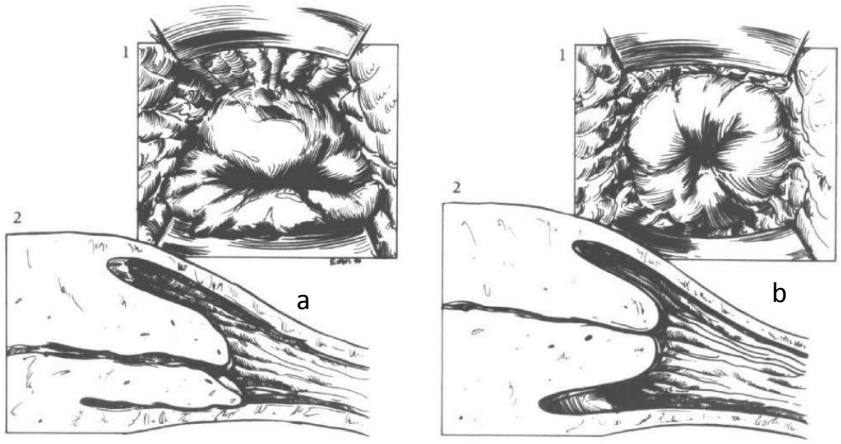


Figure 31. Vaginal portion of uterine cervix. (a) During estrus and (b) during diestrus. 1, Vaginoscopic view; 2, median section.

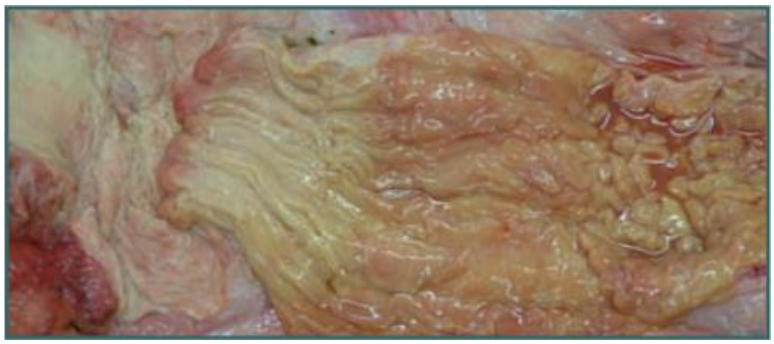


Figure 32. The internal surface of the cervix and uterus illustrating the cervical folds (centre left) which are continuous with the endometrial folds that line the uterus (centre right).

Vagina

The vagina of the mare is on average 18–23 cm long and 10–15 cm in diameter. In the well conformed mare the floor of the vagina should rest upon the ischium of the pelvis, and the walls are normally collapsed and apposed, forming the vestibular seal. The hymen, if present, is also associated with this seal and divides the vagina into anterior (cranial, nearest the mare's head) and posterior (caudal, nearest the mare's tail) sections.

In some texts the posterior vagina is referred to as the vestibule. The urethra, from the bladder, opens just caudal to the hymen. The walls of the vagina are muscular and include the constrictor vestibule muscle. The posterior vagina is lined by stratified squamous epithelium which accommodates abrasion at copulation whereas the anterior vagina is lined by columnar epithelium. In addition, both the posterior and anterior vagina is lined by

mucus-secreting cells. The muscle layer provides elasticity and its dorsal incompleteness allows the major stretching required at parturition (Figure 33).

The vagina acts as the second protector and cleaner of the system. It is largely aglandular (does not contain secretory glands) but contains acidic to neutral secretions, originating from the mucus-secreting cells, the cervix and small vestibular glands situated in the posterior vagina. These acidic secretions are bacteriocidal (kill bacteria); however, they are also spermicidal (kill sperm), necessitating that sperm are deposited into the top of the cervix/bottom of the uterus at mating, to avoid the detrimental effect of the acidic conditions. The acidic conditions also attack the epithelial cell lining of the vagina, but these cells are protected by the protective mucus layer produced by mucus secretory cells. The exact composition of vaginal secretion is controlled by the cyclical hormonal changes of the mare's reproductive cycle.

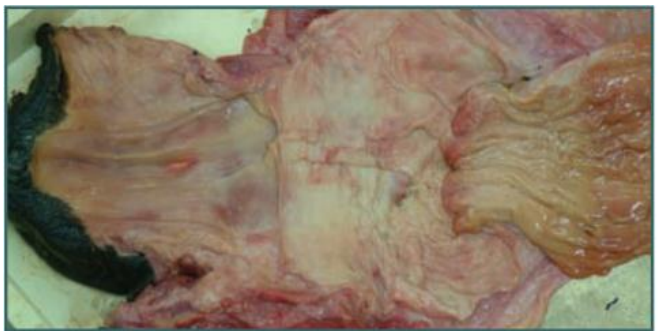


Figure 33. The internal surface of the mare's vagina illustrating from the left: the vulva; the posterior vagina lined by stratified squamous epithelium; the transverse fold (position of the hymen); the anterior vagina lined by columnar epithelium; the cervix.

Vulva

The vulva (Figure 34) is the external area of the mare's reproductive system, protecting the entrance to the vagina. The outer area is pigmented skin with the normal sebaceous and sweat glands along with the nerve and blood supply normally associated with the skin of the mare. The inner area, where the vulva is continuous with the vagina, is lined by stratified squamous epithelium plus mucus-secreting cells enabling it to accommodate abrasion at mating. The upper limit of the vulva (the dorsal commissure) is situated approximately 6–8 cm below the anus. Below the entrance to the vagina, in the lower part of the vulva (the ventral commissure), lie the clitoris, or clitoral body, and the three clitoral sinuses (one medial and two lateral; (Figure 34). These sinuses are of importance in the mare as they provide an ideal environment for the harbouring of many venereal disease (VD) bacteria, in

particular *Taylorella equigenitalis* (causal agent for contagious equine metritis, CEM), but also *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* . Hence, this area is regularly swabbed in mares prior to covering and, indeed, in the Thoroughbred industry such swabbing is compulsory (McAllister and Sack, 1990 ; Ginther, 1992 ; Horse Race Betting Levy Board, 2019). Within the walls or labia of the vulva lies the constrictor vulva muscle, running just inside the ventral part of the labia is the vestibular bulb, an enlarged area of tissue thought to assist in holding the penis in place at copulation.

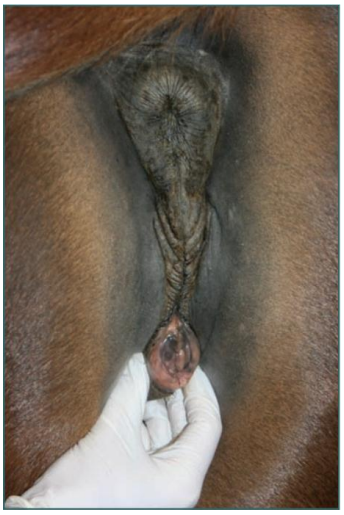


Figure 34. The vulva of the mare showing the ventral commissure within which lie the clitoral body and three sinuses, one medial and one on either side.

Selection of mares for artificial insemination

While one of the advantages of using AI is to reduce contamination and potential inflammation of the mare's uterus during breeding, it remains important to evaluate and to attempt to correct any pathological conditions of the mare's reproductive tract before proceeding with insemination. Pregnancy rates are significantly reduced when free uterine fluid and urovagina are not corrected prior to insemination.

Mares that have pathological conditions frequently become pregnant only to abort later in gestation.

Correcting urine pooling (urovagina) may be as easy as increasing the mare's body score. If weight gain fails to eliminate urine pooling a urethral extension should be considered Fluid accumulation in the uterus prior to insemination is extremely detrimental to establishment and maintenance of pregnancy and must, therefore, be corrected. Uterine fluid is diagnosed by ultrasound evaluation. The underlying cause must be identified followed by the

appropriate treatment. The nature of the fluid must first be determined. It may be urine that results from urovagina and an open cervix, an exudate that results from a uterine infection, or a transudate that is related to uterine inflammation.

Pluriparous mares with stretched uterine ligaments and older maiden mares whose cervices do not open during estrus are very susceptible to poor uterine clearance. When uterine fluid exceeds 1 cm in cross-sectional diameter during estrus, the mare should be treated with oxytocin prior to breeding and levels greater than 2 cm should be treated with drainage and appropriate diagnostic tests and treatments before breeding. (Lieux P., 1970)

Abnormal conformation of the vulva that contributes to vaginal or uterine fecal contamination or aspiration of air (Figure 35) should be surgically corrected with a Caslick's procedure (Figure 36). Abnormalities of the cervix such as adhesions, lacerations, and failure to dilate are frequently discovered during insemination. These conditions of the cervix must be corrected before a successful pregnancy can occur. Uterine infection diagnosed by cytology and culture should be treated with the indicated antibiotic and lavage.

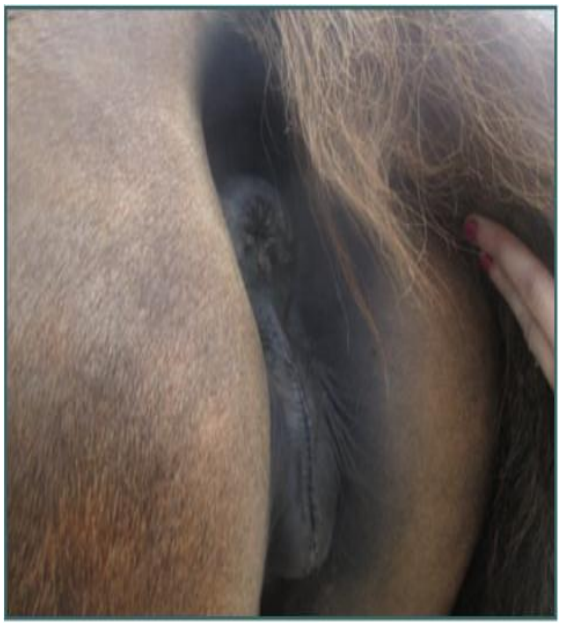


Figure 35. The vulval area of the mare: in this instance, the conformation of the perineal area is poor, with the anus sunken cranially, opening up the vulva to faecal contamination. (Photo courtesy of Ms Ria McLean.)

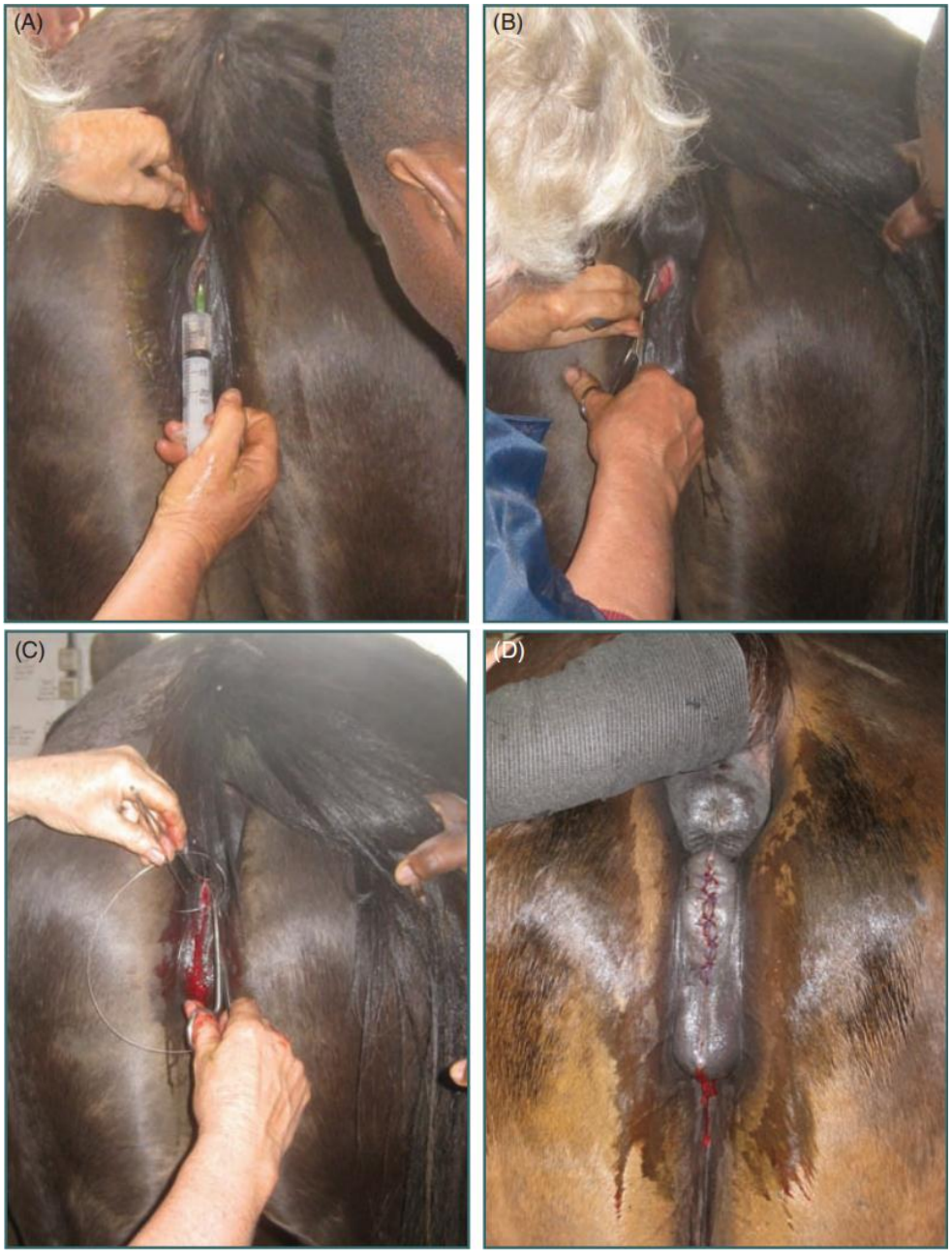


Figure 36. A Caslick operation in the mare showing (A) anaesthetizing the vulval lips; (B) cutting the vulval lips; (C) suturing together the vulval lips; and (D) the finished job.

Regardless of the insemination technique used, the preparation of the mare and the equipment are essential steps for the smooth running of the insemination and its success. The preparation of the mare will remain the same regardless of the type of insemination performed; however, the techniques for preparing and placing the semen differ.

Preparation of the mare

To perform the insemination in the best conditions, it is preferable to place the mare in a work and tie her tail so that it is raised. If this is not possible, the veterinarian can resort to

homemade isolation (box door, straw bale) or to the installation of shackles. In all cases, for reasons of safety and success, it is important to perform the insemination in a quiet place and to limit the stress of the mare. In order to facilitate the placement of the semen, the rectum can be emptied of droppings. The tail should then be placed in a plastic tail guard before cleaning the perineal region in order to limit contamination of the genital tract during insemination, and thus preserve the mare's genital tract and optimize the success of insemination (IFCE, Haras Nationaux 2014). Washing the perineal region can be done with a shower or bucket. A series of three washes with povidone-iodine is recommended, following a classic protocol:

Soaping the vulva, then its sides and under the vulva, finishing with the anus. During the third soaping, the passage over the anus is omitted. When passing over the vulva, care must be taken not to get any product inside it so as not to irritate the genital mucosa. Once the three soapings have been carried out, the vulva is carefully dried using paper towels. The mare is then ready for the placement of the semen.

Figure 37. Gauze tail wrap with tail hairs flipped up to prevent sliding of the wrap down the tail.

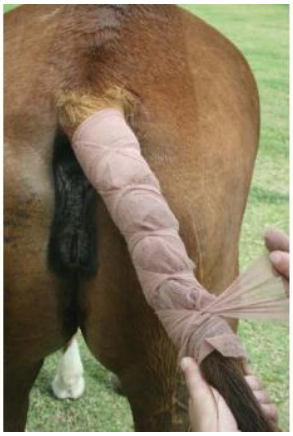


Figure 38. Gauze tail wrap twisted 360 degrees on the dorsum of the tail while the ventral aspect is

Figure 39. Tail wrap tied to the mare with quick release

Figure 40. Wrapped tail held out of the way using an elastic cord.

Figure 41. Bucket with liner to provide clean water for washing mares. All materials are disposed of between

Figure 42. Bucket with liner closed to prevent dirt from Contaminating water or to prevent water spillage when walking quickly with the bucket.

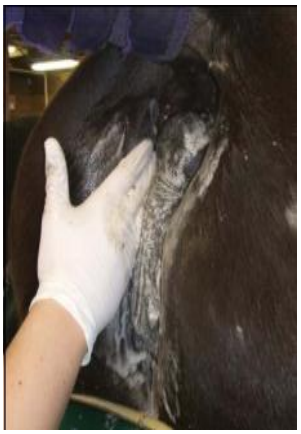


Figure 43. Washing the perineum with a gloved hand.

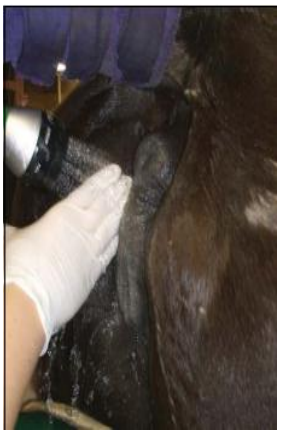


Figure 44. Rinsing the perineum with a gloved hand and a

Figure 45. After washing and rinsing the mare's external genitalia, a clean damp piece of cotton is used to check the vestibule for cleanliness.

Breeding with Fresh or Cooled Semen

Equipment and Supplies

Tail wrap, tail rope, obstetrical sleeve, obstetrical lubricant, non-irritant soap, roll cotton, stainless steel bucket, disposable liner for bucket, paper towels, exam gloves, sterile obstetrical sleeve, sterile obstetrical lubricant, semen, microscope, microscope slides, coverslips, disposable pipettes, insemination pipette, all-plastic syringes.

Pre-Insemination Technique

• Collect a semen sample.

• Filter the semen with a semen lter to remove gel and debris. Evaluate the semen for volume, concentration, and motility (total and progressive).

• Calculate a breeding dose. A minimum of 500 million progressively motile sperm (PMS) are recommended for a fresh insemination dose.

• An appropriate semen extender should always be added to the raw semen. A minimum ratio of 1:1 (semen : extender) is recommended. It is common to extend semen to a concentration of 25–50 million PMS/ml. This should result in an insemination volume of 10–20 ml.

• If cooled semen is to be used, it is recommended that a total of 1 billion PMS be packaged per dose at a concentration of 25 million PMS/ml. This should result in a total volume of 40 ml per dose. One to two doses of cooled semen are typically sent in one shipment for a given mare. Cooled semen is generally shipped by overnight courier or by counter to counter (same day) airline delivery.

• Light breed mares, such as Quarter Horses and Arabians, are typically inseminated when they are in heat with a follicle 35 mm or greater and edema is present in the uterus visible on ultrasound. The pre-ovulatory follicle of Warmblood mares and draft horse mares generally attains a significantly larger diameter and breeding decisions are adjusted accordingly.

• It is common to administer an ovulation-inducing medication at the time of breeding or possibly the day before breeding to provide a timed insemination. Options include deslorelin acetate (1.5–2.1 mg IM) or human chorionic gonadotropin (hCG) (1500–3000 IU IM or IV). Deslorelin will induce ovulation in approximately 40 hours, while hCG will induce ovulation in approximately 36 hours.

Insemination Technique

• Remove feces from the mare’s rectum.

• Place a tail wrap and tail rope on the mare.

• Wearing examination gloves, clean and dry the perineum of the mare.

• The semen sample should be thoroughly mixed prior to aspiration into an insemination all-plastic syringe and pipette.

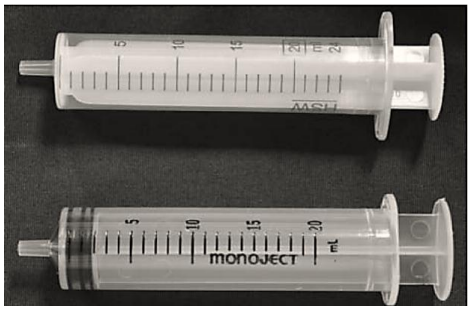


Figure 46. All–plastic syringes (top) are preferred over syringes containing rubber plunger tips (bottom) because products from the rubber can leach into the semen and damage spermatozoa.

• A notation should be made of any sediment within the semen container such as excessive smegma, urine crystals, or other organic debris that does not appear to be an opaque white-to-cream-colored semen pellet. With cooled semen, a small aliquot of semen (<1/4 ml) should be warmed for 10 minutes and examined under a microscope to evaluate sperm motility.

• A small amount of air equal to the volume of the pipette (3–5 ml depending on pipette length) may be aspirated into the syringe already containing the semen to be deposited. This will be used after insemination to clear the pipette of semen.

• Attach an insemination pipette to the syringe. Although not necessary, the pipette may be filled with semen prior to placement into the mare to avoid air from entering the uterus. Avoid losing the air previously aspirated into the syringe with the semen.

• Don a sterile obstetrical sleeve and place a sterile, water-soluble lubricant on the outside of the hand and down the arm.

• The tip of the insemination pipette is placed into the palm of the hand with the obstetrical sleeve (Figure 47).

Figure 47. Placement of an insemination pipette into the hand for placement into the mare's reproductive tract.

• The sleeved hand is inserted through the vulvar labia and advanced to the external cervical os. Typically this is to the depth of approximately mid-forearm. A slight rotational motion may be used to help spread lubricant within the reproductive tract and aid in forward movement of the arm.

• The lumen of the external cervical os is located with the index finger (Figure 48). The os of the cervix may be directed slightly downward or off to one side. Careful exploration will provide eventual access to the lumen.

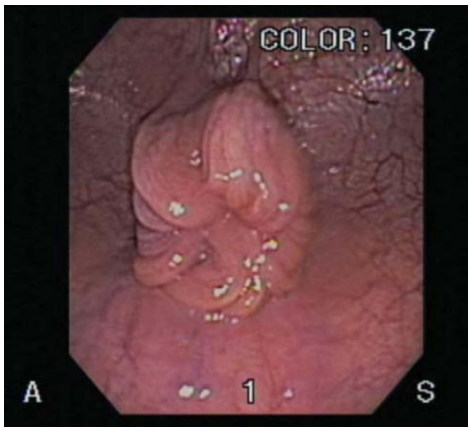


Figure 48. External cervical os. The cervical canal is located in the center of the cervix.

• Place the index finger into the cervical canal, using a probing maneuver to locate the direction of the canal. The cervical canal is not always directly straight and may slightly curve, especially downward in a multiparous mare.

• Using the hand outside the mare, holding the body of the pipette, direct the tip of the pipette into the cervical lumen past the inserted index finger (Figure 49). The pipette may be advanced a few centimeters past the indexfinger to insure placement in the uterine lumen. Sometimes downward pressure on the pipette within the cervix with the inserted index finger may help direct the pipette tip into a downward sloping cervical canal.

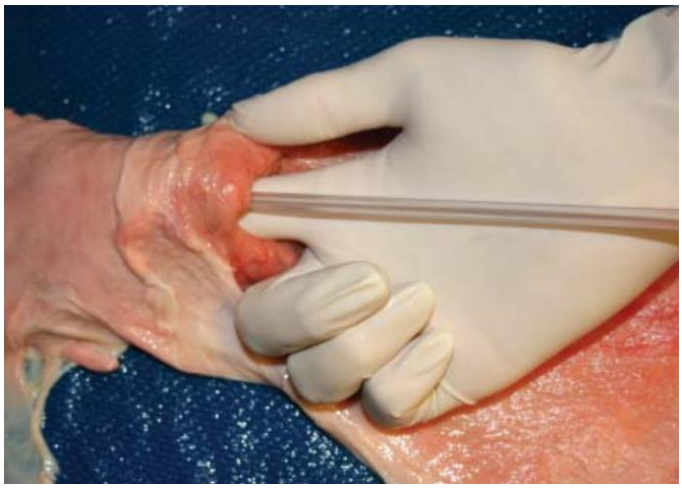


Figure 49. Introduction of an artificial insemination pipette along the index finger and through the cervical canal into the uterus.

• Start slow pressure on the syringe plunger, pushing semen through the pipette and into the uterus (Figure 50). If there is a feeling that semen is back-flowing out the cervix into the vagina, reposition the tip of the pipette further into the cervix and into the uterus.

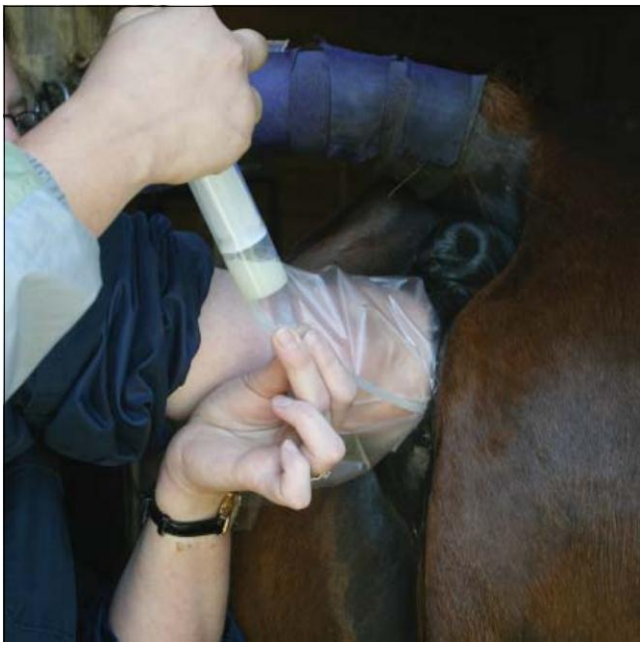


Figure 50. Vertical orientation of the syringe during insemination to allow passage of air after the inseminant.

• When getting close to depositing the entire contents of the syringe, hold the syringe more vertically so that the air bubble in the syringe is the last to enter the pipette, thus flushing the contained semen into the uterus. If no air was in the syringe, then once the insemination is complete, pull the syringe off the pipette, aspirate 3–5 ml of air, reattach, and push the remaining semen from the pipette into the uterus.

• The insemination pipette is withdrawn from the cervix along with the index finger.

• Optionally, prior to removal of the hand and pipette from the vagina, the external cervical os may be held closed for 10–15 seconds with a few fingers to prevent immediate back-flow of the inseminate.

• Lubricant is wiped off the mare's perineum and the tail wrap and tail tie removed.

• The mare should have a repeat ultrasound examination in 24 hours to determine ovulation and any accumulation of uterine fluid that may need to be treated.

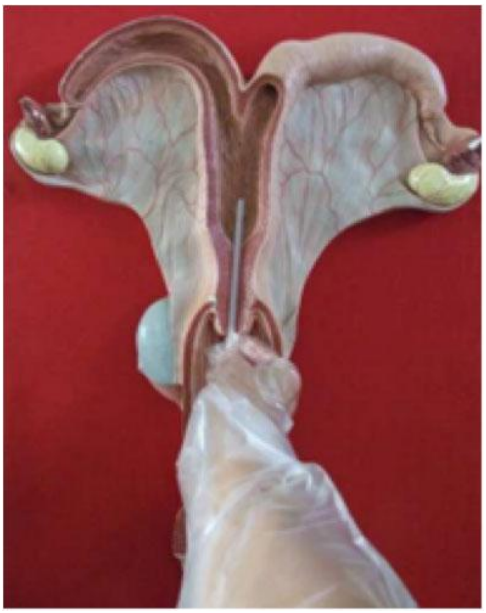


Figure 51. Illustration: classic insemination technique: The insemination pipette is located at the level of the body of the mare's uterus / Source: ©webconferenceIFCE

Interpretation

• The goal with fresh semen is to inseminate the mare within 48 hours prior to ovulation.

• The goal with cooled-stored semen is to inseminate the mare within 24–48 hours prior to ovulation.

• Compare the motility of the semen at the time of processing to the motility at the time of breeding. If there is a significant drop in motility (>30–50%) with 24 hours of storage, alternative methods for semen processing should be considered such as centrifugation, gradient centrifugation, or use of an alternative extender.

• Examine the sterile obstetrical sleeve after removal from the vagina for any discharges (purulent, bloody, etc.) or foul odor (urine, necrotic tissue) that may indicate reproductive tract pathology.

• Avoid pushing excessive amounts of air into the uterus in order to clear the pipette of semen. This may lead to uterine inflammation. Air will be hyperechoic with a ventral shadow on ultrasound especially present if the mare is examined within a few hours of breeding.

• Back-ow of semen may be more common with the use of a large volume (>60 ml) of inseminant.

• Occasionally, the mare may urinate after breeding as a consequence of having the procedure performed. This is not desirable, especially in mares with very dependent uteri as urine may enter the dilated cervix. If this occurs regularly in a particular mare with breeding, the mare may be walked for a few minutes post-insemination to prevent immediate urination.

• Significant discoloration of the semen pellet in the shipping container may indicate inadequate washing of the stallion prior to collection, with a potential increase in deposition of organic material and bacteria into the mare's reproductive tract.

Insemination Through a Vaginal Speculum

Mares can be inseminated through a vaginal speculum if the mare has had an episioplasty or Caslick procedure (i.e., "sutured"), or if for some reason it is not possible to insert the hand into the vagina. Consequently, the Caslick would not have to be opened for insemination and subsequently re-sutured. Some mares may have to be lightly sedated or restrained for this procedure.

Equipment and Supplies

Vaginal speculum, obstetrical lubricant (sterile), light source, insemination pipette, and syringe.

Technique

• The tail should be wrapped and the perineum cleansed and dried.

• A sterile vaginal speculum is lubricated and slowly inserted into the vulva initially at an upward angle. Once the speculum has passed the floor of the pelvis, the speculum is then moved to a horizontal position and inserted slowly through the vestibulo-vaginal fold into the vaginal vault.

• A penlight or other light source is used to illuminate the vaginal vault (Figure 52).

Figure 52. Viewing the cervix through a disposable speculum during insemination.

• The cervix is visually located and the insemination pipette, with the syringe containing the semen attached, is introduced through the speculum andinto the external cervical os (Figure 53).

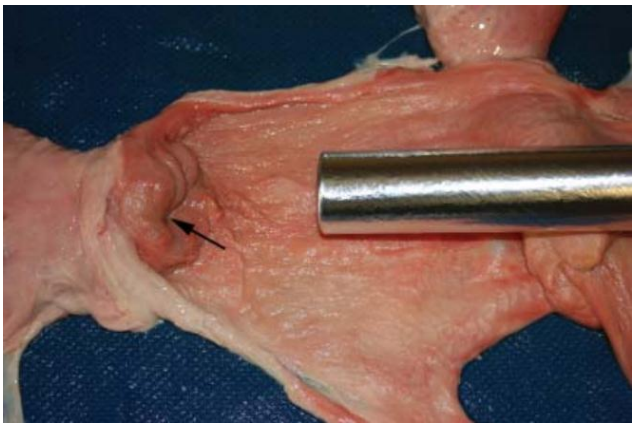


Figure 53. Position of the speculum prior to passage of the insemination pipette. Note the location of the external cervical os (arrow).

• The pipette is slowly advanced through the cervical canal into the uterine body.

• Once the insemination pipette is positioned appropriately, the semen is expelled by depressing the plunger of the syringe. The cervix should be visualized during semen deposition to make sure there is not efflux of semen from the external cervical os. If this occurs, then the pipette should be repositioned into the uterus prior to further insemination.

Additional Comments

• Mares will usually allow the passage of a vaginal speculum without issue.

• A mare with a Caslick may exhibit discomfort when the speculum is raised into a horizontal position as pressure is placed on the co-joined aspect of the dorsal commissure of the vulva.

• It may be difficult in some mares to pass the pipette into the external os of the cervix. The distal end of the speculum may be used to “guide” the pipette tip to the appropriate position. Once the tip is correctly placed, the pipette can usually be easily advanced into the uterus. A pipette tip with a bulbous end may be easier to pass forward than a narrow-tipped pipette which may get caught in tissue folds. Extending the cervix caudally may help with passage of the pipette. This would require use of long-handled forceps to gently grasp and extend the external os.

Breeding with Frozen Semen

Frozen semen use has increased in the equine industry due to the availability of commercial freezing extenders, standardized protocols for freezing semen, and the development of practical mare management

strategies for the use of frozen semen. There is often a large variation in the fertility rates between stallions with frozen-thawed semen. This can be controlled to a degree with diligent selection of stallion candidates and freezing procedures to maximize post-thaw spermatozoal viability. In addition, the selection of mares may influence the success rate of frozen semen. Pregnancy rates may be lower in mares with a history of subfertility, mares predisposed to delayed uterine clearance, and in aged mares. Proper timing of insemination is required as frozen-thawed semen has a limited viability, so that semen should be inseminated within 12 hours prior to ovulation and up to 6–8 hours after ovulation.

Equipment and Supplies

Tail wrap, tail rope, non-irritant soap, roll cotton, stainless steel bucket, disposable liner for bucket, paper towels, exam gloves, obstetrical sleeve, obstetrical lubricant, semen, microscope, microscope slides, coverslips, disposable pipettes, insemination pipette, all-plastic syringes, sterile water-soluble lubricant, sterile obstetrical sleeve, syringes, needles, ovulatory drugs, water bath, thermometer, straw cutter.

Administration of an Ovulation Induction Agent

• Light breed mares such as Quarter Horses and Arabians are typically administered an ovulation-inducing agent when they are in heat with a follicle 35 mm or greater and uterine edema is visible on ultrasound. The pre-ovulatory follicle of Warmblood mares and draft

horse mares generally attain a significantly larger diameter and breeding decisions are adjusted accordingly.

• Administration of deslorelin acetate (1.5–2.1 mg IM) will induce ovulation in approximately 40 hours (range 36–42 hours), while human chorionic gonadotropin (hCG) (1500–3000 IU IM or IV) will induce ovulation in an average of 36 hours (range 28–96 hours).

Management Strategy with One Dose of Semen Available per Cycle

• If only one dose of frozen-thawed equine semen is available, the insemination is typically performed immediately after ovulation is detected. If the mare is inseminated prior to an anticipated ovulation and the mare does not ovulate for 12–24 hours or more after insemination, pregnancy rates will be lower. The possibility of pregnancy will be non-existent if the mare is inseminated but does not ovulate at all.

• A practical mare management strategy when one dose of semen is available is to administer deslorelin acetate at 8:00 pm, with the anticipation that ovulation will occur at 12:00 pm (noon) approximately 40 hours later. The mare should be examined periodically the day after deslorelin administration and then early in the morning the day she is supposed to ovulate. If administration of the deslorelin was timed correctly, the dominant follicle will still be present in the morning and the mare will have a fresh ovulation detected when examined at approximately 12:00. The dose of semen is thawed and the mare inseminated.

• Another option is to thaw one half of the dose, if using multiple straws per dose, and inseminate prior to the anticipated ovulation. The other half of the dose is used once ovulation has been confirmed. A potential disadvantage of this strategy is if the mare fails to ovulate on schedule or fails to ovulate at all and a portion of the semen is wasted.

Management Strategy with Two Doses of Semen Available per Cycle

• If two doses of frozen semen are available for a given estrous cycle, one dose may be inseminated immediately prior to the anticipated ovulation and the second dose inseminated after ovulation has been confirmed.

• A practical mare management strategy when two doses of frozen semen are available is to administer deslorelin acetate at 8:00 am, with the anticipation that ovulation will occur at 12:00 am (midnight) approximately 40 hours later. The mare should be examined periodically the day after deslorelin administration and the mare inseminated in the evening

(approximately 32–36 hours later). The mare is examined early in the morning the next day and she is re-inseminated if a fresh ovulation is detected (approximately 46–48 hours).

• A similar management strategy using hCG would be to administer hCG at 12:00 pm with the anticipation that ovulation will occur at 12:00 am, approximately 36 hours later.

• An alternative technique would be timed inseminations at 24 and 40 hours post-ovulatory agent administration. If the mare did not ovulate between the 24- and 40-hour examinations, the second dose would be withheld until ovulation occurs. If the mare did ovulate, then the second dose is given at 40 hours. If the ovulatory agent is given at 4:00 pm, the mare would be bred at 4:00 pm the next day (24 hours) and 8:00 am the following day (40 hours).

• With these management strategies or modifications thereof, the mare is immediately inseminated if ovulation is detected earlier than anticipated.

• If the mare has not ovulated by the predicted time, she should be examined at regular intervals and a second dose inseminated after ovulation is detected.

Breeding Technique

• Prepare the mare for insemination using standard hygiene techniques. The feces of the mare should be removed from the rectum if deep-horn insemination is to be performed.

• Thaw the semen according to directions from the facility that froze the semen.

• The method used to deliver the semen into the uterus depends on the number of straws, type of straw, type of pipette, and experience of the inseminator.

• If one 0.5 ml straw is to be inseminated, the best method may be direct deposition with a pipette that allows the user to place the straw within the pipette and the use of a metal stylet to advance the cotton plug within the end of the straw and expel the semen (Figures 54 and 55). This same method can be used if multiple straws are to be inseminated. The pipette can remain in the uterus of the mare and thawed straws sequentially reloaded into the pipette for deposition into the uterus. This method is considered by many to be superior over other methods as it optimizes delivery of the entire semen dose into the uterus. If there is semen remaining in the straw, a drop should be placed on a warmed microscope slide and examined for motility.

Figure 54. End of the stylet that will be inserted into the straw inside the breeding pipette (Minitube®). The enlarged metal ball on the end of the pipette will push the cotton pledget through the straw to push semen out the pipette.

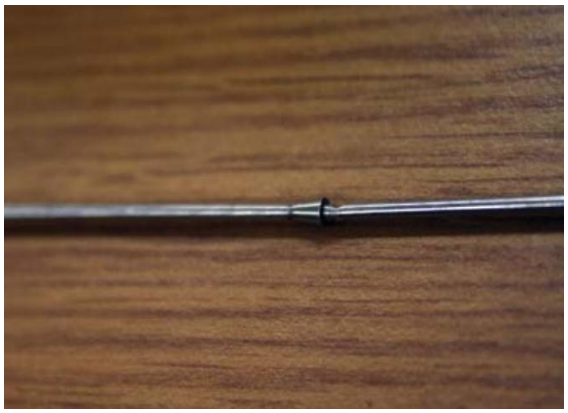


Figure 55. The cone on the stylet shaft that will catch the semen straw. Upon insertion of the stylet into the semen straw, the cone will hold the straw for removal of the straw from the pipette so an additional straw may be inserted for multiple straw breedings (Minitube®).

• An alternative technique is to dispense the thawed semen into a warmed vessel such as a test tube or centrifuge tube. This may be used when multiple 0.5 ml straws are to be thawed or with the use of 2.5–5 ml macrostraws, goblets, or packets. A prewarmed pipette is then inserted into the tube and the semen aspirated into the pipette.

• Prior to insemination a small drop of semen may be placed on a warmed microscope slide and examined for motility.

• Frozen-thawed semen may be deposited in the uterine body using standard insemination procedures (figure 51).

• Deep-horn insemination, with deposition of semen close to the utero-tubular junction (figure 56), may be the best technique for the frozen-thawed semen of some stallions to optimize pregnancy rates. This may include stallions with poor post-thaw motility, stallions with a history of low fertility, or when a limited number of straws are utilized.

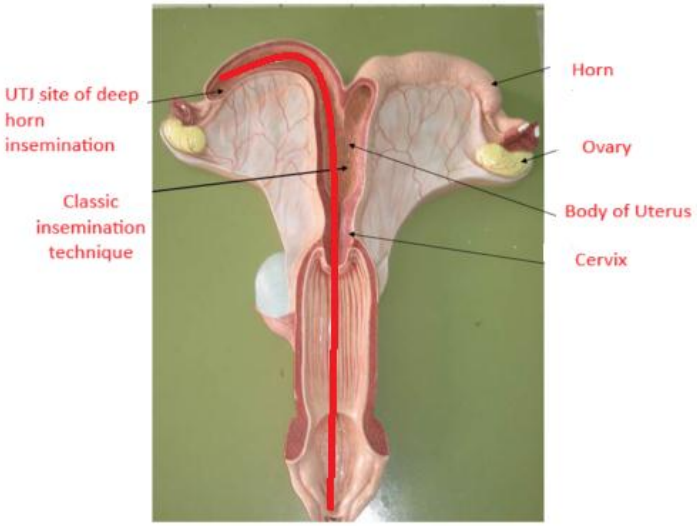


Figure 56. Illustration, Deep horn insemination, the insemination pipette is located close to the utero-tubular junction

Interpretation

• With direct insemination of straws within a pipette using a metal stylet, 92–96% of semen is deposited within the reproductive tract. With aspiration of semen into a pipette and subsequent deposition, 76–87% of semen is deposited depending on the type of insemination pipette and number of straws per dose. With the placement of semen into a syringe with deposition through a pipette, 29–79% of the dose is delivered. Loss is from wetting of surfaces that cannot be recovered when working with low volumes (0.5–2 ml) (Figure 57).

Figure 57. Semen remaining in the syringe if semen is drawn into the syringe for insemination. This technique should be avoided as it results in less than optimum semen volume delivery.

• Frozen semen may also be stored in goblets, packets, or large straws. Instructions for proper thawing of the semen should accompany delivery of the semen and should be followed to optimize post-thaw viability of the spermatozoa.

• Examine the sterile obstetrical sleeve after removal from the vagina for any discharges (purulent, bloody, etc.) or foul odor (urine, necrotic tissue) that may indicate reproductive tract pathology.

• Avoid pushing excessive amounts of air into the uterus in order to clear the pipette of semen. This may lead to uterine inflammation. Air will be hyperechoic with a ventral shadow on ultrasound especially present if the mare is examined within a few hours of breeding.

• Examine the mare the day after insemination to determine if there is any liquid accumulation and inflammation from breeding with frozen semen. The post-mating inflammatory reaction may be greater in mares bred with frozen semen than in mares bred with fresh or cooled semen due to the absence of seminal plasma. Treatment may include uterine lavage and/or administration of an ecbolic agent such as oxytocin or cloprostenol.

• Occasionally, the mare may urinate after breeding as a consequence of having the procedure performed. This is not desirable, especially in mares with very dependent uteri as urine may enter the dilated cervix. If this occurs regularly in a particular mare with breeding, the mare may be walked for a few minutes post-insemination to prevent immediate urination.

Deep Horn Insemination

Deep horn insemination is a technique that may be used to deliver spermatozoa to the tip of the uterine horn in close proximity to the utero-tubal junction. The procedure is most often used when the volume of semen is low or when frozen-thawed semen is inseminated. The insemination pipette is passed through the uterine body and up the ipsilateral horn of the ovary with the ovulatory follicle.

The pipette used should have a blunt tip, be long enough to reach the tip of the uterine horn, and be flexible. The dose to be inseminated should be aspirated into the end of the pipette if not already pre-packaged into 0.5 ml straws. There appears to be no advantage of using hysteroscopic insemination over deep horn insemination unless the number of spermatozoa is less than 5 million. Deep horn insemination requires skill and practice in the

correct placement of the pipette and transrectal manipulation of the pipette after insertion into the uterus, but the procedure can be accomplished on a farm with a little practice.

Equipment and Supplies

Tail wrap, tail rope, non-irritant soap, roll cotton, stainless steel bucket, disposable liner for bucket, paper towels, examination gloves, sterile obstetrical sleeve, obstetrical sleeve, sterile water-soluble lubricant, insemination pipette, syringes, semen.

Technique

• Remove feces from the mare's rectum.

• Place a tail wrap and tail rope on the mare.

• Wearing examination gloves, clean and dry the perineum of the mare.

• Place an insemination pipette in a warm incubator at 37◦C (99◦F). This will warm the plastic pipette, allowing it to be curled easily, if that is desired. If this is not possible, placement in the sun on a vehicle dashboard or on a vehicle dashboard with the defroster set to warm will suffice.

• Remove the pipette from the incubator (or warm area) and curl it either in a large arc or into a circle (Figure 58). Relax the pipette and it should retain the curvature (Figure 59). The curve in the pipette will help in placement into the chosen uterine horn.

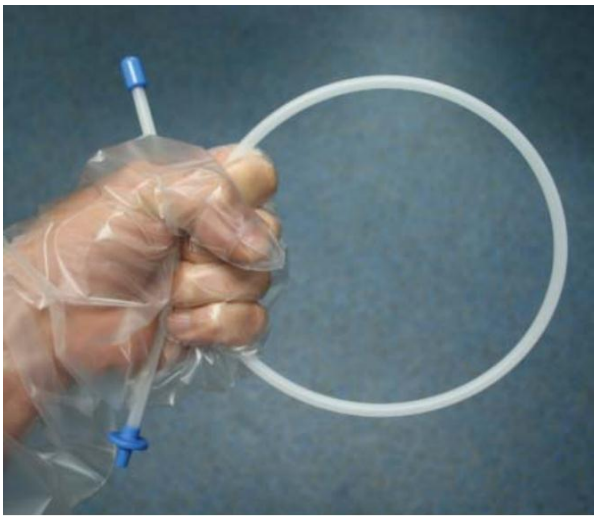


Figure 58. Bending of an artificial insemination pipette after removing from the incubator.

Figure 59. Bend that remains in the pipette after curling. This bend aids in the placement of the pipette into the correct uterine horn.

• The pipette is advanced through the cervix into the uterine body using traditional techniques.

• The gloved hand is removed from the vagina and placed into the rectum of the mare.

• The tip of the pipette is located per rectum and advanced into the correct uterine horn. The curve of the pipette and manual manipulation are used to guide the pipette.

• Lifting or straightening the uterine horn may help with passage of the pipette into and proximally up the uterine horn.

• The pipette should be advanced until it reaches the tip of the uterine horn as confirmed by palpation.

• Inseminate the mare and withdraw the pipette.

Interpretation

• When using the metal stylet within the Minitube® insemination pipette for frozen-thawed semen, the curvature of the pipette may slightly inhibit the passage of the metal stylet. Once the pipette is properly placed at the tip of the horn, some straightening of the pipette with the hand per rectum may be attempted to aid in passing the metal stylet to advance a semen straw into place.

• Excessive air should be avoided from entering the uterus during the procedure as this may result in uterine inflammation. Holding a clean gloved finger over the open end of the pipette or placement of an empty 3 cc syringe onto the pipette will prevent excessive air entry.

• When larger volumes of semen are used (>5 ml) there does not seem to be an advantage to using a deep horn insemination technique as the semen tends to gravitate toward the uterine bifurcation after insemination.

Hysteroscopic (Low Dose) Insemination

A normal fertile stallion will deposit 2–10 billion or more spermatozoa into the reproductive tract of a mare after a natural service ejaculation. In contrast, the standard minimum articial insemination dose using fresh semen is 500 million progressively motile spermatozoa (PMS). In some instances it may be necessary to inseminate a mare with a dramatically lower than usual number of sperm. This may be the case when a very limited number of frozen semen straws are available, if a stallion has a low sperm count in his ejaculate, or if the book of

mares for a given day is higher than the number of insemination doses collected from a stallion.

Acceptable pregnancy rates can be achieved using “low dose insemination techniques” in which 5–50 million or more spermatozoa, in volumes ranging from less than 0.1 ml up to 1–2 ml, are deposited into the uterus of a mare. The primary advantage of the hysteroscopic low dose insemination technique is direct visualization and precise deposition of a small volume of concentrated semen directly onto the utero-tubal junction. Disadvantages include the initial cost of the videoendoscope, the time required to set up, perform, and clean up after the procedure, and the requirement for multiple trained personnel.

Equipment and Supplies

Videoendoscope, catheter (210 cm (83 inch), syringe (3 ml), centrifuge, semen extender, glass vial, obstetrical sleeve (sterile), obstetrical lubricant (sterile), sterile saline, glutaraldehyde solution.

Technique

• Timing of a low dose insemination is an important part of the success of the process. The goal is to deposit semen immediately before ovulation is predicted to occur or immediately after ovulation has been confirmed. Induce a timed ovulation with human chorionic gonadotrophin (hCG) or deslorelin acetate.

• The mare is placed in examination stocks and sedated. A combination of detomidine hydrochloride (5 mg) plus butorphanol tartrate (5 mg) administered intravenously is usually effective for a light breed horse.

• The tail of the mare is wrapped and held out of the way.

• The mare’s perineal area is thoroughly scrubbed using a non-residual soap and rinsed with clean water and dried with disposable paper towels.

• Semen should be processed and readily available. This may entail centrifugation of semen and re-suspension in a very small volume of extender (i.e., 50–100 million PMS re-suspended in a total volume of 0.2–0.5 ml) or thawing a single straw of frozen semen.

• The endoscope should be cold sterilized before use. This may be accomplished by cold sterilization in an activated solution of 2.4% glutaraldehyde (Cidex®); the endoscope is then thoroughly rinsed with 0.9% sterile saline.

• A special catheter (210 cm) (Figure 60) is passed down the working channel of the endoscope until the tip of the catheter exits the endoscope.

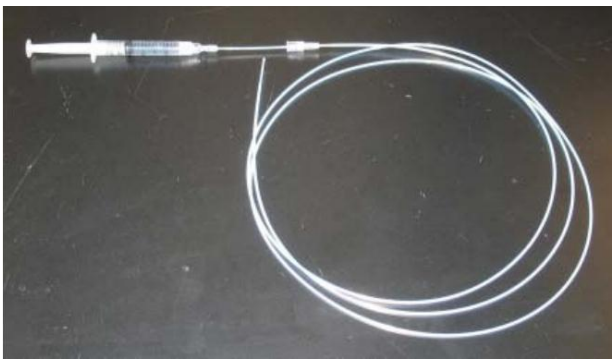


Figure 60. Catheter used to deposit semen on the uterotubular junction. The catheter is passed through the biopsy channel of a videoendoscope.

• The low volume semen sample is aspirated into the distal end of the catheter.

• The catheter is withdrawn into the endoscope.

• A sterile obstetrical sleeve is worn by the person passing the endoscope.

• A small volume of sterile obstetrical lubricant is applied to the back of the gloved hand.

• The endoscope is protected in the palm of the hand while being passed through the vulva and into the vaginal vault.

• The working end of the endoscope is passed through the cervix into the uterine body.

• The cervix is held in a closed position while the uterine lumen is inflated using the endoscope with sufficient room air to distend the lumen and allow visualization and passage of the scope up the uterine horn ipsilateral to the antral follicle.

• Once the uterus is inflated, the uterine bifurcation should be visible (Figure 61).

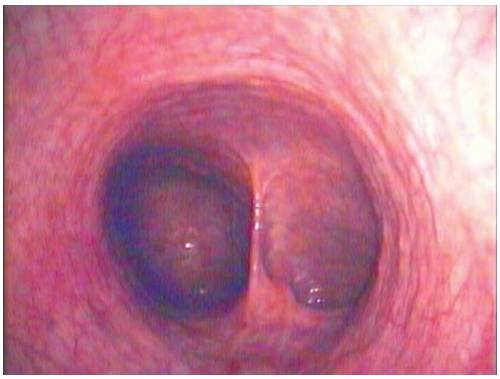


Figure 61. Uterine bifurcation as viewed through a videoendoscope.

• The endoscope is subsequently passed up the lumen of the horn adjacent to the ovary containing the pre-ovulatory follicle (Figure 62).

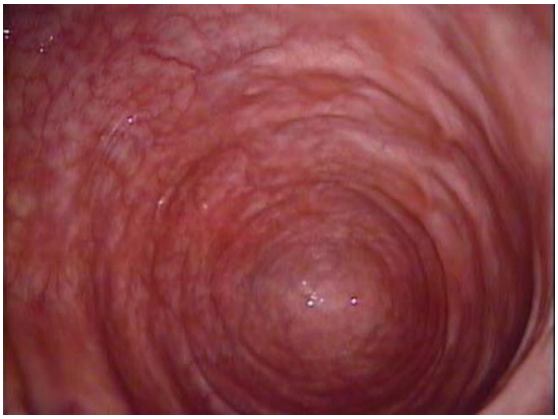


Figure 62. A dilated uterine horn as viewed through a videoendoscope.

• The utero-tubal junction is visualized at the distal end of the uterine horn (Figure 63).

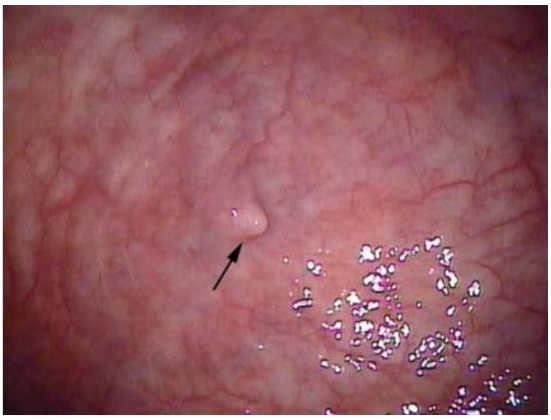


Figure 63. Uterotubular junction (arrow) viewed through a videoendoscope.

• The catheter tip is advanced out of the endoscope until the tip gently touches the papilla.

• The small volume of extended semen is then slowly deposited directly onto the papilla.

• The endoscope is withdrawn to the caudal uterine body and the air in the uterus is evacuated.

Additional Comments

Insemination of less than 100 million motile spermatozoa using a traditional technique is likely to result in a low pregnancy rate per cycle. However, pregnancy rates of 50–75% have been obtained with insemination doses of 5–25 million PMS using a hysteroscopic insemination technique.

There is no significant difference in pregnancy rate between the manual deep horn low dose technique and the hysteroscopic low dose insemination technique. Both methods can yield acceptable pregnancy rates using low numbers of spermatozoa if the stallion has good

inherent fertility. However, low dose insemination techniques may have reduced effectiveness when used with subfertile stallions or with less than 5 million total spermatozoa.

Artificial Insemination Technique: Small Ruminants

In the last decades small ruminants have become increasingly important, and nowadays sheep and goat are continuously increasing in the number of breeds and their geographic distribution. An important feature of small ruminants is that they can live and produce on land that is unfavorable for other forms of agriculture. The increase in small ruminant breeding has been supported more recently by the development and improvement of assisted reproductive technologies (ARTs). However, while some ARTs have reached widespread application, including estrus induction, estrus synchronization, and artificial insemination, other ARTs, such as superovulation and embryo transfer, in vitro embryo production, and embryo cryopreservation, are only rarely used (Ledda, S., and Gonzalez-Bulnes, A., 2018).

Artificial insemination (AI) can undoubtedly be regarded as the oldest and most widely used assisted reproductive technique/technology (ART) applied in livestock production and it is one of the most important ARTs. Fresh, fresh + diluted + chilled and frozen semen can be used for AI in small ruminants. To ensure its successful use, the AI technique must be selected on the basis of the type of semen planned to be used (Faigl, V et al., 2012).

Reproductive anatomy

Both species have similar characteristics in some anatomical aspects (a pair of nipples), gestation period (150 days), and presence of seasonal anestrus, differing in terms of magnitude and depth and presence of the male effect. However, they are completely different in feeding habits, nutrient needs, and grazing systems, with differences in terms of the female's reproductive tract, among other characteristics (Sánchez Dávila, F et Pérez Muñoz, G., 2021).

When comparing the reproductive tracts of ewes (female sheep) and goats, there are several anatomical differences and similarities due to their close genetic relationship as small ruminants. Here's a breakdown of these aspects:

Ovaries

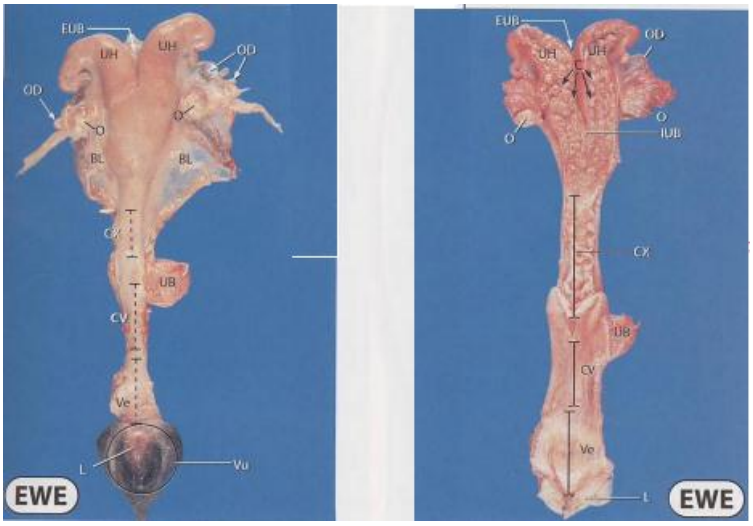
- **Ewes:** The ovaries in ewes are relatively small and are located near the ends of the uterine horns. They are oval-shaped, and their size can vary depending on the breed and the reproductive cycle stage（Hafez & Hafez, 2000).
- **Goats:** Goat ovaries are similar in shape and size to those of ewes, but they tend to be slightly larger. They also vary in size with the reproductive cycle（Hafez & Hafez, 2000).

Oviducts (Fallopian Tubes)

- **Ewes:** The oviducts in ewes are coiled and connect the ovaries to the uterine horns. They play a crucial role in transporting the ova (eggs) from the ovaries to the uterus（Noakes, Parkinson, & England, 2009）.
- **Goats:** Goats have oviducts that are quite similar in function and structure to those of ewes, with the primary role being the transportation of ova（Noakes, Parkinson, & England, 2009).

Uterus

- **Ewes:** Ewes have a bicornuate uterus, which means it has two long uterine horns that are fused at their base. The uterine horns are relatively long and coiled, providing the necessary space for carrying multiple fetuses (lambs) during pregnancy （Hafez & Hafez, 2000; Noakes, Parkinson, & England, 2009）. (Figure 64)
- **Goats:** Like ewes, goats also have a bicornuate uterus with two horns. However, the horns in goats tend to be shorter and less coiled compared to those in ewes. This is partly due to the generally smaller litter size in goats compared to sheep（Noakes, Parkinson, & England, 2009; Cattle, 2013）. (Figure 65)



BL= Broad ligament (mesometrium)
C= Caruncle
CX= Cervix
EUB= External Uterine Bifurcation
FV= Fornix Vagina
IUB= Internal Uterine Bifucation
L= Labia
O= Ovary
OD= Oviduct
UB= Urinary Bladder
UH= Uterine Horn
Ve= Vestibule
Vu= Vulva

Figure 64. Dorsal view of excesed reproductive tract of ewe
(Pathways to pregnancy and parturition, second revised edition. P.L. SENGER 2005)

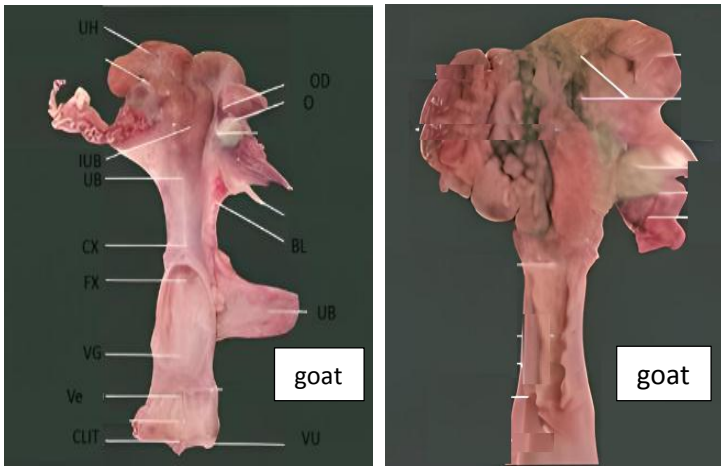


Figure 65. Dorsal view of excesed reproductive tract of goat

Cervix

- **Ewes:** The cervix of the ewe is quite long and consists of multiple interlocking folds or rings, making it difficult to traverse with a speculum during artificial insemination. This complexity in structure is believed to act as a barrier against infections and to help retain semen after mating（Hafez & Hafez, 2000; Cattle, 2013）.
- **Goats:** The goat's cervix is also long and has folds, but it is less complex than that of the ewe. The folds are fewer and less tightly interlocked, making the cervix slightly easier to navigate during artificial insemination （Noakes, Parkinson, & England, 2009）. (Figure 66)

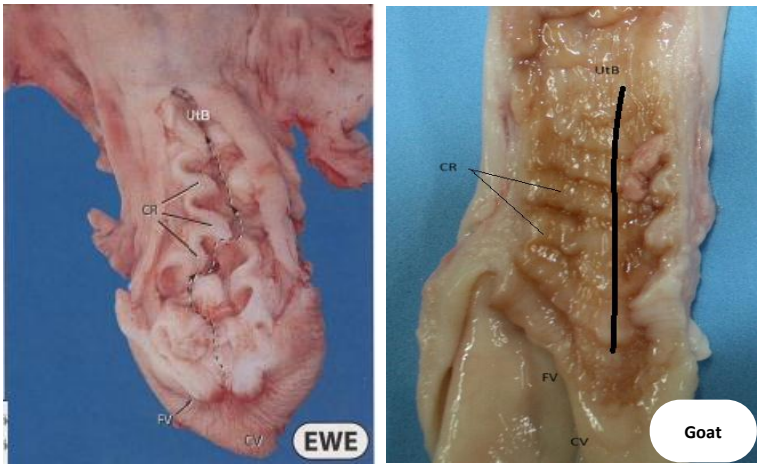


Figure 66: Excessed Cervical Tissue

Vagina

- **Ewes:** The vagina in ewes is a muscular tube that extends from the cervix to the external genitalia. It is relatively short compared to that of other large mammals（Hafez & Hafez, 2000）.
- **Goats:** Goats have a similar vaginal structure, but it tends to be slightly longer and less muscular compared to ewes（Noakes, Parkinson, & England, 2009）.

External Genitalia

- **Ewes:** The vulva in ewes is the external part of the reproductive tract, and it is generally covered by hair. The vulva's structure in ewes can vary slightly between breeds（Cattle, 2013）.
- **Goats:** The vulva in goats is similar to that of ewes, although it can be somewhat more prominent and the hair coverage may differ depending on the breed （Hafez & Hafez, 2000).

Artificial insemination techniques in small ruminants

Uterine semen deposition in small ruminants can be accomplished by a laparotomic (Silla et al., 2021), laparoscopic (Rocha et al., 2022), transcervical (Fonseca et al., 2019a), or pericervical/vaginal (Menchaca et al., 2005) technique/route. Transcervical AI may utilize either cervical fixation (a.k.a. cervical clipping) or cervical traction techniques. Cervical fixation, typically used in goats, comprises cervical immobilization with a pair of Allis forceps (Fonseca et al., 2017a, Bonato et al., 2019), whereas cervical retraction, popular in ewes,

involves the clipping of the uterine cervix with two pairs of forceps (Allis/Pozzi) and its gentle repositioning to the location where it can be transrectally palpated with fingers (Casali et al., 2017). Even though no anesthesia is required during cervical manipulations, a few studies have suggested that cervical retraction may not be innocuous to the female (Halbert et al., 1990a) and histological examinations revealed the occurrence of cervical lesions associated with cervical clipping or retraction, raising some ethical concerns (Campbell et al., 1996).

Due mainly to the complex cervical anatomy (Kershaw et al., 2005), the most widespread AI techniques in sheep are the laparoscopic (intrauterine) technique (when F/T semen is used) and vaginal insemination (with fresh/cooled/chilled semen; reviewed by Gibbons et al., 2019). Several strategies have been proposed to increase the ease of intrauterine semen deposition by the transcervical route in ewes. Even though achieving acceptable pregnancy rates is feasible after applying invasive interventions (e.g., surgical incision of cervical folds; Pau et al., 2020), such improvements have not been typically seen for cervical AI using F/T semen in sheep. With the aid of specialized instruments and/or restraint and positioning of animals (e.g., Guelph system for transcervical AI; Halbert et al., 1990a), transcervical (intrauterine) AI may result in similar pregnancy rates as laparoscopic AI [32 vs 48%, respectively, using F/T semen in Merino ewes (Windsor et al., 1994) or 42 vs 50%, respectively, using fresh semen in Corriedale ewes (Casali et al., 2017)]. Interestingly, supplementing F/T semen with seminal plasma increased the ability of spermatozoa to penetrate cervical mucus and boosted pregnancy rates after cervical AI in Merino ewes (51 vs 28% for seminal plasma-supplemented vs non-supplemented semen, respectively; Maxwell et al., 1999). The same group later demonstrated that the addition of seminal plasma to epididymal sperm improved its cervical transport, suggesting that the process might be influenced by an unknown component(s) of seminal plasma (Rickard et al., 2014). Although there are few studies reporting satisfactory outcomes after transcervical AI with F/T semen in ewes, overall fertility is low and results remain inconsistent (Candappa and Bartlewski, 2011).

Therefore, the most used insemination technique in goats is the anterior bipedal transcervical AI (a.k.a. the French technique), although laparoscopic AI is still practiced. The former has some drawbacks including incomplete intrauterine semen deposition (semen is frequently placed between the cervical rings) and discomfort to animals. The “Embrapa technique” of transcervical AI by cervical immobilization in goats (figure 67), with the female

restrained in a standing (quadrupedal) position, was designed about a decade ago (Fonseca et al., 2011) and reported internationally a few years later (Fonseca et al., 2017a). This method permits a high rate of intrauterine semen deposition (≥90%), takes ~30 s per doe to complete, is much better tolerated by the inseminated animal, and has proven feasible in field conditions (Fonseca et al., 2019a), yielding high pregnancy rates [62.5% (Fonseca et al., 2017a); 66–80% (Bonato et al., 2019); and 68% (Carvalho-de-Paula et al., 2020)] with F/T semen.

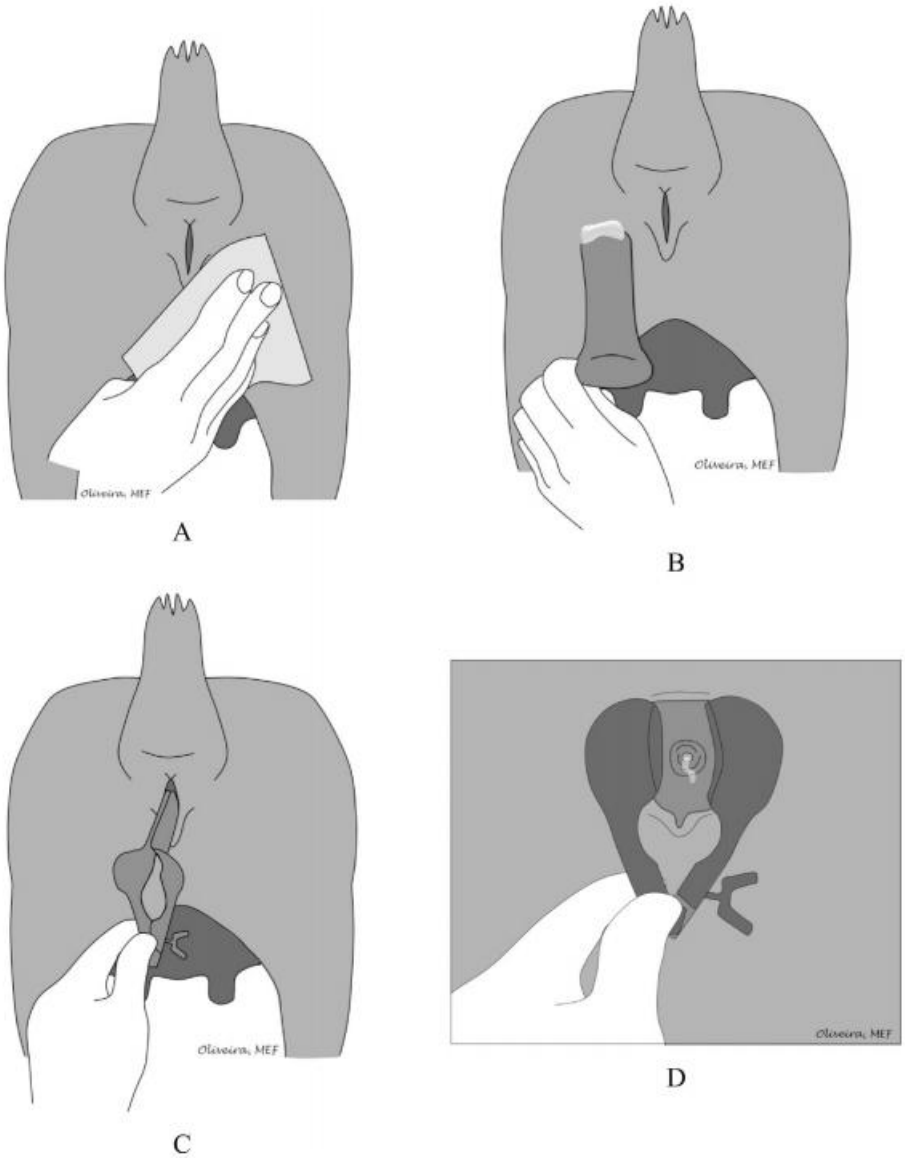


Figure 67. Embrapa AI technique. Cleaning the external genitalia of the goat with a paper towel (A); lubrication (B), and insertion (C) of the Colin vaginal speculum into the goat vagina; cervical visualization and mucus discharge (D).

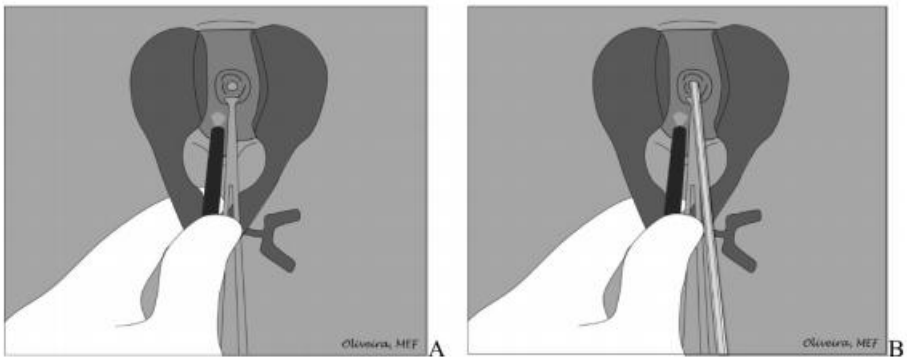


Figure 68. Cervical immobilization with Allis forceps and light source (A); cervical transposition with the semen applicator (B). Note the handling of the instruments (forceps, speculum, and light source).

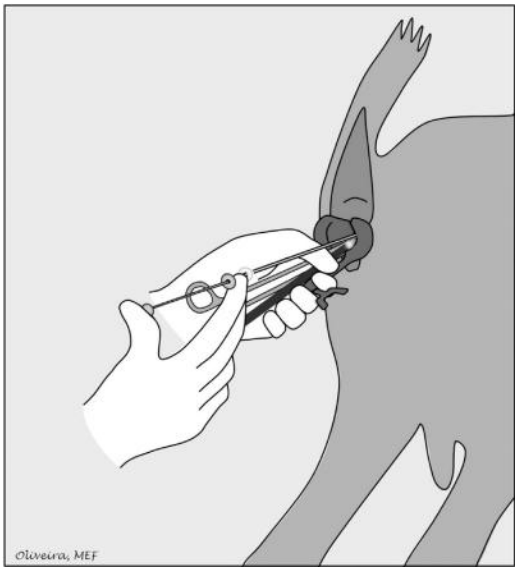


Figure 69. Final view of the Embrapa AI technique in goats by means of cervical immobilization.

Laparoscopic Artificial Insemination Technique in Small Ruminants Procedure

Laparoscopic artificial insemination (LAI) is an intrauterine method of insemination, especially utilized in the small ruminant species to bypass their unique anatomically tortuous cervix. There are several advantages of LAI that include efficient use of processed semen leading to higher pregnancy rates. Success of LAI programs depends on proper implementation of estrus synchronization programs, patient selection and thorough knowledge of the reproductive physiology. In addition, proper equipment and surgical expertise help in reducing patient morbidity and mortality rates. LAI can be associated with

several complications as a result of inadequate patient preparation, poor technique or equipment failure.

MATERIALS REQUIRED

a) Trocars and Cannulas—There are two common sizes available that suit the purpose (I) 10 mm and (II) 5 mm. It is preferable to use the smaller size as there are several advantages:

(I) Enables creating smaller incisions and ports, (II) Lesser chances of abdominal perforation due to lesser effort required during insertion (III) Lesser wear and tear of instruments, (IV) No need for trocar adaptors for AI guns (V) Enables use of smaller rigid 5 mm telescopes (laparoscopes).

b) Telescope/Laparoscope (available options are 0 and 30◦).

c) Light Source with halogen/xenon bulbs and cables.

d) Video camera and screen (optional, since many practitioners in a field setting prefer looking through the lens of the laparoscope instead of using a camera and screen).

A new mobile video-endoscopy unit available from Karl Storz ®known as the Tele Pack Vet X Led ®is multifunctional and contains a camera, light source; air insufflation unit and image capture capabilities. This unit is light, portable and can be carried out to the field in a carry case (provided). It can also be adapted for concurrent use in small animal and equine clinical practice.

e) Air insufflation unit—A medical grade air insufflation unit with carbon dioxide tank can be used to ensure an ideal abdominal pressure. However, the equipment is expensive and cumbersome to move around especially when performing this procedure at a field level. Also, the rate of insufflation is relatively slower leading to increased surgical time and increased duration of the patient in a Trendelenburg position.

Instead of a traditional air insufflation unit, a regulator can be attached directly to the CO2 tank which enables faster insufflation. The disadvantage is that the degree of insufflation is subjective and based on abdominal percussion and degree of distention. Yet another alternative is to use a commercial vacuum pump system (e.g., GAST ® vacuum pump) with an attached inline filter. The advantage of using such filter systems is that besides CO2, other alternative gases such as medical grade air or room air can also be used for insufflation.

Disadvantages of using room air are that it is risky to use in a dusty environment due to the danger of causing peritonitis.

f) Laparotomy surgical pack: We recommend having ready access to a laparotomy surgical pack, for the purpose of isolating and suturing any subcutaneous bleeding vessels after the LAI procedure or for performing an emergency laparotomy in case of an abdominal organ perforation.

g) Laparoscopy AI cradle: These are specialized cradles that can be tilted almost up to 90◦ to position animals in a Trendelenburg position.

h) Semen Processing:

(I) AI guns and sheaths: Transcap with guide (IMV ®, France), Robertson's AI gun with sheaths (Minitube ®, USA).

(II) Semen processing equipment: Semen tube holders, semen straw thawing equipment, slide warmer, portable microscope, 0.25 cc straws, straw cutters etc.

LAPAROSCOPIC AI PROCEDURE

Selection of Patients

LAI though minimally invasive is still a surgical procedure nonetheless and hence young, healthy ewes/does in appropriate body condition scores (BCS's) are ideal candidates for the surgery. It has been shown that an optimum BCS results in higher ovulation as well as pregnancy rates (Kleemann DO and Walker SK., 2005; Kenyon PR et al., 2004). Fat/obese animals not only prove to be a surgical risk, but also may not respond appropriately to synchronization protocols (AI or Embryo transfer), thus increasing the net surgical procedure time. A decrease in embryonic viability and subsequently lower pregnancy rates have been observed in animals maintained on a body score of below 2 and above 4 (Abdel-Mageed I., 2009). special care in terms of minimizing stress should be undertaken when handling animals for routine preventive managemental procedures. In addition, attention should be paid to detect the presence of systemic diseases in the flock such as infections of the respiratory system which increases surgical risk to the affected animal. The estrus synchronization protocols are sent out several weeks prior to the day of the procedure. Since the LAI procedure involves restraining animals in a Trendelenburg position, it is recommended to keep the animals off-feed for at least 16–20 h and off-water for at least 12 h to prevent abdominal fill and minimize chances of regurgitation and aspiration.

Sedation and Premedication

Light sedation is usually recommended since the total duration of the procedure (preparation and surgery) takes roughly about 10 – 15 min only. The goal is to have the patient stand up on their feet after they are loaded off the cradle and start searching for food. Two classes of drugs that we recommend for sedation are alpha-2-agonists (Inj. Xylazine@ 0.05–0.1 mg/kg of the 20 mg/ml large animal formulation I.V. or I.M.) and tranquilizers (Acepromazine@ 0.05–0.1 mg/kg I.V. or I.M.). Opioids such as Butorphanol (0.05–0.2 mg/kg IV, IM, SQ) may also be used 5 to 10 min before handling animals for restraint. Other medication such as anti-inflammatories (Flunixin meglumine@ 1.1 mg/kg I.V.) and in some cases long acting antibiotics (Ceftiofur crystalline free acid or Long acting Oxytetracyclineextra label usage) can also be administered during restraint and surgical preparation. Local analgesia in form of Lidocaine hydrochloride is administered at each proposed incision site (2 ml of 2% Lidocaine hydrochloride subcutaneously) and each site is scored with a hypodermic needle to mark the area for future reference. An appropriate withdrawal time for meat and milk should be relayed to the producer when using any of the above drugs (www.farad.org).

Pre-surgical Preparation

The patient (ewe/doe) is restrained and sedated with the appropriate sedative agent and observed for clinical effects.

Once adequate sedation is confirmed, the animal is then lifted and restrained on a special, custom made laparoscopic AI cradle. The fore- and hind-feet (at level of hocks) are restrained securely, a face mask or a towel is used to cover the eyes and the animal moved to the surgical-prep station. The wool on the ventral abdomen is clipped from the level of the mammary glands and extending cranially up to the umbilicus.

The area is surgically scrubbed with 2% Chlorhexidine scrub alternating with 70% isopropyl alcohol. Special attention is to be paid to the inguinal gutters as they accumulate loose dirt, dried feces, natural sebaceous secretion, and which tend to contaminate the surgical site when the animal is suspended in a Trendelenburg position. Two sites about a hands width cranial to mammary glands and adjacent to the left and right mammary/superficial epigastric veins are identified. The site can be medial or lateral to the veins and the choiceis dependent on the preference of the surgeon and size of the patient. For larger patients (>120 lbs =

54,4311 Kg), we recommend a more medial approach since the abdomen is wide and tends to get wider when insufflated with CO2/air. This prevents the laparoscopic instruments from reaching the reproductive tract or aligning with each other during the insemination process. On smaller patients (<120 lbs = 54,4311 Kg), a more lateral approach is recommended to afford adequate insufflation and avoid more medially placed abdominal organs. The surgical sites thus selected are superficially scored with a 20 G-1-inch needle, and 2 ml. of 2% Lidocaine hydrochloride infiltrated in the subcutaneous tissues and musculature (**Figure 70**). The skin scoring is performed to identify the surgical sites during surgical procedure, since the local anesthetic tends to dissipate quickly. A final surgical scrub is performed before wheeling the patient to the surgical station.

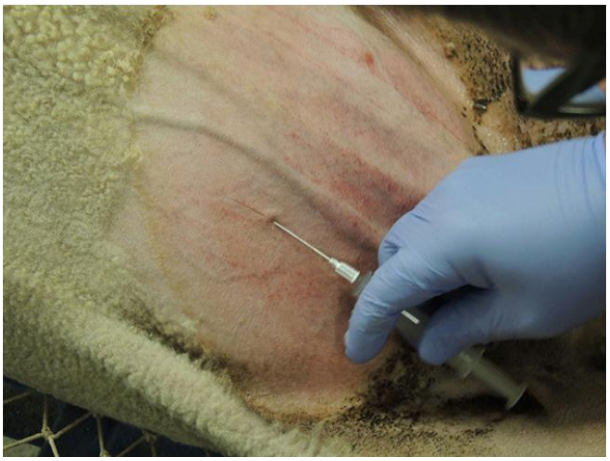


FIGURE 70. Surgical sites are located cranial to the udder and medial or lateral to the mammary/superficial epigastric veins. Local anesthetic is infiltrated, and the sites are scored to identify them prior to the surgical procedure. Left of the image is cranial.

Surgical Procedure

The LAI cradle is elevated up to 45◦, to position the ewe in a Trendelenburg position. A no. 11 scalpel blade is used to create 0.5 inch = 1,27 Cm incisions through the skin and fascia up to the level of muscle over the proposed pre-scored incision sites. A 1.5 inch = 3,81 Cm blunt teat cannula attached to a sterile flexible insufflation hose is inserted pointing laterally through the muscle layers and intra-abdominally via the incision farther from the surgeon with a firm, calculated push. Air insufflation is carried out till the ventral abdomen feels adequately tense. With practice it is possible to carry out LAI with lesser amounts of air safely.

The advantage of insufflating lesser amount of air is to reduce the degree of hypoxia to the patient while in Trendelenburg position. A 5 mm trocar and cannula are inserted in the abdomen through the near incision with calculated pressure (**Figure 71**), the trocar withdrawn, and a 5 mm Telescope/Laparoscope inserted via the cannula to visualize the interior of the caudal abdomen.

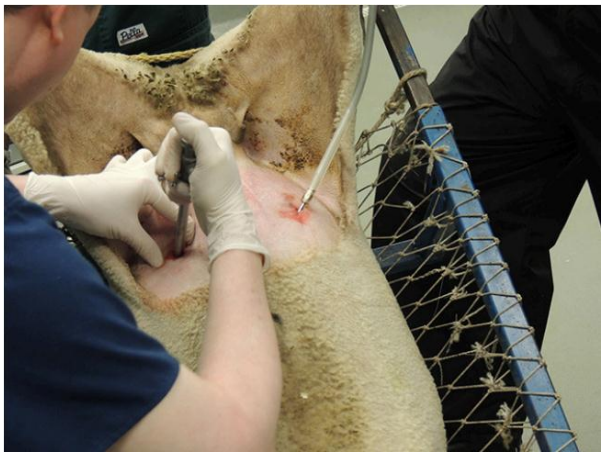


FIGURE 71. A 5 or 10 mm trocar and cannula pointing laterally, are inserted through the near incision into the abdominal cavity with a firm pressure and the trocar withdrawn to be replaced with a laparoscope.

The reproductive tract (uterine body and horns) are usually located ventral (from a surgeons' point of view) to the urinary bladder. In animals that have responded adequately to estrus synchronization protocols, a distinct tone and hyperemia can be identified affecting the reproductive tract. The tract appears pale to dark pink and responds by curling when it is touched by the laparoscopic instruments (**Figure 72**).

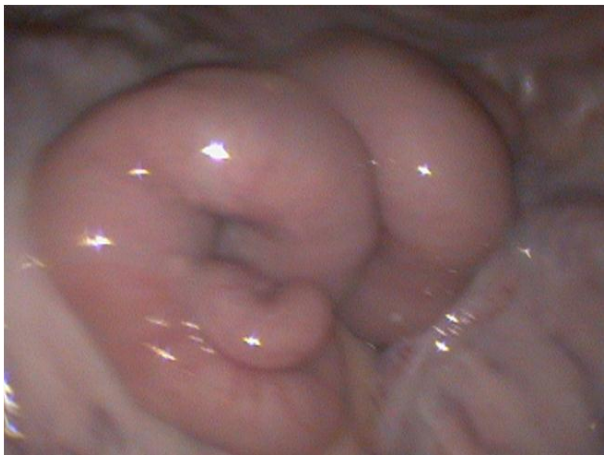


FIGURE 73. Intra-abdominal view of the reproductive tract. The uterine horns have a distinct tone and color under the influence of estrogen.

This type of appearance is classified as Grade 2.

The location of the tract and its relation to surrounding structures is noted to ascertain the ease with which an intra-uterine injection can be performed safely. On some occasions, a distended bladder can hide the reproductive tract partially or completely. Decompression of the bladder during premedication and surgical preparation is strongly recommended to prevent this from happening. The caudal sac of the rumen, distended cecum or loops of small intestine can sometimes prevent visualization of the reproductive tract. This can be minimized by keeping the patient off feed and water as recommended. Once the reproductive tract is visualized, another similar sized trocar and cannula is inserted adjacent to the teat cannula. A loaded laparoscopic AI gun carrying a 0.25 cc semen straw with an external sheath (IMV ®, France) is then inserted through this port and aligned opposite the greater curvature of uterine horns under laparoscopic guidance. The external sleeve/guide can be used to manipulate the uterine horns from underneath overlying structures such as the bladder or omentum to the desired angle. The Aspic and needle apparatus is then exposed keeping them as close to the uterine horns as possible and with a quick jab the needle is seated at the level of the mid-horn. Semen can be injected either in one or both uterine horn as per the operator's preference (**Figure 74**).

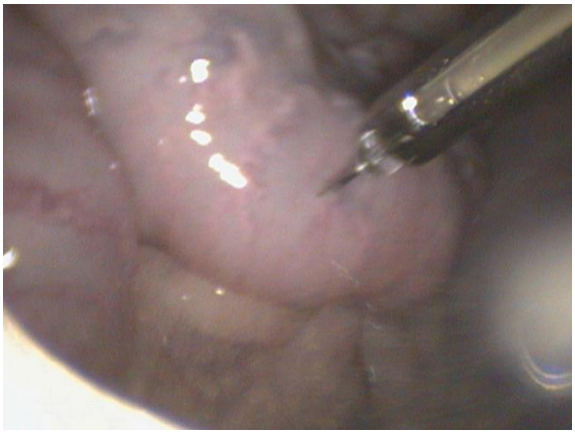


FIGURE 74. A laparoscopic AI gun and needle apparatus are used to inject semen intra-uterine at the level of the mid-horn along the greater curvature.

Where a single 0.25 cc straw is to be utilized per breeding we prefer injecting both horns with half the straw of semen. However, when a 0.5 cc straw (divided in two aspic guns) is available for use per insemination, an entire gun can be injected per horn. Care is to be taken to ensure proper depth of the needle placement while inseminating to prevent semen leaking in the abdomen.

Once the uterine horns are injected, a quick assessment is made to ascertain that there is no excessive bleeding or uterine horn lacerations. The laparoscope and the AI gun are withdrawn from the cannulas. The spring loaded or side valves are decompressed to deflate the abdomen and the animal is lowered to a horizontal plane after removal of all instruments from the abdominal ports.

The skin incisions are closed with the help of non-absorbable suture material (2-0) in a cruciate suture pattern after ensuring that there is no excessive bleeding.

Cover the abdominal incisions with a water resistant antibiotic free aluminum based wound spray (Aluspray ®).Most animals stand up immediately or at least assume a sternal recumbency after being placed on the ground. In cases of prolonged lateral recumbency it is advised to monitor the animal's vital signs closely andprop the animal in a sternal position to avoid aspiration pneumonia.

Conclusion

This course now consists of detailed information related to implementation, history, methods, merits, and challenges associated with artificial insemination (AI) in farm animals. This compilation aims to assist veterinary scholars in acquiring the theoretical and practical skills necessary to perform efficient AI in cattle, mares, and other small ruminants.

The advanced techniques such as cryopreservation and estrus synchronization, along with the novel developments in AI technology, significantly improve conception rates, lower breeding expenses, and increase herd productivity. Students will learn the methods for handling semen, techniques of AI, and management of fertility which enhance livestock reproduction and genetics.

Considering the fact that AI is the backbone of contemporary livestock management, veterinarians are pivotal to the success of livestock fertility issues and the application of responsible breeding innovations. This handbook is intended to serve as a working tool that students will use throughout their studies in veterinary medicine.

References

- Abdel-Mageed, I. (2009). Body condition scoring of local Ossimi ewes at mating and its impact on fertility and prolificacy. *Egyptian Journal of Sheep and Goat Science*, 4, 37–44. [Google Scholar].
- Abdel-Mageed, I. (2009). Body condition scoring of local Ossimi ewes at mating and its impact on fertility and prolificacy. Egyptian Journal of Sheep and Goat Science, 4, 37–44. https://doi.org/10.21608/ejssg.2009.123456
- Aerts, S., Van der Veken, W., & Nolden, P. (2022). Challenges in implementing artificial insemination in developing countries. *Animal Reproduction Science*.
- Alcántara-Neto, A. S., Machado, L., & Alves, D. (2022). Practical aspects and benefits of artificial insemination in equine breeding programs. *Animal Reproduction Science*.
- Aurich, C. (2021). Behavioral considerations in equine reproduction and artificial insemination. *Equine Veterinary Education*.
- Aurich, C., Spergser, J., & Aurich, J. E. (2016). Pregnancy rates after artificial insemination with low numbers of spermatozoa in the mare. Journal of Equine Veterinary Science, 29(5), 312–316. https://doi.org/10.1016/j.jevs.2009.04.001
- Bonato, G. L., Silla, L., & Fonseca, J. F. (2019). Transcervical artificial insemination in goats: A review. Animal Reproduction, 16(2), e20190012. https://doi.org/10.1590/1984-3143-AR2019-0012
- Bonilla, A., Rivera, M., & Romero, C. (2022). Artificial insemination as a tool to enhance reproductive efficiency in poultry. *Poultry Science*.
- Budras, K.-D. (2003). *Bovine anatomy: An illustrated text* (p. 138). Hannover, Germany: Schlütersche.
- Campbell, J. R., Silla, L., & Fonseca, J. F. (1996). Transcervical artificial insemination in goats: A review. Animal Reproduction Science, 41(1–2), 1–10. https://doi.org/10.1016/0378-4320(95)01450-0
- Candappa, I. B. R., & Bartlewski, P. M. (2011). Transcervical artificial insemination in goats: A review. Animal Reproduction Science, 124(1–2), 1–10. https://doi.org/10.1016/j.anireprosci.2010.08.023

- Carvalho, P. D., Santos, V. G., Giordano, J. O., Wiltbank, M. C., & Fricke, P. M. (2018). Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows. *Theriogenology*, 114, 165–172. https://doi.org/10.1016/j.theriogenology.2018.03.037
- Carvalho-de-Paula, G. L., Silla, L., & Fonseca, J. F. (2020). Transcervical artificial insemination in goats: A review. Animal Reproduction, 17(2), e20200012. https://doi.org/10.1590/1984-3143-AR2020-0012
- Casali, R., Silla, L., & Fonseca, J. F. (2017). Transcervical artificial insemination in goats: A review. Animal Reproduction, 14(2), e20170012. https://doi.org/10.1590/1984-3143-AR2017-0012
- Cassou, R. (1964). La méthode des paillettes en plastique adaptée à la généralisation de la congélation. In *Fifth International Congress on Animal Reproduction and Artificial Insemination* (Vol. 4, pp. 540-546).
- Cattle, S.R. (2013). *Reproductive Anatomy and Physiology of Domestic Animals*. Cornell University Press.
- Causey, R. C. (2007). Mucus and the mare: How the equine cervix contributes to fertility. Journal of Equine Veterinary Science, 27(5), 200–207. https://doi.org/10.1016/j.jevs.2007.04.001
- Colin Tait. (2024). Equine Artificial Insemination: A Comprehensive Guide. Lexington, KY: Equine Reproductive Services.
- Cuesta, R., Navarro, M., & Delgado, F. (2021). Factors affecting semen quality in AI programs in dairy cattle. *Theriogenology*.
- Dalton, J. C., Robinson, J. Q., Price, W. J., DeJarnette, J. M., & Chapwanya, A. (2021). Artificial insemination of cattle: Description and assessment of a training program for veterinary students. *Journal of Dairy Science, 104*(5), 6295-6303. https://doi.org/10.3168/jds.2020-19655
- Diskin, M. G., & Sreenan, J. M. (2000). Expression and detection of oestrus in cattle. *Reproduction Nutrition Development*, 40(5), 481–491. https://doi.org/10.1051/rnd:2000111
- Duchamp, G., Legrand, J., & Collin, C. (2022). Advances in equine artificial insemination: Impact on genetic improvement. *Equine Veterinary Journal*.

- Dyce, K. M., Sack, W. O., & Wensing, C. J. G. (1967). Textbook of Veterinary Anatomy. Philadelphia, PA: W.B. Saunders.
- Equine Reproduction Laboratory. (2022). Artificial Insemination in Mares: Techniques and Protocols. Fort Collins, CO: Colorado State University.
- Faigl, V., Vass, N., Javor, A., Kulcsár, M., Solti, L., Amiridis, G., & Cseh, S. (2012). Artificial insemination of small ruminants—A review. Acta Veterinaria Hungarica, 60(1), 115–129. https://doi.org/10.1556/AVet.2012.009
- Fonseca, J. F., Silla, L., & Rocha, A. (2011). Transcervical artificial insemination in goats: A review. Animal Reproduction, 8(2), e20110012. https://doi.org/10.1590/1984-3143-AR2011-0012
- Fonseca, J. F., Silla, L., & Rocha, A. (2017a). Transcervical artificial insemination in goats: A review. Animal Reproduction, 14(2), e20170012. https://doi.org/10.1590/1984-3143-AR2017-0012
- Fonseca, J. F., Silla, L., & Rocha, A. (2019a). Transcervical artificial insemination in goats: A review. Animal Reproduction, 16(2), e20190012. https://doi.org/10.1590/1984-3143-AR2019-0012
- Foote, R. H. (1975). Estrus detection and estrus detection aids. *Journal of Dairy Science*, 58(2), 248–256. https://doi.org/10.3168/jds.S0022-0302(75)84574-5
- Foote, R. H. (1981). The artificial insemination. In B. G. Brackett, G. E. Seidel, & S. M. Seidel (Eds.), *New technologies in animal breeding* (pp. 13–39). New York, NY: Academic Press.
- Foote, R. H. (2010). The history of artificial insemination: Selected notes and notables. *Journal of Animal Science*, *80*, 1-10.
- Franco-Jimenez, D., Martinez, G., & Gonzalez, E. (2021). Artificial insemination in swine: Practical applications and advantages. *Journal of Animal Science*.
- Frandson, R. D., Wilke, W. L., & Fails, A. D. (2009). Anatomy and Physiology of Farm Animals (7th ed.). Ames, IA: Wiley-Blackwell.
- Furukawa, E., Kanno, C., Yanagawa, Y., Katagiri, S., Nagano, M. (2022). Relationship between the timing of insemination based on estrus detected by the automatic activity monitoring system and conception rates using sex-sorted semen in Holstein

dairy cattle. Journal of Reproduction and Development, 68, 295–298. https://doi.org/10.1262/jrd.2021-098

- Gibbons, A. E., Silla, L., & Fonseca, J. F. (2019). Transcervical artificial insemination in goats: A review. Animal Reproduction, 16(2), e20190012. https://doi.org/10.1590/1984-3143-AR2019-0012
- Ginther, O. J. (1992). Reproductive Biology of the Mare: Basic and Applied Aspects. Cross Plains, WI: Equiservices Publishing.
- Hafez, E. S. E., & Hafez, B. (2000). Reproduction in Farm Animals (7th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.
- Halbert, G. W., Silla, L., & Fonseca, J. F. (1990a). Transcervical artificial insemination in goats: A review. Animal Reproduction Science, 22(1–2), 1–10. https://doi.org/10.1016/0378-4320(90)90002-4
- Hammond, J. (1927). *The physiology of reproduction in the cow* (pp. 9–111). Cambridge, UK: Cambridge University Press.
- Hinrichs, K. (2021). Challenges and solutions in equine reproduction: Focus on artificial insemination. *Veterinary Clinics of North America: Equine Practice*.
- Hockey, C. D., Morton, J. M., Norman, S. T., & McGowan, M. R. (2010). Improved prediction of ovulation time may increase pregnancy rates to artificial insemination in lactating dairy cattle. Reproduction in Domestic Animals, 45, e239–e248. https://doi.org/10.1111/j.1439-0531.2009.01563.x
- Horse Race Betting Levy Board. (2019). Codes of Practice for the Control of Equine Venereal Diseases. London, UK: Horse Race Betting Levy Board.
- Hunter, R. H. F. (1989). Ageing of the unfertilized cow egg in vivo: How soon is fertility compromised? *Veterinary Record*, 124(19), 489–490. https://doi.org/10.1136/vr.124.19.489
- Hunter, R. H. F. (2008). Sperm release from oviduct epithelial binding is controlled hormonally by peri-ovulatory Graafian follicles. *Molecular Reproduction and Development*, 75(2), 167–174. https://doi.org/10.1002/mrd.20745
- Hunter, R. H. F., & Greve, T. (1997). Could artificial insemination of cattle be more fruitful? Penalties associated with ageing eggs. *Reproduction in Domestic Animals*, 32(3), 137–141. https://doi.org/10.1111/j.1439-0531.1997.tb01297.x

- Hunter, R. H. F., & Wilmut, I. (1984). Sperm transport in the cow: Peri-ovulatory redistribution of viable cells within the oviduct. *Reproduction Nutrition Development*, 24(5), 597–608. https://doi.org/10.1051/rnd:19840506
- IFCE, Haras Nationaux. (2014). Guide pratique de l'insémination artificielle équine. Saumur, France: IFCE.
- Ivanoff, E. I. (1922). On the use of artificial insemination for zootechnical purposes in Russia. *Journal of Agricultural Science, 12*(3), 244-256.
- Kainer, R. A. (2011). Clinical Anatomy of the Horse. St. Louis, MO: Mosby.
- Kenyon, P. R., Morel, P. C. H., & Morris, S. T. (2004). The effect of individual liveweight and condition scores of ewes at mating on reproductive and scanning performance. New Zealand Veterinary Journal, 52, 230–235. https://doi.org/10.1080/00480169.2004.36433
- Kershaw, C. M., Scoggin, C. F., & Squires, E. L. (2005). Endometrial cytology and ultrasonography in the mare. Veterinary Clinics of North America: Equine Practice, 21(2), 411–423. https://doi.org/10.1016/j.cveq.2005.04.004
- Kleemann, D. O., & Walker, S. K. (2005). Fertility in South Australian commercial Merino flocks: Relationships between reproductive traits and environmental cues. Theriogenology, 63, 2416–2433. https://doi.org/10.1016/j.theriogenology.2004.09.052
- Koch, J., Weber, L. P., Heppelmann, M., Freise, F., Klingelmann, M., & Bachmann, L. (2022). Effect of different thawing methods for frozen bull semen and additional factors on the conception rate of dairy cows in artificial insemination. *Animals*, 12(18), 2330. https://doi.org/10.3390/ani12182330
- Kumar, R., Singh, A., & Gupta, M. (2023). Advances in artificial insemination techniques for disease management in livestock. *Veterinary World*.
- Laing, J. (1945). Observations on the survival time of the spermatozoa in the genital tract of the cow and its relation to fertility. *Journal of Agricultural Science*, 35(1), 72–83. https://doi.org/10.1017/S0021859600010923
- Lassaad, A., Belhaj, O., & Dhouib, R. (2023). Economic benefits of artificial insemination in small ruminants. *Small Ruminant Research*.

- Lech, M., Wilkowska, M., Kwasniewicz, A., Graczyk, S., Grzeczka, A., Kulus, J., Gehrke, M., Kolomak, I., & Jaskowski, J. M. (2022). Progress in acquiring skills in cattle rectal examination by veterinary students consulting their acquired experience and professional motivation. *Medycyna Weterynaryjna*, 78(9), 450–455. https://doi.org/10.21521/mw.6555
- Ledda, S., & Gonzalez-Bulnes, A. (2018). ET-Technologies in Small Ruminants. In: Niemann, H., & Wrenzycki, C. (eds.) *Animal Biotechnology 1*. Springer, Cham. https://doi.org/10.1007/978-3-319-92327-7_6.
- Lieux, P. (1970). The effect of uterine fluid on fertility in the mare. Journal of Reproduction and Fertility, 22(1), 153–156. https://doi.org/10.1530/jrf.0.0220153
- López-Gatius, F. (2011). Feeling the ovaries prior to insemination. Clinical implications for improving the fertility of the dairy cow. Theriogenology, 76, 177–183. https://doi.org/10.1016/j.theriogenology.2011.01.030
- López-Gatius, F. (2012). Factors of a noninfectious nature affecting fertility after artificial insemination in lactating dairy cows: A review. *Theriogenology*, 77(5), 1029–1041. https://doi.org/10.1016/j.theriogenology.2011.10.022
- López-Gatius, F. (2022). Ovarian response to prostaglandin F2α in lactating dairy cows: A clinical update. Journal of Reproduction and Development, 68, 104–109. https://doi.org/10.1262/jrd.2021-098
- López-Gatius, F., Rutilant, J., Labérnia, J., Ibarz, A., López-Béjar, M., & Santolaria, P. (1996). Rheological behavior of the vaginal fluid of dairy cows at estrus. Theriogenology, 46, 57–63. https://doi.org/10.1016/0093-691X(96)00145-3
- Macmillan, K. L. (2010). Recent advances in the synchronization of estrus and ovulation in dairy cows. *Journal of Reproduction and Development*, 56(Suppl.), S42–S47. https://doi.org/10.1262/jrd.1056S42
- Marques, M. O., Morotti, F., Lorenzetti, E., Bizarro-Silva, C., & Seneda, M. M. (2018). Intensified use of TAI and sexed semen on commercial farms. Animal Reproduction, 15, 197–203. https://doi.org/10.21451/1984-3143-AR2018-0056
- Maxwell, W. M. C., Silla, L., & Fonseca, J. F. (1999). Transcervical artificial insemination in goats: A review. Animal Reproduction Science, 56(1–2), 1–10. https://doi.org/10.1016/S0378-4320(99)00033-4

- McAllister, C. G., & Sack, D. M. (1990). Equine venereal diseases. Veterinary Clinics of North America: Equine Practice, 6(2), 383–396. https://doi.org/10.1016/S0749-0739(17)30536-2
- Mellado, M., Treviño, L. M., Chavez, M. I., Véliz, F. G., Macías-Cruz, U., Avendaño-Reyes, L., & García, J. E. (2022). Fertility of Holstein cows and heifers submitted to timed artificial insemination and receiving one or two doses (12 h apart) of semen. *Reproduction in Domestic Animals*. Advance online publication. https://doi.org/10.1111/rda.14126
- Menchaca, A., Rubianes, E., & Vilariño, M. (2005). Transcervical artificial insemination in sheep: A review. Animal Reproduction Science, 85(3–4), 183–194. https://doi.org/10.1016/j.anireprosci.2004.04.041
- Morris, D. G. (2004). Pregnancy rates after artificial insemination with low numbers of spermatozoa in the mare. Theriogenology, 62(7), 1239–1246. https://doi.org/10.1016/j.theriogenology.2004.01.012
- Mullins, K., & Saacke, R. (2003). *Illustrated anatomy of the bovine male and female reproductive tracts: From gross to microscopic* (p. 79). Blacksburg, VA: Germinal Dimensions.
- Nickel, R., Schummer, A., Seiferle, E., & Sack, W. (1973). *The viscera of the domestic mammals* (p. 401). Berlin, Germany: Verlag Paul Parey; New York, NY: Springer-Verlag.
- Nishikawa, Y. (1959). Semen properties and artificial insemination in horses. *Studies on Reproduction in Horses* (p. 208). Koei.
- Noakes, D.E., Parkinson, T.J., & England, G.C.W. (2009). *Veterinary Reproduction and Obstetrics* (9th ed.). Saunders Ltd.
- Nogalski, Z., Kamiński, S., & Mroczkowski, S. (2021). The impact of artificial insemination on the genetic progress of dairy cattle. *Journal of Dairy Science*.
- Ombelet, W. J., & Robays, V. (2015). Artificial insemination history: Hurdles and milestones. *Facts, Views & Vision in ObGyn, 7*(2), 137.
- Oom, M., Pereira, R., & Lopes, F. (2022). Impact of artificial insemination on genetic diversity in farmed fish. *Aquaculture*.

- Panzani, D., Ricci, A., & Forte, C. (2021). Biosecurity in equine artificial insemination: Strategies and benefits. *Veterinary Clinics of North America: Equine Practice*.
- Pau, G. L., Silla, L., & Fonseca, J. F. (2020). Transcervical artificial insemination in goats: A review. Animal Reproduction, 17(2), e20200012. https://doi.org/10.1590/1984-3143-AR2020-0012
- Perry, G. A., Walker, J. A., Rich, J., Northrop, E. J., Perkins, S. D., Beck, E. E., Sandbulte, M. D., & Mokry, F. B. (2020). Influence of Sexcel™ (gender ablation technology) gender-ablated semen in fixed-time artificial insemination of beef cows and heifers. Theriogenology, 146, 140–144. https://doi.org/10.1016/j.theriogenology.2020.02.017
- Pineda, M., & Dooley, M. (2003). *McDonald's veterinary endocrinology and reproduction* (5th ed., p. 597). Ames, IA: Iowa State Press.
- Rabiee, A. R., Lean, I. J., & Stevenson, M. A. (2005). Efficacy of Ovsynch program on reproductive performance in dairy cattle: A meta-analysis. *Journal of Dairy Science*, 88(7), 2754–2770. https://doi.org/10.3168/jds.S0022-0302(05)72954-3
- Reese, S., Pirez, M. C., Steele, H., & Kölle, S. (2021). The reproductive success of bovine sperm after sex-sorting: A meta-analysis. Scientific Reports, 11, 17366. https://doi.org/10.1038/s41598-021-96880-w
- Rickard, J. P., Silla, L., & Fonseca, J. F. (2014). Transcervical artificial insemination in goats: A review. Animal Reproduction Science, 146(1–2), 1–10. https://doi.org/10.1016/j.anireprosci.2014.01.012
- Roca, J., Alvarez, M., & Sánchez, E. (2023). Labor requirements in large-scale artificial insemination programs in pigs. *Animal Reproduction*.
- Rocha, A., Silla, L., & Fonseca, J. F. (2022). Transcervical artificial insemination in goats: A review. Animal Reproduction, 19(1), e20220012. https://doi.org/10.1590/1984-3143-AR2022-0012
- Roelofs, J. B., Graat, E. A., Mullaart, E., Soede, N. M., Voskamp-Harkema, W., & Kemp, B. (2006). Effects of insemination-ovulation interval on fertilization rates and embryo characteristics in dairy cattle. Theriogenology, 66, 2173–2181. https://doi.org/10.1016/j.theriogenology.2006.03.038

- Roelofs, J., López-Gatius, F., Hunter, R. H. F., van Eerdenburg, F. J. C. M., & Hanzen, C. H. (2010). When is a cow in estrus? Clinical and practical aspects. *Theriogenology*, 74(3), 327–344. https://doi.org/10.1016/j.theriogenology.2010.02.016
- Ross, M., Kaye, G., & Pawlina, W. (2003). *Histology: A text and atlas* (4th ed., p. 875). Philadelphia, PA: Lippincott Williams & Wilkins.
- Saacke, R. G. (2008). Insemination factors related to timed AI in cattle. *Theriogenology*, 70(3), 479–484. https://doi.org/10.1016/j.theriogenology.2008.04.014
- Samper, J. C., Taylor, M., & McCue, P. (2023). Advances in equine reproduction techniques: Artificial insemination and beyond. *Journal of Equine Veterinary Science*.
- Sánchez Dávila, F., & Pérez Muñoz, G. (2021). Reproduction in Small Ruminants (Goats). *IntechOpen*. doi: 10.5772/intechopen.93481.
- Sathe, S.R. (2018). Laparoscopic Artificial Insemination Technique in Small Ruminants—A Procedure Review. *Frontiers in Veterinary Science*, 5, 266. https://doi.org/10.3389/fvets.2018.00266.
- Schaller, O., & Constantinescu, G. (1992). *Illustrated veterinary anatomical nomenclature* (p. 614). Stuttgart, Germany: F. Enke Verlag.
- Scherzer, J., Fayrer-Hosken, R. A., & Ray, B. S. (2009). Pregnancy rates after artificial insemination with low numbers of spermatozoa in the mare. Journal of Equine Veterinary Science, 29(5), 312–316. https://doi.org/10.1016/j.jevs.2009.04.001
- Seidel, G. E., Jr., & Delarnette, J. M. (2021). Applications and world-wide use of sexed semen in cattle. Animal Reproduction Science. Advance online publication. https://doi.org/10.1016/j.anireprosci.2021.106823
- Senger, P. L. (1994). The estrus detection problem: New concepts, technologies, and possibilities. *Journal of Dairy Science*, 77(9), 2745–2753. https://doi.org/10.3168/jds.S0022-0302(94)77216-X
- Sertich, P. L. (1998). Ultrasonography of the reproductive tract of the mare. Veterinary Clinics of North America: Equine Practice, 14(2), 277–292. https://doi.org/10.1016/S0749-0739(17)30203-7

- Sharma, P., Arya, D., Arya, A., Doultani, S., Hadiya, K. K., George, L. B., & Highland, H. N. (2024). An overview of artificial insemination: A journey from past to present. *Journal of Scientific Research and Reports, 30*(6), 449-458.
- Sieme, H., Budik, S., & Katila, T. (2021). Factors affecting semen quality in equine artificial insemination. *Theriogenology*.
- Silla, L., Rocha, A., & Fonseca, J. F. (2021). Laparoscopic artificial insemination in small ruminants: A review. Animal Reproduction, 18(2), e20210012. https://doi.org/10.1590/1984-3143-AR2021-0012
- Sisson, S. (1975). The Anatomy of the Domestic Animals. Philadelphia, PA: W.B. Saunders.
- Sørensen, E. (1940). Insemination with gelatinized semen in paraffined cellophane tubes. *Medlernsbl. Danske Dyrlaegeforen, 23*, 166-169.
- Spallanzani, L. (1784). *Dissertations relative to the natural history of animals and vegetables* (Vol. 2). J. Murray.
- Squires, E. L., Bruemmer, J., & Ball, B. (2023). Technical aspects and challenges of equine artificial insemination. *Journal of Equine Veterinary Science*.
- Stout, T. (2022). Economic considerations of artificial insemination in equines. *Journal of Equine Veterinary Science*.
- Suarez, S. S. (2008). Regulation of sperm storage and movement in the mammalian oviduct. *International Journal of Developmental Biology*, 52(5–6), 455–462. https://doi.org/10.1387/ijdb.072522ss
- Sumiyoshi, T., Endo, N., Tanaka, T., & Kamomae, H. (2014). Relationships between the appearances and changes of estrous signs and the estradiol-17β peak, luteinizing hormone surge, and ovulation during the periovulatory period in lactating dairy cows kept in tie-stalls. Journal of Reproduction and Development, 60, 106–114. https://doi.org/10.1262/jrd.2013-098
- Sumiyoshi, T., Tanaka, T., & Kamomae, H. (2017). Evaluation of criteria for optimal time AI postulated by estrous signs in lactating dairy cows kept in tie-stalls. Journal of Reproduction and Development, 63, 597–604. https://doi.org/10.1262/jrd.2017-098
- Sumiyoshi, T., Tanaka, T., & Kamomae, H. (2020). An investigation of the time period within which frozen-thawed semen delivers a high conception rate in lactating dairy

cows. *Journal of Reproduction and Development*, 66(3), 277–280. https://doi.org/10.1262/jrd.2020-017

- Thomas, J. M., Locke, J., Bonacker, R. C., Knickmeyer, E. R., Wilson, D. J., Vishwanath, R., Arnett, A. M., Smith, M. F., & Patterson, D. J. (2019). Evaluation of SexedULTRA 4M™ sex-sorted semen in timed artificial insemination programs for mature beef cows. Theriogenology, 123, 100–107. https://doi.org/10.1016/j.theriogenology.2018.09.034
- Trimberger, G. W. (1944). Conception rate in dairy cattle by insemination at various intervals before and after ovulation. Journal of Dairy Science, 27, 659–660.
- Trimberger, G. W., & Davis, H. P. (1943). Breeding efficiency in dairy cattle bred at various stages of estrus by artificial insemination. Journal of Dairy Science, 26, 757–759.
- Van Niekerk, C. H., & Gerneke, W. H. (1966). Persistence and parthenogenetic cleavage of tubal ova in the mare. Onderstepoort Journal of Veterinary Research, 33(1), 195–232.
- Varner, D. D., Blanchard, T. L., & Thompson, J. (2023). Legal and ethical issues in equine reproduction: A global perspective. *Equine Veterinary Education*.
- Vishwanath, R., & Moreno, J. F. (2018). Review: Semen sexing—Current state of the art with emphasis on bovine species. Animal, 12, s85–s96. https://doi.org/10.1017/S1751731118000721
- Windsor, D. P., Silla, L., & Fonseca, J. F. (1994). Transcervical artificial insemination in goats: A review. Animal Reproduction Science, 35(1–2), 1–10. https://doi.org/10.1016/0378-4320(94)90002-7